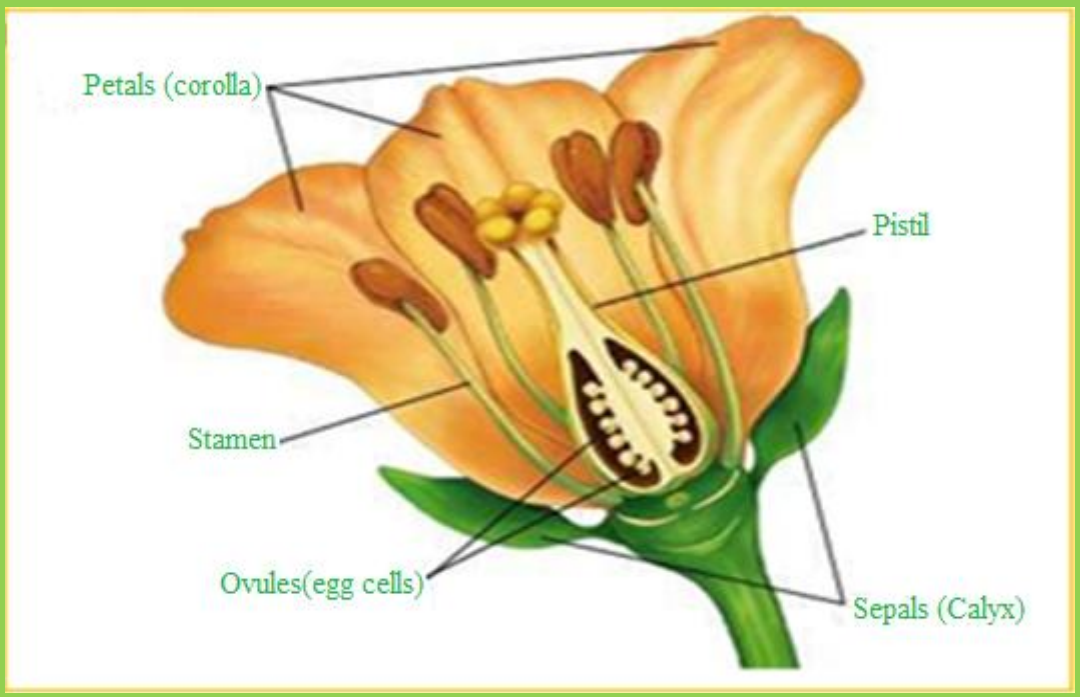
BSCBO- 204

B.Sc. II YEAR

Laboratory Course-II



DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

BSCBO-204

LABORATORY COURSE-II

SCHOOL OF SCIENCES
DEPARTMENT OF BOTANY
UTTARAKHAND OPEN UNIVERSITY

Phone No. 05946-261122, 261123
Toll free No. 18001804025
Fax No. 05946-264232, E. mail info@uou.ac.in
htpp://uou.ac.in

Expert Committee

Board of Studies

Programme Coordinator

Unit Written By:	Unit No.

1. Dr. Manish Belwal
Asst Prof., Department of Botany
Govt. Post Graduate College
Gopeshwar (Chamoli)

1, 2 & 3

2. Dr. G.K. Dhingra
Associate Prof., Department of Botany
Pt. LMS Govt. PG College,
Rishikesh

4

3. Dr. Prem Prakash
Asst Prof., Department of Botany
Govt. PG College, Dwarahat

5, 6, 7 & 8

4. Prof. N.P.Melkania
Dept. of Environmental Science,
School of Vocational Studies and Applied Sciences,
Gautam Buddha University,
Greater Noida
and
Monali Varun
Scientist EIA studies, Enviro Infra Solutions Pvt. Ltd.
Ghaziabad

9, 10 & 11

5. Dr. Pawan Agarwal
Associate Professor
Department of Biotechnology
GBPIET, Ghurdauri, Pauri,

12

Course Editor

Prof. N.S. Bisht
Head, Department of Botany
HNB Garhwal Central University
Pauri Campus, Pauri

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CONTENTS

BLOCK-1 DIVERSITY OF ANGIOSPERMS

UNIT-1 IDENTIFICATION OF LOCALITY OF AVAILABLE PLANTS BELONGING TO THE FAMILIES AND THEIR DESCRIPTION IN SEMI TECHNICAL LANGUAGE

1.1 OBJECTIVES

After reading this section you will know, how to-

- Carry out plant identification
- Describe the locality of available plants
- Describe the meaning of semi technical language
- Describe the families in semi technical language.

1.2 INTRODUCTION

The flowering plants, also known as Angiospermae or Magnoliophyta, are also considered to be the most diverse group of land plants constituting about 443families. Angiosperms are distinguished from gymnosperms on the basis of specific characteristics including flowers, endosperms and the fruit production. An angiospermic plant produces seeds within an enclosure (a fruit). The term "angiosperm" comes from the Greek word (*angeion*, "case" or "casing", and *sperma*, "seed") meaning "enclosed seeds".

Angiosperms might be differentiated from other seed plants in several ways. The characteristic feature of angiosperms is the flower. Flowers show remarkable variation in form and elaboration, and provide the most trustworthy external characteristics for establishing relationships among angiosperm species. Mostly, the floral apparatus arise terminally on a shoot or from the axil of a leaf. Occasionally, a flower arises singly in the axil of an ordinary foliage leaf. Typically, the flower-bearing portion of the plant forms a more or less elaborate branch system called inflorescence.

Traditionally, the flowering plants are divided into two groups as: Dicotyledoneae or Magnoliopsida and Monocotyledoneae or Liliopsida.

Among 443 families of flowering plants, 42 families are referred as the most-diversified on the basis of species. These 42 families are Asteraceae, Fabaceae, Rubiaceae, Poaceae, Lamiaceae, Euphorbiaceae, Melastomataceae, Myrtaceae, Apocynaceae, Cyperaceae, Malvaceae, Araceae, Ericaceae, Gesneriaceae, Apiaceae, Brassicaceae, Piperaceae, Acanthaceae, Rosaceae, Boraginaceae, Utricaceae, Ranunculaceae, Lauraceae, Solanaceae, Companulaceae, Arecaceae, Annonaceae, Caryophyllaceae, Orobanchaceae, Amranthaceae, Iridaceae, Aizoaceae, Rutaceae, Phyllanthaceae, Scrophulariaceae, Gentianaceae, Convolvulaceae, Proteaceae, Sapindaceae, Cactaceae, and Araliaceae.

Plant identification is the process of matching a specimen plant to a known taxon. With the help of dichotomous keys or multi-access keys the plant identification is made. Plant identification has been evolved over hundreds of years and depends to a large extent on what criteria and whose system is used. Plant identification implies comparisons of certain characteristics and then assigning a particular plant to a known taxonomic group, ultimately arriving at a species. **Taxonomy is the branch of botany which deals with plant identification, nomenclature and classification.** The term, first coined by French botanist A. P. de Candolle (1813). Carl Linnaeus used the term 'Systematics' which now includes identification, nomenclature and evolutionary relationships.

1.3 LOCALITY OF AVAILABLE PLANTS

Locality of available plants can be described on the basis of place, spot, or district etc. with reference to plant's availability. As we very well know that innumerable plants grow on the earth. At first we do not know the names of plants growing around us. In fact, most of us never bother to even look around. But we should have a curiosity to know about the variations of these plants. For this, first we have to follow the identification of these plants.

1.3.1-Identification

Usually Identification is a basic activity and one of the primary objectives of systematics. Practically, it involves both classification and nomenclature. Identification is simply the determination of the similarities or differences between two species, i.e., two species are the same or they are different. Here first we will try to compare an unknown plant with a named specimen and then determine that both are the same or showing differences by following classification. If unknown belongs to the same group (species, genus, family. etc.) as a known specimen, the information is stored in classification systems which become available and applicable to the material at hand. Therefore we can say that identification and classification involve comparison and judgment and require a definition of criteria of similarities .

In terms of reliability or accuracy the best method of identification is expert determination. Here let us discuss methods of plant identification:

Methods of Plant Identification:

Here let us know the methods which are used for the description and identification offlowering plants:

First Method:

In this method we have to follow the determination of the families to which the unknown plant belongs. By knowing the name of the family we can turn the keys to genera for determining the generic name and then for the specific identity of the plant to the species key.

Second Method:

In this method we will utilize the latest floras and check list of the particular region. These comprise usually an index to the plants known for the locality and generally provide pertinent habit, distribution and frequency of plants.

Third Method:

The identification of plant is done by studying monographs or revisionary works for the particular family or genus.

Plant Characters before its identification are studied on the following lines:

1. Habit and habitat of plant is studied in natural surroundings only. Then the plant nature is studied whether is herbaceous, or woody; annual or perennial.

2. Plant roots are observed(Tap root or adventitious root/ Branched or unbranched)
3. Leaf phyllotaxy and its venation
4. Inflorescence type – Racemose or cymose or special type as Capitulum (e.g. Asteraceae), Cyathium (e.g. Euphorbiaceae), Verticillaster (e.g. Lamiaceae) etc.
5. Flower – actinomorphic or zygomorphic.
6. Presence of epicalyx (e.g. Malvaceae).
7. Number of sepals and petals or tepals, their aestivation.
8. Petals free (e.g. Polypetalae) or fused (e.g. Gamopetalae).
9. Number of stamens and their position – antipetalous (e.g. Chenopodiaecae) alternipetalous or obdiplostamonous, (e.g. Caryophyllaceae). Staminal tube (e.g. Malvaceae).
10. Number of carpel/carpels, free or fused, style – gynobasic (e.g. Lamiaceae); shape of stigma, ovary unilocular or multilocular .
11. Type of placentation.
12. Kind of fruit .
13. Seed of particular plant is also studied (with reference to Number of seeds in fruit.; Endospermic or non-endospermic; Number of cotyledons.

Different parts of the flower are represented by different symbols which form a formula called floral formula.

Various parts of flower in floral formula are represented as follows:

Br	=	Bracteate
Ebr	=	Ebracteate
Brl	=	Bracteolate
⊕	=	Actinomorphic
Φ	=	Zygomorphic
♂	=	Male
♀	=	Female
⚥	=	Hermaphrodite
K	=	Calyx
C	=	Corolla
P	=	Perianth
A	=	Androecium
G	=	Gynoecium

Here we will also learn about few more representations as if the number of sepals are 5 represented as K_5(polysepalous). $K_{(5)}$ represents that 5 sepals are fused and termed as gamosepalous. $C_5A(\alpha)$ indicates that many fused stamens are epipetalous. For representation of superior ovary a line should be below the gynoecium i.e. $\underline{G}$ and the inferior ovary is

represented by **G** Different whrols of floral parts can be described as C_{5+5} means 10 petals are arranged in two whrols of 5 each.

Floral Diagram

The floral diagram is the most essential and most important after drawing the other necessary diagrams (like a part of the plant, structure of the flower, L.S. of the flower, a stamen, carpel, and T.S. of the ovary) for the given plant. It expresses the number, fusion, symmetry and other similar characteristics of the floral parts in a flower.

Different floral parts in a flower are always expressed in a circular manner. In the different concentric circles of a floral diagram, the sepals should be drawn in the outermost circle. The sepals are followed by petals, stamens and carpels towards inner sides, respectively. Gamosepalous or polysepalous condition is made by joining the sepals or making them free. Same is the case with petals. Position of sepals and petals must be drawn in the respective circles corresponding to their actual position in section. A small circle above the floral diagram represents the mother axis. In zygomorphic flower the mother axis is shown as Φ while in actinomorphic flower it is drawn as 0. The bract, if present, is drawn below the floral diagram while the bracteoles are shown on the sides.

For drawing the floral diagram it is necessary to note whether a sepal or space between two sepals stands towards mother axis. We must start from this particular sepal and mark the position of other sepals. Petals must be drawn alternate to sepals. In actinomorphic flowers all sepals and petals must be of same size. But in case of zygomorphic flowers unequal sized sepals or petals are drawn. Spur in a sepal or petal must be drawn in the form of a loop.

In case of epipetalous condition, the stamens must be joined to petals with a line. Stamens are shown by transverse section of anthers. In obdiplostamonous condition the stamens of outer whorl must be drawn opposite to petals. Introrse stamens are shown facing towards gynoecium while extrorse towards petals. Use a cross (x) or asterisk (*) sign in place of a staminode (sterile stamen), if present. The gynoecium must be drawn in the form of transverse section of the ovary.

1.3.2-Description in Semi Technical Language

After learning the methods for describing and identification of the flowering plants in our locality we will discuss the taxonomic description of families in semi technical language for the identification of locality available plants.

1.3.2.1-Taxonomic Description of Families

1- Ranunculaceae (Butter cup or Crow Foot family)

Ranunculus scleratus L. (Fig.1.1.)

Vegetative Characters:

Habit: Annual herb

Root: Tap roots, branched

Stem: Erect, stout, branched, glabrous and solid, cultivated varieties posses reduced stem.

Leaf: Simple, petiolate, exstipulate (stipules if present are membranous), trilobed, multicostate reticulate venation.

Floral characters:

Inflorescence: Cymose

Flower: Bracteate, bracteolate (2), complete, hermaphrodite, pedicellate, spirocyclic, actinomorphic, pentamerous, hypogynous.

Calyx: Sepals 5, polysepalous, petaloid, imbricate or quincunical aestivation. Sepals are reflexed from the base.

Corolla: Petals 5, polypetalous, bright yellow, spreading from the base, imbricate aestivation. At the base of each petal there is the presence of greenish pocket shaped nectar.

Androecium: Stamens numerous, spirally arranged over the receptacle, polyandrous. Filaments are long and yellow. Anthers are long, basifixed, dithecous, extrose.

Gynoecium: Polycarpellary, apocarpous. Carpels are arranged spirally over an oblong receptacle. Superior ovary, unilocular.Single ovule in each locule with basal placentation. Style is very much reduced or absent and fimbriate stigma.

Fruit: An etario of achenes.

Floral formula:

Br, Brl, $\oplus$, δ, K_5, C_5, Aα,G$\underline{\alpha}$.

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons in each seed
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**

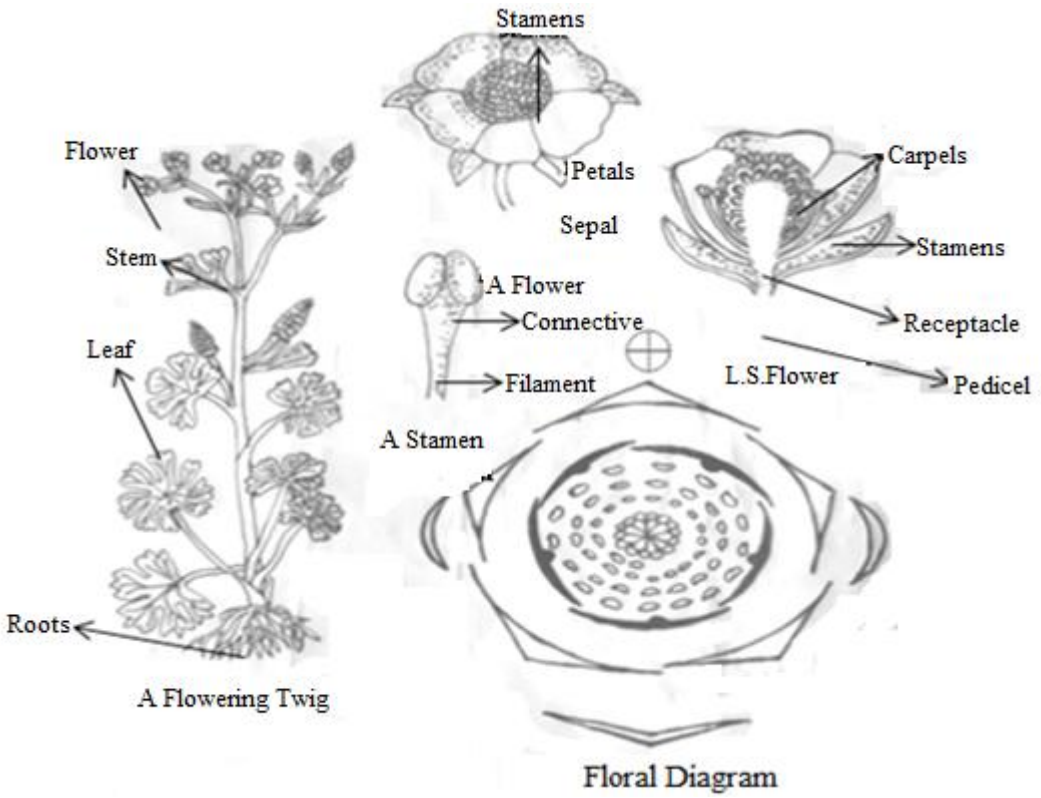

Fig.1.1 ***Ranunculus scleratus*** **L.**

c. 1. Petals are free---------- **Polypetalae**
d. 1. Dome shaped thalamus
2. Superior ovary----------**Thalamiflorae**
e. 1. Many stamens
2. Carpels are many and free---------- **Ranales**
f. 1. Herbaceous habit
2. Leaves exstipulate
3. Numerous spirally arranged stamens---------**Ranunculaceae**
g. 1. Many free spirally arranged stamens
2. Basal placentation----------***Ranunculus***

2-Caryophyllaceae (The pink family)

Stellaria media **Cyrill. (The Chick weed) (Fig.1.2)**

Vegetative Characters:

Habit: A wild, annual herb

Root: Tap roots, branched

Stem: Herbaceous, aerial, erect or decumbe nt, cylindrical, branched, solid having swollen nodes, hairy when young, 6" to 2' long and green.

Leaf: Ramal and cauline, simple, opposite decussate, exstipulate, upper sessile but lower petiolate, entire linear or lanceolate, reticulate venation.

Floral characters:

Inflorescence: Dichasial cyme.

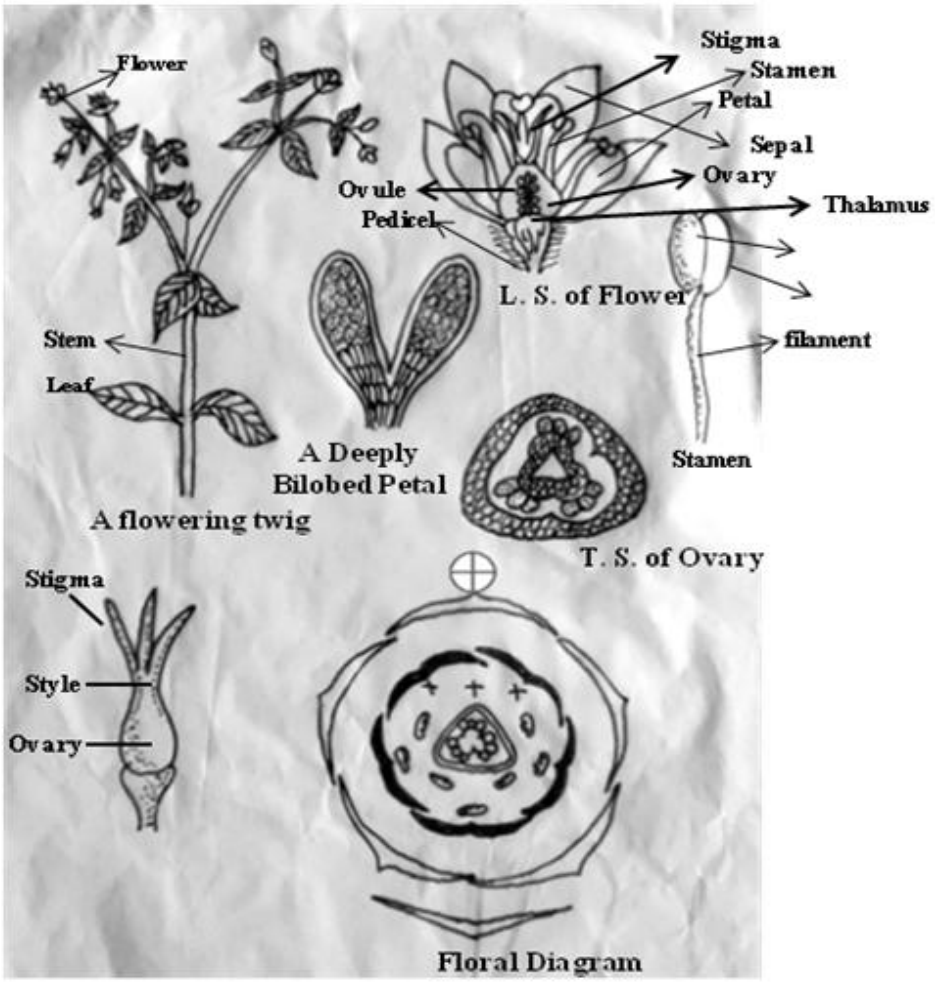

Fig.1.2: *Stellaria media* cyrill.

Flower: Bracteate, complete, hermaphrodite, pedicellate, pedicel glabrous, cyclic, actinomorphic, pentamerous, hypogynous, small and white.

Calyx: Sepals 5, polysepalous, oblong or lanceolate, quincuncial aestivation. Sepals are acute, hairy and green.

Corolla: Petals 5, polypetalous, deeply bilobed, white, imbricate or valvate aestivation.

Androecium: Stamens 10 or are reduced to 5 or 8 and rest abortive into staminodes. These are arranged in two whorls, polyandrous, obdiplostamonous. Filaments slender and are of equal length, dithecous, basifixed or sometimes dorsifixed, introrse. In some flowers all the 5 stamens of outer whorl are reduced or absent.

Gynoecium: Tricarpellary, syncarpous. The ovary is superior, unilocular with many ovules. Free central placentation is found. Style is reduced and stigma three.

Fruit: A capsule.

Seed: Brown, flat, endospermic

Floral formula:

Br, Brl, $\oplus$, δ, K_5, C_5, A_{5+5}, $\underline{G_{(3)}}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons in each seed
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Petals are free---------- **Polypetalae**
d. 1. Dome shaped thalamus
   2. Superior ovary----------**Thalamiflorae**
e. 1. Stamens definite
   2. Free central placentation ----------**Caryophyllinae**
f. 1. Opposite decussate leaves
   2. Inflorescence dichasial cyme
   3. Obdiplostamonous condition---------**Caryophyllaceae**
g. 1. White coloured small flowers
   2. Tricarpellary gynoecium
   3. Reduced stamens and style----------***Stellaria media***

3-Rutaceae (The Orange family)

***Citrus aurantifolia* Swing** *(C. mediaVar acida)* **(Fig.1.3)**

Vegetative Characters:

Habit: A cultivated tree.

Root: Tap roots, branched

Stem: The stem is woody, erect, branched, solid and cylindrical. Stem is green in colour.**Leaf:** Mostly compound, opposite and alternate, exstipulate. The leaves are characterized by oil glands. In *Citrus* the leaf is apparently simple but the winged petiole is articulated to the lamina, which indicates that it is really one leaflet of a compound leaf.

Floral characters:

Inflorescence: Axillary cyme or solitary axillary. The flowers may also remain arranged in axillary or terminal corymbs.

Flower: A prominent disc present below ovary, ebracteate, complete, hermaphrodite, pedicellate, actinomorphic, pentamerous, hypogynous, cyclic. White coloured scented flowers are developed.

Calyx: Consist of 5 sepals, gamosepalous and valvate aestivation is found.

Corolla: Petals 5, polypetalous, sometimes are fused at base. Aestivation is imbricate. Petals are white in colours.

Androecium: Stamens are indefinite and attached to disc (Polyadelphous). Bases of filaments are fused. Anthers are two celled, dorsifixed or basifixed, introrse.

Gynoecium: Penta to multicarpellary, syncarpous. The ovary is superior, multilocular one or more ovules in each locule. Axile placentation is found. Style is single and short with yellow and capitate stigma. A nectariferous disc is present below the ovary.

Fruit: Hesperidium.

Seed: Non-endospermic

Floral formula:

Ebr, $\oplus$, $\mathbb{Q}$, $K_{(5)}$, C_5, Aα(Polyadelphous),$G_{\underline{(5 \text{ to } \alpha)}}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**

b. 1. Presence of two cotyledons in each seed
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**

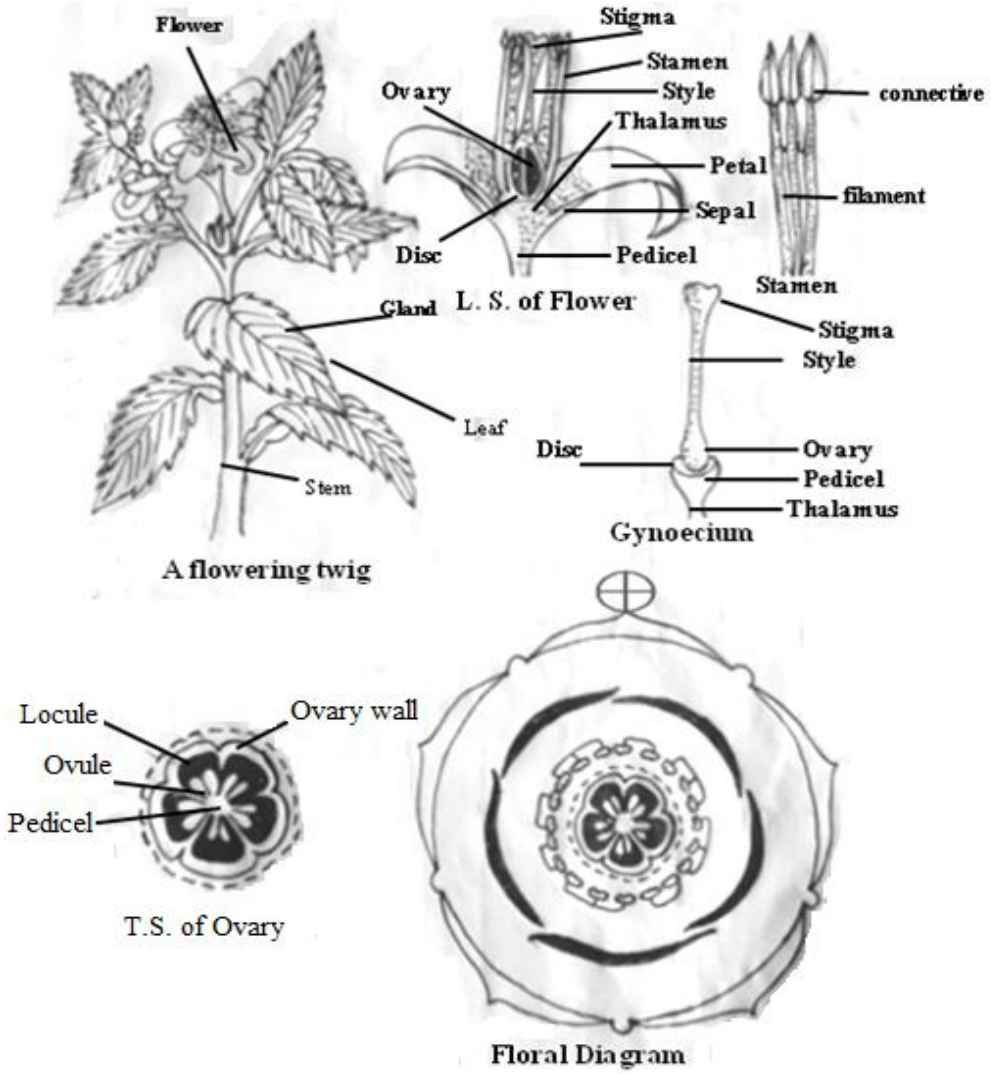

Fig.1.3: *Citrus aurantifolia* Swing

c. 1. Petals are free---------- **Polypetalae**

d. 1. Discoid thalamus
   2. Flowers are hypogynous----------**Disciflorae**

e. 1. Ring shaped disc below the ovary
   2. Pendulous ovules
   3. Non endospermic seed----------**Geraniales**

f. 1. Usually shrubs or trees
   2. Leaves with aromatic glands
   3. Many polyadelphous stamens
   4. Fruit hesperidium----------**Rutaceae**

g. 1. White coloured flowers
   2. Leaf with swollen leaf base----------***Citrus***

4-Rosaceae (The Rose Family)

***Prunus persica* L. (Peach = Aru) (Fig.1.4)**

Vegetative Characters:

Habit: A cultivated small tree.

Root: Tap roots, branched.

Stem: The stem is woody but herbaceous at upper portions, erect, branched, cylindrical, smooth and solid. Upper portion of Stem is green in colour but lower brownish.

Leaf: Usually simple or compound, opposite, alternate, exstipulate, subsessile, oval to lanceolate, acute, unicostate reticulate venation.

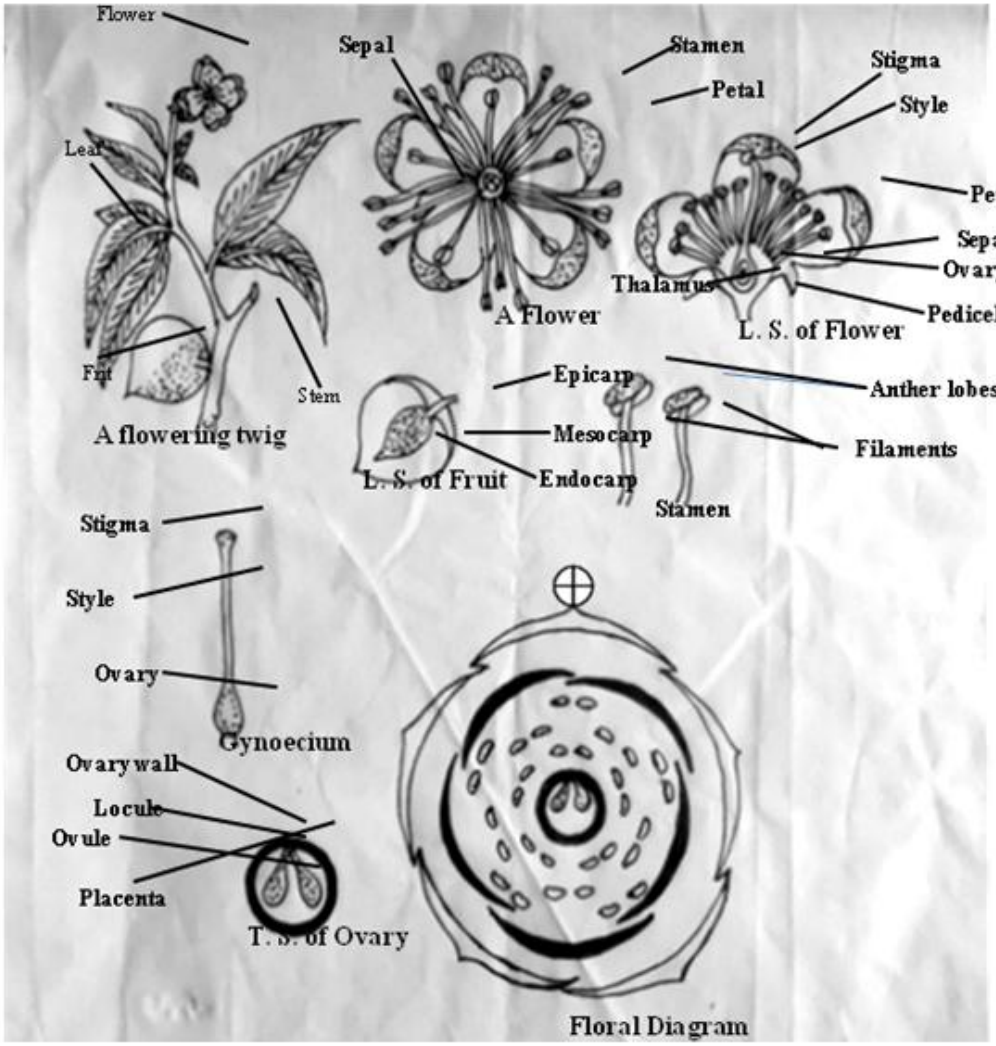

Fig.1.4 *PrunusPersica L.*

Floral characters:

Inflorescence: Solitary axillary or in cymose clusters, crowded towards dwarf branches.
Flower: A cup shaped thalamus is found. Ebracteate, subsessile or pedicellate, complete, hermaphrodite, actinomorphic, pentamerous, perigynous pink coloured flower.
Calyx: Consist of 5 sepals, gamosepalous and quincuncial. The valvate aestivation is found. The calyx tube becomes campanulate, green.
Corolla: Petals 5, polypetalous, rosaceous, pink. Aestivation is imbricate.
Androecium: Polyandrous, dithecous, dorsifixed, introrse. Consist of many stamens (15-60) which are arranged in whorls. The stamens are antisepalous in outermost whorl.
Gynoecium: Monocarpellary. The ovary is semi-inferior, unilocular, two ovules (pendulous) in the locule. Marginal placentation is found. Style is long and with capitate stigma.
Fruit: Drupe.
Seed: One, Non-endospermic
Floral formula:

Ebr, $\oplus$ ♂ $K_{(5)}$, C_5, Aα,G1 –

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Petals are free---------- **Polypetalae**
d. 1. Cup shaped thalamus
   2. Semi inferior ovary----------**Calyciflorae**
e. 1. Alternate simple leaves
   2. Gamosepalous calyx----------**Rosales**
f. 1. Rosaceous corolla
   2. Perigynous flowers
   3. Many stamens arranged in whorls----------**Rosaceae**
g. 1. Fruit is drupe
   2. Two pendulous ovules
   3. Pink flowers----------***Prunus persica***

5-Fabaceae (The Pea Family)

***Pisum sativum* (Pea) (Fig.1.5)**

Vegetative Characters:

Habit: Annual climbing herb, cultivated in gardens as an ornamental.
Root: Branched, nodulated, tap roots bearing nitrogen fixing bacteria in the roots.
Stem: The stem is herbaceous, aerial, weak, branched, flattened, climbing, green and glabrous.

Leaf: Simple or pinnately compound, imparipinnate, alternate, petiolate. Each leaflet is opposite, sessile, ovate, entire, acute and showing unicostate reticulate venation. Upper leaflets are modified completely into tendrils.

Floral characters:

Inflorescence: Usually of racemose or solitary axillary.

Flower: Zygomorphic, bisexual, complete, pentamerous, bracteate, hypogynous or perigynous.

Calyx: Consist of 5 sepals, gamosepalous. The valvate aestivation is found.

Corolla: Petals 5, polypetalous, papilionaceous. There is a large upper posterior petal (largest) called standard or vexillum, two lateral petals called the wings and two anterior or innermost petals more or less fused to form a boat shaped structure called the keel or carina. This kind of corolla is called the papilionaceous or butterfly shaped corolla. The descending imbricate aestivation is found. Petals are beautifully coloured.

Androecium: Stamens 10, usually diadelphous (into two groups i.e. 9+1). Nine fuse to form a sheath round the pistil while 10^{th} is free.

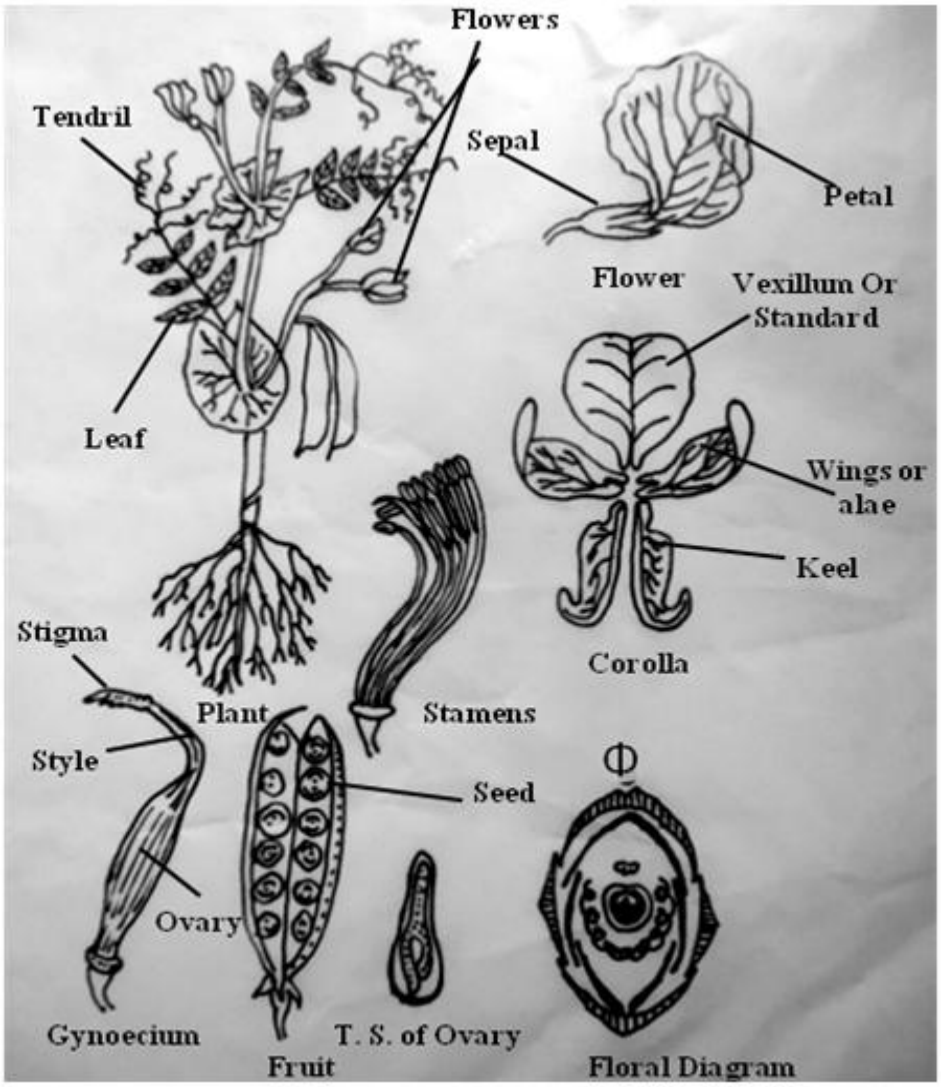

Fig. 1.5: ***Pisum sativum***

Gynoecium: Monocarpellary. The ovary is superior, unilocular. Marginal placentation is found. Style is long and bent at base, stigma flattened and hairy.
Fruit: Legume or pod.
Seed: Non-endospermic
Floral formula:

Br, %, ♂, K_5, $C_{1+2+(2)}$, $A_{9+1}G\underline{1}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Petals are free---------- **Polypetalae**
d. 1. Calyx gamosepalous
   2. Cup shaped thalamus----------**Calyciflorae**
e. 1. Alternate and stipulate leaves
   2. Diadelphousstamens----------**Rosales**
f. 1. Climbing plant
   2. Zygomorphic flowers
   3. Papilionaceous corolla----------**Papilionaceae**
g. 1. Fruit is legume or pod
   2. Presence of tendrils
   3. White flowers----------***Pisum sativum***

6-Asclepiadaceae (The Milk Weed Family)

***Calotropis procera* (Ait.) R. Br. (Fig.1.6)**

Vegetative Characters:

Habit: A shrubby weed, covered with soft, white, wooly tomentum and contains milky latex.
Root: Well branched, tap root.
Stem: The stem is herbaceous but lower portion soft and woody.Stem is erect, branched, cylindrical, solid, covered with white wooly tomentum; contains milky latex.

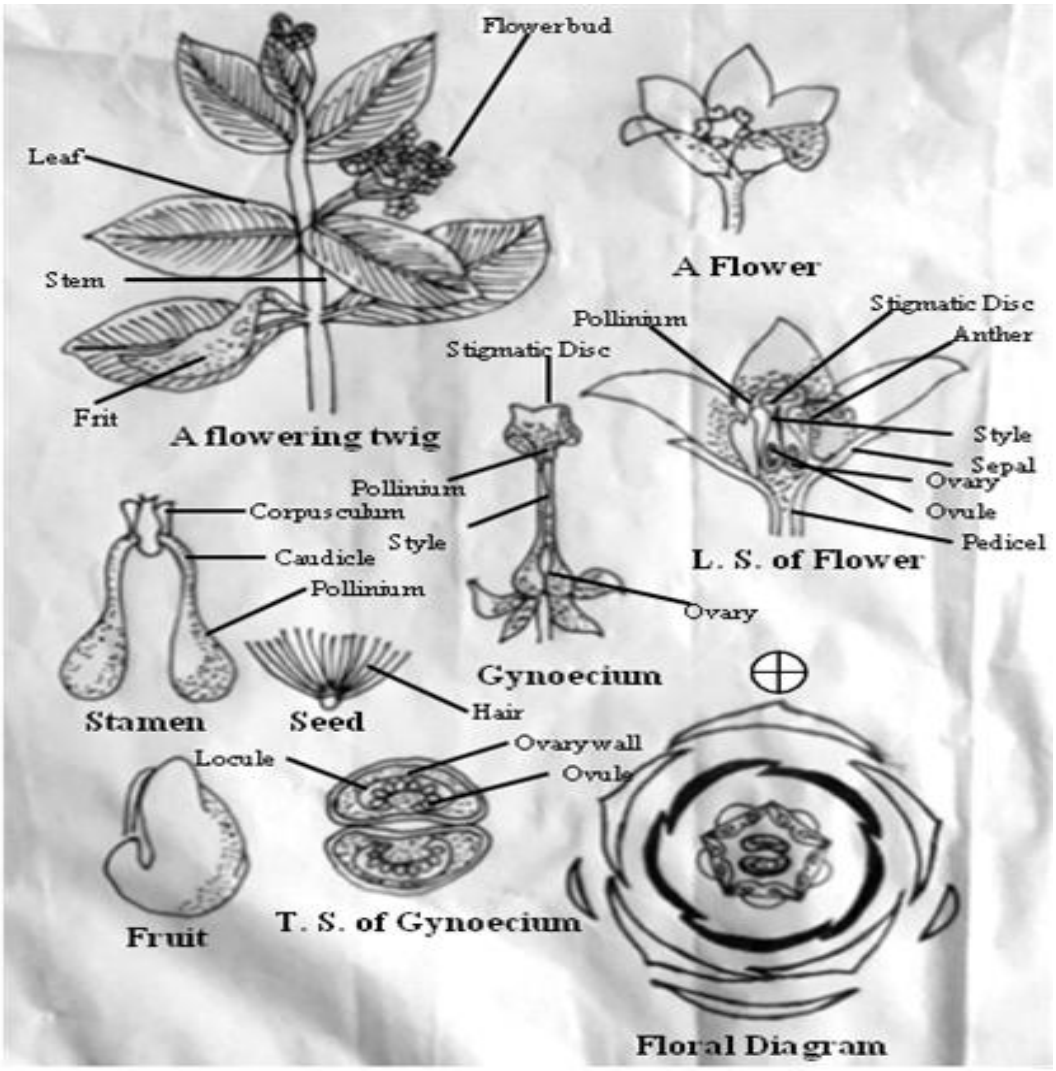

Fig.1.6: *Calotropis procera* (Ait.) R.Br.

Leaf: Ramal and cauline, simple, opposite decussate, exstipulate, sessile or subsessile; ovate to oblong, base auriculate. Leaves are entire, acute; under surface covered with wooly tomentum contain milky latex and showing unicostate reticulate venation.

Floral characters:

Inflorescence: Axillary umbellate cyme.

Flower: Bracteate, two bracteoles, pedicellate, complete, hermaphrodite, actinomorphic, pentamerous and hypogynous. Flowers are purplish red to white and having very strong smell.

Calyx: Consist of 5 sepals, polysepalous and are slightly fused at the base.

Corolla: Petals 5, gamopetalous, campanulate. The valvate but sometimes twisted aestivation is found. Petals are beautifully coloured with pink or whitish with purplish spots.

Androecium: Stamens 5, sometimes epipetalous and are connected with the stigma which is thus known as gynostegium. Each stamen is in the form of two pollinia. Stamens form translators which consist of two pollinia connected with the corpusculum with the help of

their individual retinaculae or caudicles. A fleshy scale like laterally compressed coronary outgrowth arises from the back of each stamen.

Gynoecium: Bicarpellary, superior.Ovaries are separated at the base and many ovules in each locule. Marginal placentation is found. Two styles are united apically with the stigma to form a pentangular disc called stigmatic disc.

Fruit: Follicle

Seed: Many, hairy and endospermic

Floral formula: Br, Brl, $\oplus, \delta$, K_5, $C_{(5)}$, $A_{(5)}$,$\underline{G_2}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Gamopetalous condition----------**Gamopetalae**
d. 1. Bicarpellary, superior ovary----------**Bicarpellatae**
e. 1. Opposite decussate leaves.
   2. ***5*** lobed calyx and corolla----------**Gentianales**
f. 1. Leaves and stem with latex
   2. Presence of gynostegium
   3. Marginal placentation----------**Asclepiadaceae**
g. 1. Fruit is follicle
   2. Presence of translators
   3. Plant parts covered with soft hair----------***Calotropis procera***

7-Solanaceae (The Potato Family)

Datura stramonium **(Fig.1.7)**

Vegetative Characters:

Habit: Annual herb

Root: Tap roots, branched, not very deep.

Stem: The stem is herbaceous, erect, green, dichotomously branched

Leaf: Usually simple, alternate but opposite in upper parts, exstipulate, petiolate, ovate, entire, acute.

Floral characters:

Inflorescence: Solitary terminal or solitary axillary.

Flower: Bracteate, pedicellate, complete, hermaphrodite, pentamerous, actinomorphic, hypogynous, large.

Calyx: Consist of 5 sepals, gamosepalous persistent. The valvate or twisted aestivation is found.

Corolla: Petals 5, gamopetalous and modified into trumpet shape, white. Aestivation is twisted.

Androecium: Consist of 5 stamens, epipetalous, polyandrous. Filaments are long and anthers basifixed.

Gynoecium: Bicarpellary, syncarpous, bilocular. Carpels are obliquely placed. The placenta is swollen with superior ovary and false septum provides a tetralocular appearance. There are many ovules in each locule. Axile placentation is found. Dome shaped stigma with long style.

Fruit: Spiny capsule opening by four valves.

Seed: Many, Nacrotic and poisonous

Floral formula:

Ebr, ⊕, ♂, $K_{(5)}$, $C_{(5)}$, A_5,$\underline{G(2)}$

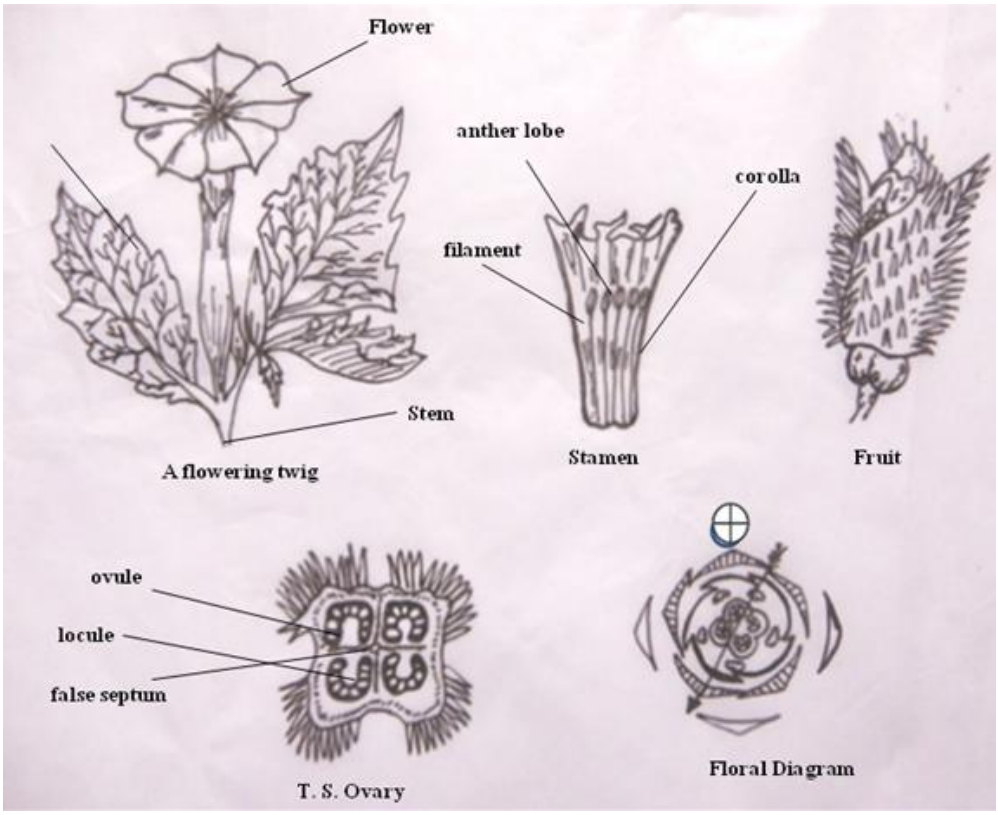

Fig.1.7: ***Datura stramonium***

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. **Reticulate venation**----------**Dicotyledons**
c. 1. Gamopetalous condition---------- **Gamopetalae**
d. 1. Bicarpellary, syncarpous, superior gynoecium
   2. Axile placentation ----------**Bicarpellatae**
e. 1. Leaves exstipulate
   2. Epipetalous stamens----------**Polemoniales**
f. 1. Carpels are obliquely placed

2. Swollen placenta
3. Dome shaped stigma with long style ----------**Solanaceae**

g. 1. Spiny capsule opening by four valves
2. Many ovules in each locule
3. Nacrotic and poisonous ----------***Datura stramonium***

8-Acanthaceae (The Acanthus Family)

***Adhatoda vesica* Nees. (Fig.1.8)**

Vegetative Characters:

Habit: Annual herb

Root: Tap roots, branched.

Stem: The stem is herbaceous, erect, branched, cylindrical, solid, swollen nodes, green to pale-green in colour.

Leaf: Ramal and cauline, simple, exstipulate, opposite, decussate, petiolate, lanceolate to ovate, entire, pale green in colour. Unicostate reticulate venation is found.

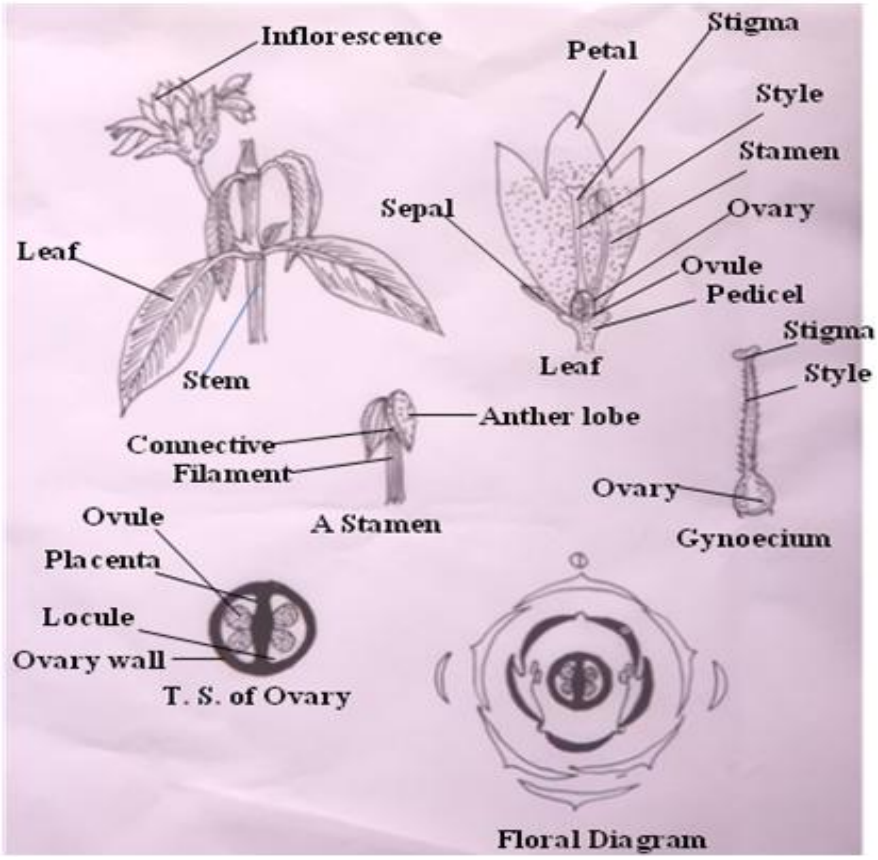

Fig.1.8: *Adhatoda vesica* Nees.

Floral characters:

Inflorescence: Axillary racemose spike.

Flower: Bracteate (leafy bracts), bracteolate (the bracteoles are leafy and enclosed the bud), sub sessile, complete, hermaphrodite, pentamerous, zygomorphic, hypogynous, large and white.

Calyx: Consist of 5 sepals, polysepalous but are slightly conate at the base, quincunical, pale green in colour. The aestivation is mostly imbricate.
Corolla: Petals 5, gamopetalous, Petals are conate in bilipped corolla, 2/3 bilabiate personate, consisting of a posterior curved lip of two petals and an anterior lip of three petals. Anterior most middle petal of anterior lip is raised and strongly nerved, white in colour.
Androecium: Consist of 2 stamens, epipetalous, polyandrous, dithecous, basifixed and introrse. External anther lobe is higher than inner.
Gynoecium: Bicarpellary, syncarpous, bilocular. Carpels are medianly placed. There are one to two ovules in each locule Axile placentation is found. Slightly bifid stigma with simple long hairy style.
Fruit: Capsule
Seed: Large non endospermic seed with hooks also called jaculators.
Floral formula:

Br, Brl, Φ, ♂♀ , $K_{(5)}$, $\overset{\frown}{C_{(2/3)}}$, A_2,$\underline{G(2)}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Gamopetalous condition---------- **Gamopetalae**
d. 1. Bicarpellary, superior ovary
   2. Axile placentation ----------**Bicarpellatae**
e. 1. Zygomorphic flowers
   2. Bilabiate personate corolla ----------**Personales**
f. 1. Opposite decussate leaves
   2. Flowers are bilipped
   3. Seeds with jaculators ----------**Acanthaceae**
g. 1. Inflorescence racemose spike
   2. Stamens are two in number
   3. Unequal sized anther lobes----------***Adhatoda vasica***

9-Lamiaceae (The Mint Family)

***Ocimum basilicum* Linn. (Ban Tulsi) (Fig.1.9)**

Vegetative Characters:

Habit: A cultivated, aromatic, tall herb
Root: Tap roots, branched.
Stem: The stem is herbaceous but becomes woody below, quadrangular, erect, branched, hairy, green, aromatic.
Leaf: Ramal and cauline, simple, exstipulate, opposite decussate, petiolate, ovate, serrate, hairy. Unicostate reticulate venation is found.

Floral characters:

Inflorescence: Verticillaster.

Flower: Bracteate, ebracteolate, pedicellate, complete, hermaphrodite, zygomorphic, hypogynous, bilabiate, small, aromatic.

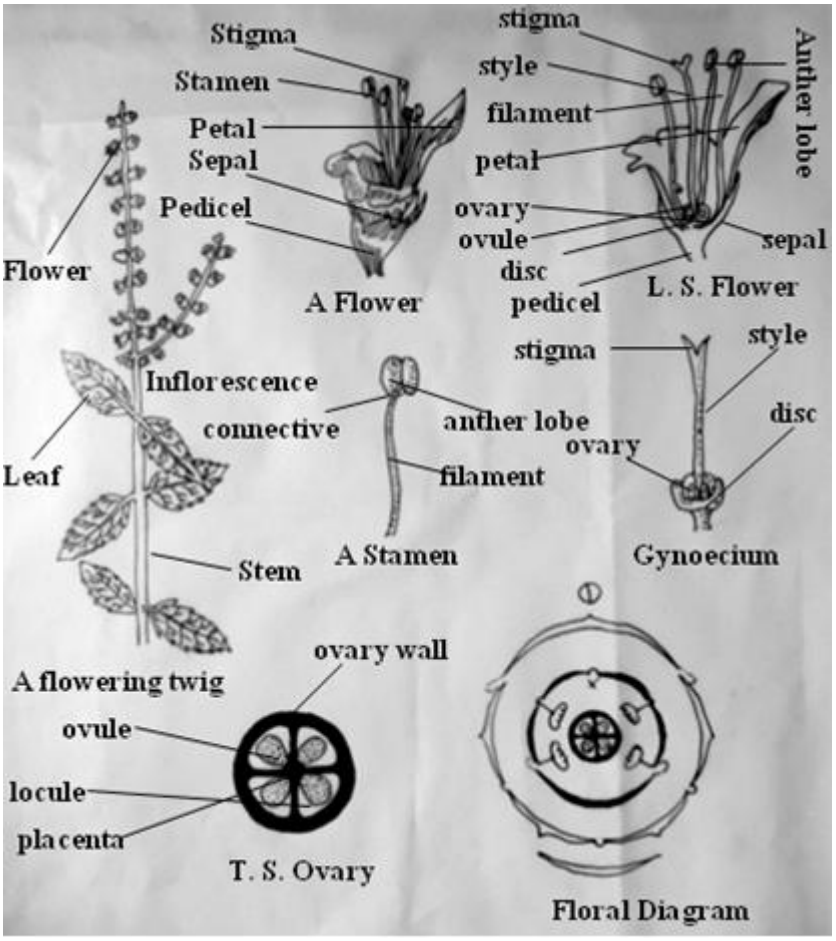

Fig.1.9: *Ocimum basilicum* Linn.

Calyx: Consist of 5 sepals, ¼ bilipped consisting of upper posterior lip of one big lobe and anterior lip of 4 lobe; gamosepalous and violet green in colour. The aestivation is valvate.

Corolla: Petals 5; arranged in 4/1 form, bilabiate, consisting of upper posterior lip of 4 lobes and lower anterior lip of 1 lobe; gamopetalous, white or pink coloured. Valvate aestivation is found.

Androecium: Consist of 4 stamens, epipetalous, polyandrous and didynamous. The posterior stamen is lacking. Anther lobes broad and are slightly separated, dithecous, dorsifixed, introrse.

Gynoecium: Bicarpellary, syncarpous, bilocular in very early stages but later on becomes quadrilocular due to the formation of false septum. Each locule with one locule and axile placentation is found. Ovary is superior and 4 lobed. Style is long and gynobasic and come up between 4 parts of the ovary. Stigma is bifid. A four-lobed hypogynous disc is present below the ovary.

Fruit: Schizocarpic (carcerulus), made up of four nutlets.

Seed: Four- non endospermic.

Floral formula:

Br, Ebrl,Φ , ♂/♀, $K_{(1/4)}$, $\overset{\frown}{C_{(4/1)}}$, A_{2+2},$\underline{G(2)}$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of two cotyledons
   2. Flowers are pentamerous
   3. Reticulate venation----------**Dicotyledons**
c. 1. Gamopetalous condition---------- **Gamopetalae**
d. 1. Bicarpellary, syncarpous, superior gynoecium
   2. Axile placentation ----------**Bicarpellatae**
e. 1. Plant is herb
   2. Leaves are opposite decussate
   3. Flowers are zygomorphic, bilipped
   4. Fruit Schizocarpic ----------**Lamiales**
f. 1. Angular stem
   2. Scented or aromatic leaves
   3. Inflorescence verticillaster----------**Lamiaceae**
g. 1. Stamens didynamous
   2. Gynobasic style
   3. Ovary tetralocular
   3. Calyx ¼ and corolla 4/1---------- ***Ocimum basilicum***

10-Orchidaceae (The Orchid Family)

Orchis latifolia **(Fig.1.10)**

Vegetative Characters:

Habit: Mostly terrestrial succulent, herbs, epiphytic or saprophytic.

Root: Adventitious roots, tuberous.

Stem: Normally the stem is erect, sometimes climbing or trailing.

Leaf: Simple, entire alternate or rarely opposite to whorled, oval or linear, parallel venation, often fleshy.

Floral characters:

Inflorescence: Racemose, mostly spike.

Flower: Bracteate often very showy, hermaphrodite, zygomorphic, epigynous, A few modifications are due to hyper trophy, adhesion or suppression.

Perianth: Six tepals in two whorls, free or variously combined usually fleshy.The posterior segment of inner whorl is always more developed and known as labellum. Mostly labellum remains provided with a spur, secreting nectar. Labellum comes to the anterior because of the twisting ovary and serves as the landing stage for insects.

Androecium: Consisting of 3 stamens uniting with the pistil to form the gynoecium. There is one functional stamen, anther two celled, opened by longitudinal slits. The pollen grains are united to form pollina.

Gynoecium: Tricarpellary (consist of three carpels), syncarpous, ovary inferior and unilocular (rarely trilocular). Parietal placentation is found. Three stigma, out of which two lateral ones receptive or fertile, third one is sterile and transformed into small beaked outgrowth the restillum.

Fruit: Capsule

Seed: Minute non endospermic.

Floral formula:

$\Phi, ♂ , P_{3+3}$ or $_{(3+3)}, A_{3or\ 2,}G(\underline{3})$

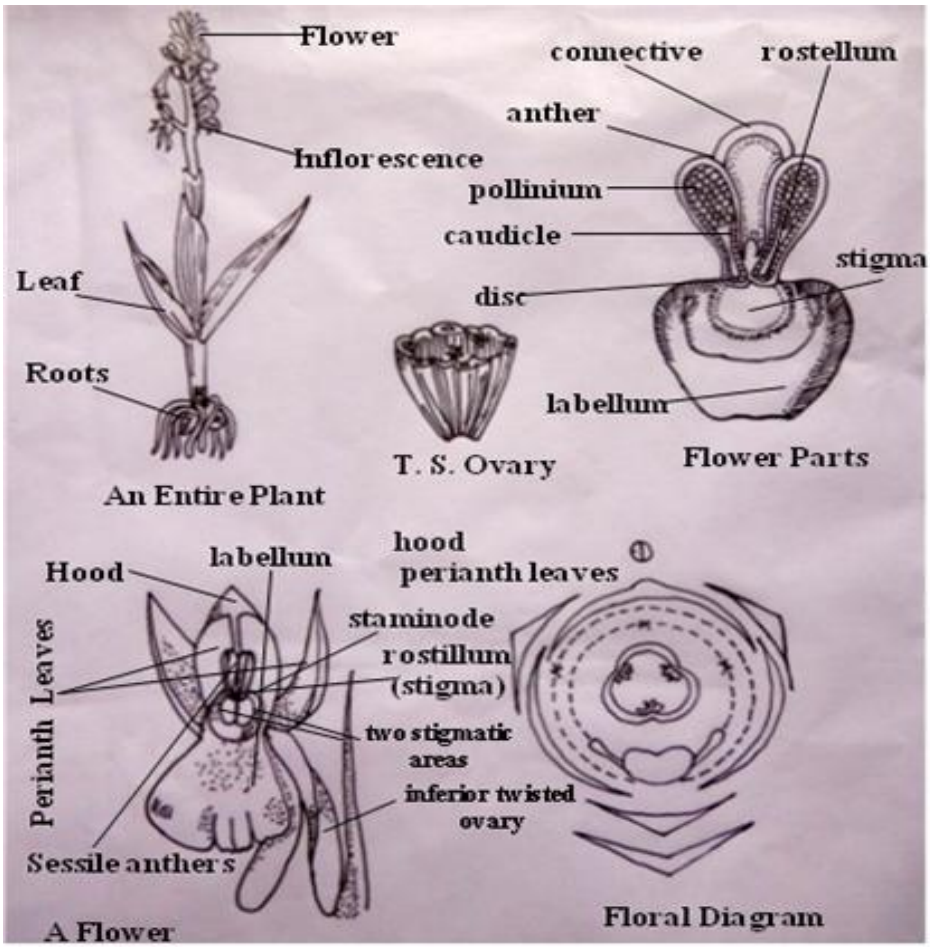

Fig.1.10: ***Orchis latifolia***

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**

b. 1. Presence of one cotyledons
   2. Flowers are trimerous
   3. Parallel venation----------**Monocotyledons**

c. 1. Perianth leaves 3+3

2. Petalloid perianth----------**Coronarieae**

d. 1. Leaves are alternate or rarely opposite to whorled
2. Racemose inflorescence----------**Orchidales**

e. 1. The stem is erect, sometimes climbing
2. Fleshy leaves
3. Anther two celled, opened by longitudinal slits --------**Orcihdaceae**.

g. 1. Stamens uniting with the pistil to form the gynoecium
2. The pollen grains are united to form pollina.
3. Ovary inferior and unilocular
4. Minute non endospermic ---------- ***Orchis latifolia***

11-Poaceae (The Grass Family)

***Triticum aestivum* L. (Fig.1.11)**

Vegetative Characters:

Habit: An annual cultivated, cereal crop.

Root: Fibrous, adventitious roots.

Stem: Herbaceous, erect, cylindrical, unbranched but rarely branched. Nodes and internodes are very clear, fistular, rough and green.

Leaf: Simple, sessile, alternate have long linear blade. A membranous ligule is present at the junction of blade and leaf base. Linear to lanceolate. Multicostate parallel venation.

Floral characters:

Inflorescence: Spike of spikelets. Each spikelet consists of a pair of glumes. There are many inferior palea or lemma, superior palea and enclosing the lodicules, stamens and gynoecium.

Flower: Bracteate (lemma or inferior palea), bracteolate (superior palea), sessile, complete, hermaphrodite, zygomorphic, hypogynous, small and inconspicuous. Lemma is prolonged into a long **awn**.

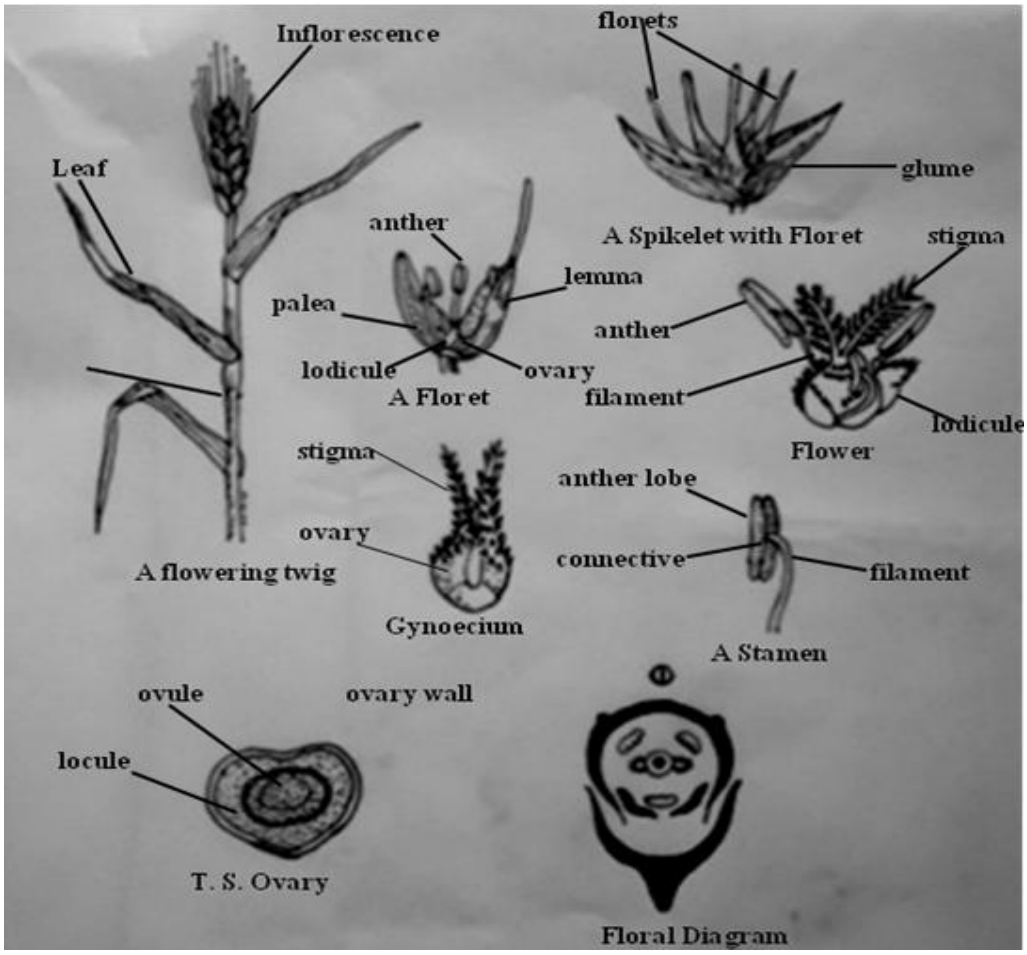

Fig.1.11: ***Triticum aestivum*** **L.**

Perianth: Perianth absent, however the lodicules may be considered as highly reduced perianth.

Androecium: Consisting of 3 stamens, polyandrous, one anterior and two posterolaterally placed. Long filament is come out of the flower, dithecous, versatile, introrse.

Gynoecium: Monocarpellary, syncarpous, ovary superior. Unilocular with single ovule. There are two styles. There are two feathery stigmas arising from lateral parts of the pistil.

Fruit: Caryopsis.

Seed: Endospermic.

Floral formula:

$$Br, Brl, \Phi, \female, P_{2}, A_{3,} \underline{G}_{1}$$

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**

b. 1. Presence of one cotyledons
   2. Flowers are trimerous
   3. Parallel venation----------**Monocotyledons**

c. 1. Sessile, naked flowers
   2. Highly reduced perianth

3. Presence of glumes----------**Glumiflorae**

d. 1. Inflorescence is spike of spikelets
2. Fruit caryopsis
3. Awned flowers---------- ----**Poaceae**.

g. 1. Stem is jointed and hollow
2. Leaves with ligule
3. Inflorescence is spike
4. Feathery stigma---------- ***Triticum aestivum***

12-Liliaceae (The Lily Family)

Asphodelus tenuifolius **(Fig1.12)**

Vegetative Characters:

Habit: An annual herb.

Root: Fibrous, adventitious roots.

Stem: Condensed, underground and reduced, fistular.

Leaf: Simple, Radical, sessile, exstipulate, leaf base sheathing, long, acicular, entire, acute, fleshy, hollow, multicostate parallel venation.

Floral characters:

Inflorescence: Racemose raceme with its erect flower, cylindrical, fleshy, long peduncle or scape.

Flower: Bracteate, ebracteolate, pedicillate, complete, hermaphrodite, actinomorphic, trimerous, small and white.

Perianth: 6 Tepals, arranged in two whorls of 3 each, polyphyllous, petalloid, oblong. Tepals are white in colour and midrib brownish and ridged. Valvate aestivation is found.

Androecium: Consisting of 6 stamens, arranged in two whorls of 3 each, polyandrous, epiphyllous and are attached just opposite to each perianth lobe. Filaments are of unequal size as the larger of outer whorl than that of inner whorl, dithecous, versatile, introrse, white.

Gynoecium: Tricarpellary, syncarpous, trilocular superior ovary. There are two ovules in each locule. Trifid yellowish stigma on filiform style

Fruit: A capsule

Seed: Endospermic.

Floral formula:

Br, EBrl⊕ ,♂, P_{3+3}, $A_{3+3,}$G (3)

Identification and Systematic Position:

a. 1. Seeds enclosed within ovary wall---------- **Angiosperms**
b. 1. Presence of one cotyledons
2. Flowers are trimerous
3. Parallel venation----------**Monocotyledons**
c. 1. Ovary superior
2. Petalloid perianth----------**Coronarieae**

d. 1. Leaves are radical
2. Racemose raceme with flowers on long peduncle
3. Perianth leaves 3+3
4. Stamens 3+3 ----------**Liliaceae**

g. 1. Leaves are acicular and fleshy
2. White coloured flowers
3. Fibrous adventitious roots---------- ***Asphodelus tenuifolius***

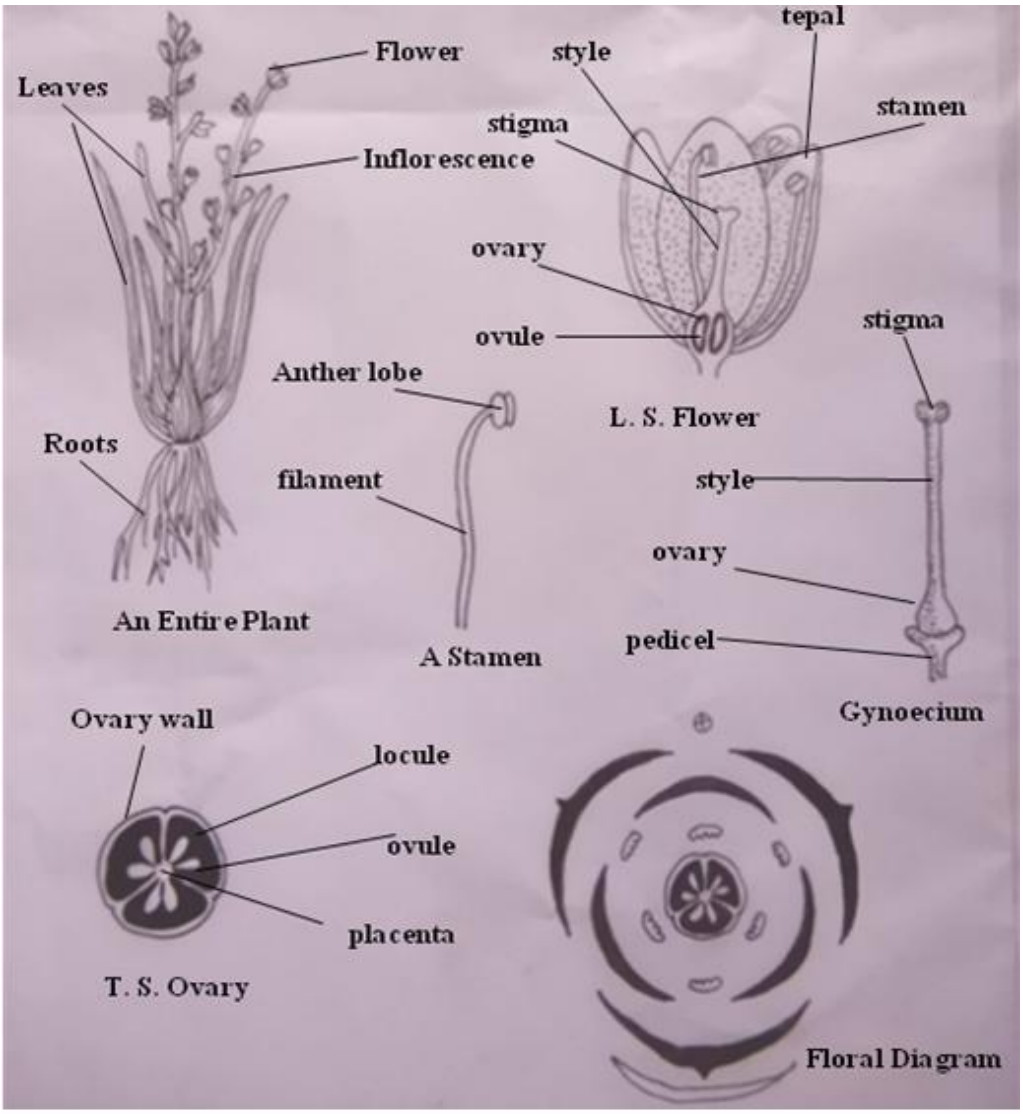

Fig.1.12: ***Asphodelus tenuifolius***

1.4 SUMMARY

1. With the huge variety of plants surrounding us, it is extremely essential to pinpoint a particular plant of our interest by noting the similarities or differences with other plants. Thus it becomes extremely necessary that the plant is first identified, given a proper name so that we can communicate our ideas about it, and also know the group to which the plant belongs.

2. Taxonomy means classification following certain rules or, principles. But it is very important to note that a plant's name is the key to its literature, and grouping can only be possible when its identity is revealed and is named for the sake of convenience and communication of ideas about it.
3. The term taxonomy was first introduced to the plant science in 1813 by A. P. de Candolle, which was about the plant classification. But later this term became more inclusive and at present it includes identification of plants, their nomenclature and classification. Traditionally taxonomy was based largely on gross morphological features of a plant.
4. The three functions of taxonomy include, identification, nomenclature and classification. Its main aim is to provide a convenient method of identification and communication about a taxon and provide a classification which is based on natural affinities of plant as far as possible.
5. The word **'taxon'** (taxa) was first used by a German Biologist Adolf Meyer in 1926 for animal groups. It was later proposed for the plant system in 1948 by Herman J. Lam. It is a taxonomic group of any rank, e.g. family, genus, species, subspecies, etc.
6. Identification of a taxon is a prerequisite for any study based on it. It is the determination of a taxon based on overall similarities and differences with other taxa. Identification is generally done by comparing representative specimen of a given taxon with the help of key descriptions, illustrations, etc.
7. In the present chapter we have described 12 families in semi technical language and now we are going to summarize the identification of these 12 families as per in the syllabus as following:-
   - **Ranunculaceae:** Herbs or climbing shrubs; Tetra-Pentamerous flowers; Gynoeciumα-1; fruit simple or etario of achenes
   - **Caryophyllaceae:** Herbs; stem with swollen nodes; leaves sessile, opposite; Flowers bracteate, bracteolate, pentamerous; Actinomorphic, sepals persistent; corolla caryophyllaceous; Androecium$(A)_{5+5}$; free central placentation
   - **Rutaceae:** Shrubs, trees; leaves with glandular dots; stamens 8-10, obdiplostamonous or many and polyadelphous; disc nectar secreting; fruit hesperidium
   - **Rosaceae:** Alternate and stipulate, pinnately compound leaves; flowers pentamerous; calyx persistent; stamens many; Rosaceous corolla; perigynous; polycarpellary, apocarpous gynoecium; fruit etario of achenes
   - **Fabaceae:** Climbing plants; zygomorphic flowers; papilionaceous corolla; monocarpellary ovary; marginal placentation; Androecium- A(9)+1, diadelphous
   - **Asclepiadaceae:** Leaves opposite, Leaves and stems with latex; Actinomorphic flowers; epipetalous stamens; presence of gynostegium; marginal placentation; corolla not hypocrateriform; staminal corona is present
   - **Solanaceae:** Leaves alternate, exstipulate; actinomorphic flowers; carpels obliquely placed; swollen placenta; corolla rotate or campanulate; several ovules in each locule, axile placentation; fruit berry
   - **Acanthaceae:** Opposie decussate leaves; flowers bilipped; corolla bilabiate personate; Androecium – A_2 or 2+2; seeds with jaculators (hooks)

- **Lamiaceae:** Angular stem; scented and aromatic leaves; zygomorphic flowers; inflorescence verticillaster; style gynobasic; ovary four locular; stamens didynamous
- **Orchidaceae:** Perennial herbs with unbranched stem; zygomorphic flowers; presence of labellum; anther highly modified; presence of gynostegium.
- **Poaceae:** Stem with two stichous ligulate leaves, inflorescence spike or spikelets, awned flowers, flowering glumes2 as lemma and palea, G(2), stigma 2, fruit caryopsis.
- **Liliaceae:** Leaves radical, inflorescence racemose raceme with flowers on long peduncle, perianth leaves 3+3, stamens 3+3, some with staminodes, fruit capsule.

1.5 GLOSSARY

Achene – A small, dry, one-seeded, indehiscent fruit (one that doesn't split open)
Acuminate – The shape of a tip (apex) or base of a leaf or perianth segment where the part tapers gradually and often in a concave manner.
Acute – Evenly narrowed into a point at an angle of less than 90 degrees.
Adventitious – Arising from an unusual or irregular position, such as roots along a stem.
Alternate – Arrangement of leaves or parts one at a node, as leaves on a stem. For comparison, opposite or whorled
Ament – A catkin, or scaly spike.
Angiosperm – Having seeds borne within a pericarp.
Anther – Pollen-bearing part of a stamen, borne at the top of a filament.
Apetalous – Without petals, e.g. flowers of grasses.
Apex – The tip or terminal end.
Apical – Describes the apex or tip.
Auriculate – Having ear-like appendages, as the projections of some leaf and petal bases.
Axil – The angle between a stem and an attached leaf.
Axis – The main stem.
Axillary – Borne or carried in the axil.
Berry – A fleshy, indehiscent, pulpy, multi-seeded fruit resulting from a single pistil, e.g. tomato.
Bipinnate – Twice pinnate, the primary leaflets being again divided into secondary leaflets.
Bloom – A waxy coating sometimes found on a stem, leaf, flower or fruit surface, usually of a grayish cast and easily removed.
Bract – A much-reduced leaf, often scale-like and usually associated with a flower or inflorescence
Caducous – Falling off very early as compared to similar structures in other plants.
Calyx – The outer whorl of perianth, composed of the sepals, usually green in color and smaller than the inner set.
Capsule – A dry dehiscent fruit produced from a compound pistil,
Catkin – A spike-like inflorescence, comprised of scaly bracts subtending unisexual flowers, often somewhat flexuous and pendulous,

Ciliate – Marginally fringed with hairs, often minutely so, and then termed "ciliolate."
Compound leaf – A leaf of two or more leaflets.
Connate – Describing similar structures united or fused together.
Corolla – Inner whorl of the perianth, between the calyx and the stamens; a collective term for the petals of a flower.
Cotyledon – The primary leaves of the embryo, present in the seed. One of the first leaves to appear after germination (there may be more than 1).
Cyme – A more or less flat-topped determinate inflorescence whose outer flowers open last. **Dehiscent** – Splitting open. The term is commonly applied to anthers or seed pods.
Determinate – Describes an inflorescence in which the terminal flower blooms first, thereby halting further elongation of the flowering stem.
Dimorphic – Having two forms.
Drupe – A fleshy, indehiscent fruit whose seed is enclosed in a stony endocarp, e.g. date, cherry.
Entire – Having a margin without teeth or lobes.
Filiform – Long and very slender; thread-like.
Fruit – Technically a ripened ovary with its adnate parts, the seed-containing unit characteristic of all Angiosperms.
Genus – A group of species possessing fundamental traits in common but differing in other lesser characteristics; a taxonomic grouping of similar species (pl. genera); similar genera are grouped into families.
Glabrous – Not hairy.
Glandular – Bearing glands.
Hairy – Pubescent with long hairs.
Imperfect – A flower that lacks either stamens or pistils.
Inferior – Beneath, below; said of an ovary when situated below the apparent point of attachment of stamens and perianth.
Inflorescence – The arrangement of flowers on the axis.
Lanceolate – Much longer than wide, broadest below the middle and tapering to the apex.
Latex – Milky sap.
Margin – The edge of a leaf.
Marginal – Pertaining to the margin.
Native – Inherent and original to an area; pre European influence in the United States..
Node – A joint on a stem, represented by point of origin of a leaf or bud; sometimes represented by a swollen or constricted ring, or by a distinct leaf scar.
Nut – A dry, indehiscent, 1-celled, 1-seeded fruit having a hard and bony mesocarp, the outermost endocarp may be fibrous or slightly fleshy.
Opposite – Describing leaves that are situated in pairs at a node along an axis.
Ovate – Egg-shaped in outline, broadest below the middle.
Pedicel – Stalk of a single flower in an inflorescence.
Peduncle – Stalk of a flower or inflorescence.
Perianth – A collective term embracing both the calyx and corolla

Pericarp – A term used by some to designate a fruit; technically, the ovary wall.
Pinna – The leaflet of a compound leaf; in ferns, the primary division attached to the main rachis; feather-like.
Raceme – A simple indeterminate inflorescence, having a single long axis, with pedicelled flowers.
Reflexed – Bent abruptly backward or downward.
Schizocarp – A dry dehiscent fruit that splits into two halves.
Sepal – A single segment of a divided calyx.
Sessile – Without a stalk.
Simple – Said of a leaf when not compound, of an inflorescence when unbranched.
Solitary – Borne singly, not paired or clustered.
Species – A natural group of plants composed of similar individuals which can produce similar offspring; usually including several minor variations.
Spike – A unbranched, elongated, simple, indeterminate inflorescence whose flowers are sessile; the flowers may be congested or remote.
Spikelet –The floral unit, or ultimate cluster, of a grass inflorescence comprised of flowers and their subtending bracts.
Stamen – Male or pollen-bearing organ of a flower, composed of filaments and anthers.
Stipule – A basal appendage of a petiole, usually one at each side, often ear-like and sometimes caducous.
Tendril – A modified stem or leaf, usually filiform, branched or simple, that twines about an object providing support.
Tepal – A segment of perianth not differentiated into calyx or corolla
Terminal – At the tip or distal end.
Umbel – An indeterminate inflorescence, usually but not necessarily flat-topped with the pedicels and peduncles (termed rays) arising from a common point, resembling the stays of an umbrella.
Undulate – Wavy, as in a leaf margin.
Unisexual – Bearing either stamens or pistils but not both.
Valvate – Meeting at the edges without overlapping, as leaves or petals in the bud.
Whorl – Arrangement of three or more structures arising from a single node.
Woolly – Having long, soft, more or less matted hairs; like wool.

1.6 SELF ASSESSMENT QUESTION

1.6.1 Short Answer Type Questions:

1. Who coined the term classification for the first time?
2. Who used the term systematics?
3. What K (5) stands for?
4. What does the mean of C_{5+5}?
5. Which family is known as crow foot family?
6. Which type of placentation is found in *Ranunculus*?

7. Which family is known as the orange family?
8. In which year the taxonomy was first introduced to the plant science?
9. Who used the term Taxon for the plant system previously?

1.6.2 Fill in the Blanks:

1. Traditionally taxonomy was based largely on ----------of a plant.
2. The three functions of taxonomy include-------**,** ---------- **and**----------**.**
3. Tetra-Pentamerous flowers is the characteristic feature of ----------**.**
4. In Fabaceae the --------placentation is found.
5. Presence of gynostegium is the feature of----------.
6. ⚥ stands for----------**.**
7. Superior ovary is represented by ----.
8. *Stellaria media* Cyrill. is commonly known as----------**.**
9. Nine stamens fuse to form a sheath round the pistil while 10^{th}stamenis free might be represented by -----.
10. Presence of two cotyledons is the characteristic feature of ----------
11. In Papilionaceae corolla are.
12. Flowers of ***Orchis latifolia*** **are----------.**

1.6.1 Answers Key:

1. A. P. de Candolle **2.** Carl Linnaeus, **3.** Five sepals are fused (gamosepalous).
4.Ten petals are arranged in two whrols of 5 each, **5.** Ranunculaceae
6. Basal placentation, **7.**Rutaceae, **8.**1813, **9.** Herman J. Lam

1.6.2. Answers Key: 1. Morphological features, 2. Identification, nomenclature and classification, 3. Ranunculaceae, 4. Marginal, 5. Orcidaceae, 6. Hermaphrodite, 7. G 8. The Chick weed, 9. A_{9+1}., 10. Dicotyledons, 11. Butterfly shaped, 12. Zygomorphic

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1.9 TERMINAL QUESTIONS

1. Describe the flowering plants with special reference to their availability in Uttarakhand.
2. Describe the process of identification.
3. With the help of floral diagram and formula describe following families:
    a. The chick weed family
    b. The orange family
    c. The pea family
    d. The rose family
    e. The milk weed family
4. Describe the floral description of available plant of Acanthus family in Uttarakhand.
5. Describe the Mint family.
6. Define placentation. Write some important points for following placentation types:
    a. Basal placentation
    b. Marginal placentation
    c. Axile placentation
7. Describe the vegetative and floral characters of orchid family member of your locality.

UNIT-2 COLLECTION OF PLANT SPECIMENS-HERBARIUM AND LIVE SPECIMENS

2.1 OBJECTIVES

After reading this section you will know-

- What is plant specimen
- How to collect plant specimens
- What is Herbarium
- How to prepare Herbarium
- The description, collection and preservation techniques of specimens for herbarium
- To describe, collect and maintain the live plant specimens for both pure and applied studies

2.2 INTRODUCTION

In this unit we will try to know about plant specimen and its types, which are mostly used as an example of a species or a type for scientific study or display. The specimens may be in the form of whole plant or plant parts. These are usually in dried form by mounting on a sheet of paper but depending upon the material, may also be stored in boxes or kept in preservatives. The specimens in a herbarium are often used as reference material in describing plant taxa.

Plant collection is the prerequisite for preparing the plant specimens for the purposes of research, cultivation, or as a hobby. Plant specimens may be kept alive, but are more commonly dried and pressed to preserve the quality of the specimen. Plant collection is an ancient practice with records of a Chinese botanist collecting roses over 5000 years ago. Herbaria are collections of preserved plants samples and their associated data for scientific purposes. The largest Herbarium in the world exist at the **National Museum of Natural History** in **Paris, France**.

The main museum is located in Paris, on the left bank of the River Seine. It was founded in 1793 during the French Revolution, but was established earlier in 1635. As of 2017, the museum has 14 sites throughout France, with four in Paris, including the original location at the royal botanical garden; the Garden of Plants (The Jardin des Plantes) is the main botanical garden in France.

Plant samples in herbaria typically include a reference sheet with information about the plant and details of collection. This detailed and organized system of filing provides horticulturist and other researchers alike with a way to find information about a certain plant, and a way to add new information to an existing plant sample file.

The collection of live plant specimens from the wild, sometimes referred to as **plant hunting**, is an activity that has occurred for centuries. The earliest recorded evidence of plant hunting was in 1495 BC when botanists were sent to Somalia to collect incense trees for Queen Hatshepsut. In historical past botanical adventurers were made to explore the world to find exotic plants and their domestication often at considerable personal risk. These plants usually ended up in botanical gardens or the private gardens of wealthy collectors.

A herbarium is a collection of pressed and dried plant specimen, mounted on sheets bearing a label, arranged according to a sequence and available for reference study. The specimens kept by a herbarium may be whole plants or parts of plants. Each dried plant is labeled with essential data usually descriptive and ecological collection data, as well as the name of the collector(s) and the date of collection. Herbarium specimens are stored in protective cabinets in a dry location. They are classified and arranged to allow easy access.

A herbarium of a particular region represents the diversity and distribution of the region's vegetation and its history. At a herbarium, we can identify plants by matching unnamed plants with named specimens in the collection. We can also compare different species from one area, or individuals of the same species, from a range of different sites.

A Botanical garden is a place for growing wide range of plants labelled with their name and also is an educational institute for scientific workers and general public or laymen to awake their interest in plant life .In reality the botanical garden may be an independent institution or affiliate of an organization or research institution for carrying out botanical researches and dissemination of scientific knowledge. In botanical garden there should be a herbarium, library, art and photographic studies, lecture theater and should have recreational facilities.

2.3 COLLECTION OF PLANT SPECIMENS

The collection encompasses all major groups of plants (bryophytes, ferns, gymnosperms and angiosperms) as well as algae and lichens. The scope of the collection is worldwide, but with special strengths in the neotropics, North America, Pacific oceanic islands, the Philippines, and the Indian subcontinent. Many of the plant groups represented in National Herbarium rank among the finest and/or largest in the world. The flowering plant families of Acanthaceae, Asteraceae, Bromeliaceae, Gesneriaceae, Melastomataceae, andPoaceae have benefited from a long history of research and study as well as current specialist support. Other flowering plant groups that enjoy active support include Araceae, Commelinaceae, Onagraceae, Passifloraceae, Sapindaceae, Sterculiaceae, Theaceae, and Zingiberales.

We should be very clear during the collection of plant specimens with some important points as given below:

1. Select vigorous and typical specimens.
2. Avoid insect-damaged plants.
3. Choose individuals that show the variation in leaf, flower and fruit size.
4. It may be important to show morphological variation, involving the collection of individuals of different sizes or ages.
5. Collect at least two sets of specimens (duplicates) and give number to each set.
6. Keep one set for your reference, and send the duplicate numbered set to the Herbarium for identification or as a voucher if required.

A good specimen includes stems, leaves, flowers and fruits. Basal parts of grasses, sedges, ferns and bulbous plants are essential for identification. Underground parts e.g. tubers, rhizomes are important for some plant groups. The plant material should be fertile i.e. in flower or fruit (both if possible), as these characteristics are often vital for identification.

2.3.1-Herbarium

A herbarium consists of preserved plant specimens, each with a label bearing documentary information. Specimens are used as references for comparison and identification with unknown samples.

Herbarium (plural: **herbaria**), the term can also refer to the building or room where the specimens are housed, or to the scientific institute that not only stores but uses them for research (live also).

The credit goes to an Italian taxonomist Luca Ghini (1490-1556) for his initiative efforts with reference to explore the Herbarium concept. The oldest traditions of making herbarium collection have been traced to Italy. During 1532, Luca Ghini and his student Gherardo Cibo created herbarium which is also kept in Rome in the form of oldest preserved herbarium. During this period Luca Ghini visited various parts of Italy for collecting many plant specimens and the first herbarium of world was established in 1545 in University of Padua, Italy. Most of the early herbaria were prepared with sheets bound into books. It was continued till the time of Carolus Linnaeus who came up with the idea of maintaining them on free sheets that allowed their easy re-ordering within cabinets. Nowadays the plants are mounted on single sheet and arranged according to the classification. Main objectives of collecting plants in the field and preserving them in herbarium with their proper documentation including notes that give maximum information about the plants.

2.3.1.1 Specimen preparation for Herbarium

The technique of specimen preparation for herbarium is as follows:

2.3.1.1.1 Plant Collection

Specimens must be collected in every stage of their growth and reproduction as well as from different localities and habitats (Fig.2.1). We should be very conservative during collection and collect only what we need. It is a good idea to use the following rule of thumb:

1. Never collect a plant when we can see fewer than 6 individuals in the area.
2. Select vigorous, typical specimens.
3. Avoid insect-damaged plants.
4. Make sure the plant has flowers and/or fruits.
5. It may be a good idea to collect extra flowers and fruit for identification purposes.
6. Sterile plants are very difficult to identify.
7. Roots, bulbs, and other underground parts of herbaceous (non-woody) plants should be carefully dug up, and the soil removed with care.
8. When collecting shrubs and trees, clip one or two small branches.
9. Plants too large for a single sheet may be divided and pressed as a series of sheets. It is good practice to collect in duplicate. This means that if possible, we should collect sufficient material to make more than a single herbarium specimen.

Fig. 2.1 Selection of a specimen (Try to collect both flowers and fruit also if available)

For collection of the plants one should go out on excursion several times in a season. We should have commonly used equipment during excursion practice. A list of these equipments is being given below:

- scissor to cut and trim specimens(Fig.2.2).
- A khurpi for digging up roots and underground stems
- A knife

Fig.2.2 For collection secateurs are used for clean cut of the stem

- A vasculum for keeping the collected plants and their twigs
- A pair of forceps
- Day press that is light enough to carry around. This should include a few cardboard corrugates, and sheets of newspaper.
- A field press with many more corrugates and more newspapers.
- Spare corrugates and newspaper and some sheets of foam for pressing bulky items
- GPS for recording an accurate latitude and longitude. Alternatively, mark the position on a topographic map.

- A field notebook and pencil. This can be a pocket-sized notebook or a book of pre-printed specimen labels may be used.
- Large and small plastic bags to hold specimens temporarily
- Small brown paper bags for collecting fruits, seeds, bryophytes and lichens
- A hand lens
- Tie-on tags, often called jeweller's tags
- Felt tipped pens and pencils for numbering collection and writing notes
- A camera/phone for photographing the form of the plant, flower colour and its natural habitat.

2.3.1.1.2 Pressing of Specimens

After the collection procedure it is necessary to quickly dry the plants under firm pressure to retain plant colors and the plant arrangement (Fig.2.3).

Fig.2.3 Procedure of pressing the specimen

The specimen should be carefully displayed on the pressing sheets (blotters or newspaper sheets) just to avoid the folding or hiding of parts (Fig.2.4). This process is to be carried out immediately because once a plant wilts; it will not make an attractive mount. A supply of corrugated cardboard sheets (cut to fit your press) is also needed. As we fill our press, alternate the cardboard sheets and folded paper (beginning and ending with a sheet of cardboard) to keep the specimens flat and speed the drying process (sometime blotter sheets can be placed between the newspaper and cardboard to speed the drying process).

Fig.2.4 Procedure of putting the specimen in day press

We should check the plant closely to make sure all soil is removed from the roots and remove excess moisture with a paper towel. If the plant is less than 12 inches long, place it in the folded newspaper. Arrange the stems, leaves, roots and flowers exactly as you want them to appear on the mount. Flowers should be pressed open. Both the upper and lower surfaces of flowers and leaves should be displayed. If the plant is longer than 12 inches, it will be necessary to fold the plant in the shape of a V, N or W.

Re-examine the plant after it has been pressed for 24 hours. This will be our last opportunity to do some rearranging while the plant is still flexible. Proper observations are needed by changing the newspaper or blotter paper every day until the plant is thoroughly dry. We should remember one important thing that succulent (fleshy) plants will take much longer time to press. Plants can be removed from the press in seven to 10 days. Keep the plants in folded newspaper until you are ready to mount them.

2.3.1.1.2 Mounting of Specimens

After drying, the specimens are ready for mounting. For mounting, herbarium sheets, standard (white) tag or poster board are recommended. Although herbarium sheets of standard size (29 x 41±1cm) usually have to be ordered, poster board can be purchased at most stores selling office and school supplies. Herbarium sheets are usually made up of heavy hand made card sheets that are well known for their long durability. Several adhesives (A transparent glue i.e. Elmer's glue) are used for attaching specimens to the sheet. For holding heavy or woody specimens, strips of brown gummed paper might be used as additional aid.

The specimen should be placed upright with the roots near the bottom and should provide an attractive look. We can also use small strips of gummed cloth for this purpose. Scotch tape (Cellulose tape) is not recommended. An example of the label that should be used on mounts (and the instructions on how to fill it out) is given below.

2.3.1.1.3 Herbarium Labels

After mounting the specimen a label is glued on the lower right hand corner of sheet. The label provides information taken from the field note book. The format and size of label varies but usually it is about 4.5x7.5 cm. Label have all the information about Botanical name, Local name, Locality, Time, Characters, collector's name etc. In addition, the label should include at least the following data:

1. A heading indicating the name of institution from where specimen is originated and the region of collection
2. The name of the family
3. The botanical name of the plant with authority
4. The locality of collection
5. The data of collection (***Nearest landmark, Elevation, Aspect*** **etc.)**
6. The habitat
7. The name of collector
8. The collectors field number
9. The venacular name and local uses

2.3.1.1.3 Filing of Herbarium Sheets

The mounted and properly identified plant specimens are usually stored according to an accepted classification (Bentham and Hooker's classification) in special wooden or steel cabinets (Fig.2.5) with special concern to protect them from dust and insects.

The plants should be filed in a logical order that makes it easy to find a specific specimen. By filing all specimens according to family and arranging the family members in alphabetical order by genus and species, it will be much easier for us to find a specific specimen whenever it is required for further study. As we have learned that the herbarium specimens are permanent collections, therefore they require proper care. Herbarium specimens must be protected against damages caused by fungi and insects. Therefore such damages can be controlled completely by periodical fumigation with chemicals or poisoning the responsible factors with a solution of mercuric chloride or lauryl pentachlorophenate. It might be usually a good idea to store a few moth balls with plant specimens to protect them from insects. Dichloro-diphenyl-trichloroethane (DDT) can also be used as an insect repellent.

Fig.2.5 Storage the mounted and properly identifies plant specimens

2.3.1.2 Important Herbaria

The greatest herbarium of the world is well known as the Royal Botanic Garden at Kew, England, possessing about six million specimens. A few good herbaria are also there in our country. The biggest herbarium of our country is known as the Indian Botanic Garden, Calcutta, possessing about one million specimens. The herbarium of the Forest Research Institute, Dehradun has about 3, 00,000 specimens. The herbaria of Agricultural College and Research Institute, Coimbatore and National Botanical Gardens, Lucknow, have about 200,000 and 40,000 specimens respectively. There are about 25,000 specimens in the herbarium of the Divisions of Mycology and Plant Pathology at Indian Agricultural Research Institute, New Delhi. The herbarium of the Division of Botany at I.A.R.I. New Delhi, contains about 3000 specimens.

Here we will discuss in brief about some important herbaria of India.

2.3.1.2.1 Forest Research Institute (FRI), Dehradun:

Today, it comprises of three sections, viz., systematic botany, wood anatomy and plant physiology. This division today maintains a botanical garden, an arboretum, having one of the richest live collections of both indigenous and exotic tree species, and a bambusetum,

containing germ plasm of forty species of indigenous and exotic bamboos. It was started by Gamble (1890), and today the herbarium of the FRI has grown to become one of the largest herbarium of Asia. Today it holds 3,25,000 authenticated plant specimens, including 1300 type specimens, as well as a carpological collection.

2.3.1.2.2 Herbarium of the Indian Botanic Gardens, Calcutta:

It was established in 1787. It is directed by the State of West Bengal, Department of Agriculture, Animal Husbandry and Forest. It is also known as Central National Herbarium Kolkata. More than 2,500,000 species mainly phanerogams and ferns of India and neighbouring countries of South and South East Asia are kept in this herbarium .

2.3.1.2.3 Herbarium of the National Botanic Gardens, Lucknow (N.B.R.I):

It was founded in 1948 and taken over by the Council of Scientific and Industrial Research (CSIR), New Delhi, Government of India in 1953. The number of specimens is about half million. The garden has been established by C.S.I.R. as a Central Garden for India with the number of species about 1, 00,000.

2.3.1.2.4 Herbarium of the Division of Botany, Indian Agricultural Research Institute, (I.A.R.I.), New Delhi:

It was established in 1901 and maintained by Government of India. The number of specimens is about 5, 000mainly from North India. There are introduced plants of economic value and wild relatives of crop plants.

2.3.1.2.5. Herbaria of Botanical Survey of India:

- Eastern circle Herbarium, Shillong of BSI was established in 1956. It has the number of specimens about 10,000,00.
- Southern circle Herbarium, Coimbator of BSI was established in 1874 and has the number of specimens about 2, 00,000.
- Western circle Herbarium, Pune of BSI was established in 1956. In this herbarium the number of specimens is about 50,000.
- Northern Circle Herbarium, Dehradun was established in 1956 and holds the about 60,000specimens.
- Central Circle Herbarium, Allahabad was established in 1955. It has the number of specimens about 45,000.

2.3.2-Live Specimens

Long before the term “biodiversity” was used, botanical gardens carried out activities that are now associated with biodiversity. For the collection of live specimens botanical gardens are being established. Botanical gardens took part in describing new species and studied them to discover their potential uses in industry, horticulture or for research. Gardens also conserved species of rare wild plants (or *ex situ* conservation, meaning outside of their natural habitat).

In botanical gardens we can conserve endangered plant species through live collections as well as through seed banks. These benefit pollinators like butterflies,

honeybees, bats, and birds, which play an important role in the pollination of crops. According to the International Agenda for Botanic Gardens in Conservation (IABGC) (2000), ***Botanic gardens*** are institutions holding documented collections of living plants for the purposes of scientific research, conservation, display and education."

A botanical garden must be a public institution committed to long-term maintenance of its collections. Botanical gardens have a unique environment to raise public awareness and help people understand the importance of biodiversity, educate people about the threats it currently faces and make them realize that nature conservation is everyone's job. This is why it is so important for gardens to maintain interpretation programs, host school groups and present exhibitions. The major role of botanical gardens in biodiversity conservation is ***ex situ* conservation**. *Ex-situ* conservation (growing wild plants outside their natural environment) has many advantages, but should not be seen as an objective in itself. It is referred to be as one important element of a comprehensive strategy to conserve species in their environment. *Ex situ* conservation helps to attain this objective by providing material to reintroduce plants into degraded areas or to reinforce existing populations.

Botanical gardens have three main objectives:

- The first and best known objective is recreation. Exhibitions, plant sales, picnics under the trees and relaxing in a natural environment are some of the possibilities that botanical gardens offer both residents and tourists.
- The second very important objective of botanical gardens is education. This includes summer camps for kids, school group tours, interpretation, classes and seminars as well as publications and other ways of sharing information between botanical gardens and horticulture and botany professionals.
- Finally, gardens have a scientific objective. Today, fields of study are even broader, from molecular research in the lab to ecological field work. Conservation and study of local plants should also be given emphasis.

At present there are more than 600 botanical gardens all over the world. Major botanical gardens of the worlds and India are being described here under:

2.3.2.1.1 Royal Botanical Garden, Kew

The Royal Garden at Kew was founded in 1759, initially as part of the Royal Garden set aside as a physic garden. William Aiton (1741–1793) was the first curator. Initially, Royal Botanic Garden, Kew (1759) was set up to cultivate new species returned from expeditions to the tropics. In 1841 William J. Hooker was appointed as Director and under his guidance the garden was extended from 20 to 250 acres. At present it has been extended upto 300 acres. It contains herbarium including 5,000,000 specimens

2.3.2.1.2 The Royal Botanic Garden Sydney Australia

The first botanical garden in Australia was founded early in the 19th century. The Royal Botanic Garden, Sydney is a major botanical garden located in the heart of Sydney, New South Wales, Australia. It was founded in 1816 and the garden is the oldest scientific institution in Australia as well as one of the most important historic botanical institutions in the world.

2.3.2.1.3 The Dunedin Botanic Garden, New Zealand

The Dunedin Botanic Garden is New Zealand's first botanic garden and holds the status of six star Garden of International significance. It was established in 1863. In 2010 it was recognised as a Garden of International significance for its excellence as a public garden as well as for its botanical collections. An important aspect of The New Zealand Native plant collection is the cultivation of rare and endangered native plant species.

2.3.2.2 Indian Botanical Gardens

2.3.2.2.1 The Acharya Jagadish Chandra Bose Indian Botanic Garden

The Acharya Jagadish Chandra Bose Indian Botanic Garden (previously known as Indian Botanic Garden) is situated in Shibpur, Howrah near Kolkata. It is commonly known as the Calcutta Botanical Garden, and previously as the Royal Botanic Garden, Calcutta. The garden exhibits a wide variety of rare plants and a total collection of over 15,000 live specimens spread over 273 acres of land. It is now under Botanical Survey of India.The garden was also called the East India Company's Garden or the 'Company Bagan'.

The great Banayan Tree is the main attraction of the garden that **forms the second largest canopy in the world,** which is about 250 years of age and have over 1600 aerial roots. It is well known for a palm house, orchid house, medicinal plants, ferns and cacti. *Victoria regia*, a giant water lilly is also the beauty of the garden.

2.3.2.2.2 Lloyd's Botanical Garden

Lloyd's Botanical Garden, or Darjeeling Botanical Garden, is abotanical garden in Darjeeling in the Indian state of West Bengal. It was established in 1878 as an extension of Royal Botanical Garden Calcutta on 40 acres (160,000 m^2) of land. The land was donated by William Lloyd, in whose name the botanical garden has been named as, Lloyd's Botanical Garden. The Darjeeling Botanical Garden preserves several species of bamboo, oak, magnolia, wild geranium, rhododendron etc. It has a rock garden, orchidarium and separate sections for conifers and indegenous plants.Gardens is well known for its collection of orchids (in the Orchid House) and different herbs.

2.3.2.2.3 Lalbagh Botanical Garden

Lalbagh or Lalbagh Botanical Garden, meaning *The Red Garden* in English or Mysore State Botanical Garden, Bangalore is one of the best botanical garden in South India. The garden was named as Lalbagh by Hyder Ali, the ruler of Mysore in 1760. During 1799-1819 maximum exotic plants were introduced in this garden by Major Waugh. Lalbagh was given the status of a Government Botanical Garden in 1856.

The botanical garden is enriched with numerous native and exotic flora of wide ranging diversity. Today, nearly 673 genera and 1,854 species of plants are present in this garden.

2.3.2.2.4 National Botanical Garden Lucknow

This Botanical Garden is situated in the heart of Lucknow, the capital of Uttar Pradesh province, and covers an area of 25 ha along the southern bank of the River Gomti. The

garden was established by Nawab Saadat Ali Khan (1784-1814) as a Royal Garden. It was established in its new form in 1946 by Prof. K. N. Kaul and today is well known as NBRI(National Botanical Research Institute) Lucknow. It shows the diversity of plants, comprising a collection of 6,000 indigenous, ornamental and exotic taxa.

The plant wealth of the Botanic Garden is displayed in the arboretum, conservatory, cactus and succulent house, palm house, bonsai section, fern house and new conservatory.

2.3.2.2.5 Saharanpur botanical garden

The Saharanpur botanical garden (presently known as Horticultural Experiment and Training Centre, Saharanpur) is a very beautiful garden since British period. John F. Duthie (1845-1922) an English botanist and explorer collected plants from Kumaun, Kashmir and gangetic plain. He compiled the "Flora of upper gangetic plains" with the help of his garden staff.

2.4 SUMMARY

1. The term herbarium was given by Linnaeus. Plant samples can be dried or preserved in liquid. Plant samples can also be kept alive in greenhouse or garden.
2. A herbarium consists of preserved plant specimens, each with a label bearing documentary information. Herbaria are repositories for vascular plants, bryophytes, lichens, algae, and fungi.
3. Specimens are used as references for comparison and identification with unknown samples.
4. The method of preparation and storage depends on the type of plant being processed. Most specimens are mounted on standard herbarium sheets.
5. They include reproductive and vegetative organs, features critical to identification.
6. Some herbarium specimens are known as a 'voucher specimens'. Voucher specimens serve as a basis of scientific study.
7. Voucher specimens are collected from taxa that are the subject of research or investigation, generally resulting in a publication in a scientific journal or report. The herbarium specimens bear labels with adequate data on habit and habitat, common name, native uses etc.
8. Herbarium specimens are permanent records of a particular locality. Therefore, we should be more careful during the selection and collection of plant samples.
9. Thereafter herbarium specimens should be properly prepared, preserved and maintained.
10. For the collection of live specimens botanical gardens are being established.
11. In botanical gardens we can conserve endangered plant species through live collections as well as through seed banks.
12. A botanical garden must be a public institution committed to long-term maintenance of its collections.
13. Botanical gardens have a unique environment to raise public awareness and help people understand the importance of biodiversity.
14. Gardens carry out interpretation programs, host school groups and present exhibitions.
15. The major role of botanical gardens is biodiversity conservation as ***ex situ*** **conservation**.

2.5 GLOSSARY

Plant specimen: An individual animal, plant, piece of a mineral, etc. used as an example of its species or type for scientific study or display.
Herbarium: a reference collection of pressed, dried (preserved), botanical specimens.
Preservation: The act of keeping something the same or of preventing it from being damaged
Maintenance: the process of preserving a condition or situation or the state of being preserved.
Species: the narrowest taxonomic grouping; a group of closely related animals or plants that are capable of interbreeding.
Mounting: A backing, setting, or support for something.
Acquisition: An asset or object bought or obtained, typically by a library or museum.
Cultivation: The process of promoting the growth of a biological culture.
Ancient practice: The practice was more common in ancient times than it is now
Jardin des Plantes: Garden of plants is the main botanical garden in France.
Botanical garden: A garden for the exhibition and scientific study of collected plants, usually in association with green houses, herbaria, laboratories etc.
Filing: A filing is when a legal document becomes part of the public record.
Domestication: The process of adapting wild plants for human use.
Reference material: Reference materials are various sources that provide background information or quick facts on any particular topic.
Vigorous: grows with great enthusiasm.
Vascular plant: a plant with vascular tissues (xylem and phloem)
Bulbous plant: plant growing from a bulb
Repositories: a place where things are stored and can be found.
Sterile plants:the plant does not produce seeds
Conservation: The protection of animals, plants, and natural resources
Voucher specimens: a specimen maintained in a collection or herbarium that is associated with specific research or referred to in a report; may include type specimens, or specimens from a flora or consultant

2.6 SELF ASSESSMENT QUESTIONS

2.6.1 Short Answer Type Questions

1. What is the name of largest Herbarium in the world?
2. How plant specimens can be kept alive.
3. Where a label must be mentioned in each mount.
4. What procedure is to be followed after the collection?
5. Why specimens should be displayed on the pressing sheets?
6. What standard size should be of herbarium sheets?
7. What basic information should be mentioned in herbarium label.

8. Which chemical might be used as an insect repellent for the safety of herbarium.

2.6.2 Fill in the Blanks

1. ----------may be in form of whole plants or plant parts.
2. ----------is the acquisition of plant specimens for the purposes of research
3. ----------was founded in 1793 during the French Revolution
4. -------is a collection of preserved plants, usually in dried form, used for botanical research.
5. ---------and his student created herbarium which is also kept in Rome in form of oldest preserved herbarium.
6. --------is used for digging up roots and underground stems
7. ----------is used for recording an accurate latitude and longitude.
8. The greatest herbarium of the world is well known as the---------- at Kew, England.
9. The biggest herbarium of our country is known as the ---------.
10. For the collection of live specimens ----------are being established.
11. The ----------is a major botanical garden located in the heart of Sydney, New South Wales, Australia.
12. ----------, a giant water lilly is also the beauty of the Calcutta Botanical Garden
13. John F. Duthie was appointed as Superintendent of ----------

2.6.1 Answers Key:

1. National Museum of Natural History in Paris.
2. Plant specimens can be kept alive in botanical gardens.
3. A label must be in the lower right hand corner of each mount.
4. Pressing is to be followed after the collection.
5. Specimens should be carefully displayed on the pressing sheets (blotters or newspaper sheets) just to avoid the folding or hiding of parts.
6. Standard size of herbarium sheets should be of 29 x 41±1cm.
7. Basic informations should be mentioned in herbarium label with specimens such as Botanical name, Local name, Locality, Time, Characters and collectors name
8. Dichloro-diphenyl-trichloroethane (DDT) is used as an insect repellent for the safety of herbarium.

2.6.2 Answers key: 1. The specimens; 2. Plant collecting**; 3.** National Museum of Natural History**; 4.** A herbarium**;** 5. Luca Ghini**; 6.** A khurpi**; 7.** GPS**; 8.** Royal Botanic Garden; 9. Indian Botanic Garden, Calcutta; 10. Botanical gardens**; 11.** Royal Botanic Garden Sydney; 12. *Victoria regia;* **13.** Saharanpur Botanical Garden

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2.9 TERMINAL QUESTIONS

1. Write an essay on the collection of plant specimens.
2. Describe the collection and preservation techniques of specimens for herbarium.
3. Write a detailed account of herbarium.
4. Write a short essay on the major botanical gardens of world.
5. Describe the role of botanical gardens.
6. Describe the Indian botanical gardens.

UNIT-3 T.S. OF ANTHER

3.1 OBJECTIVES

After reading this unit, students will be able-

- To study the structure of Flower
- To study the male reproductive part of Flower
- To study the T. S. of Anther
- To understand the procedure for preparing the T. S. of Anther

3.2 INTRODUCTION

The **angiosperms** are seed-bearing plants that produce flowers. The seeds, which contain the plant embryo, are produced in the flower. All the parts of a flower are actually modified leaves that are specialized for their roles in the reproductive process. Let us try to understand a typical flower with the help of Fig.3.1.

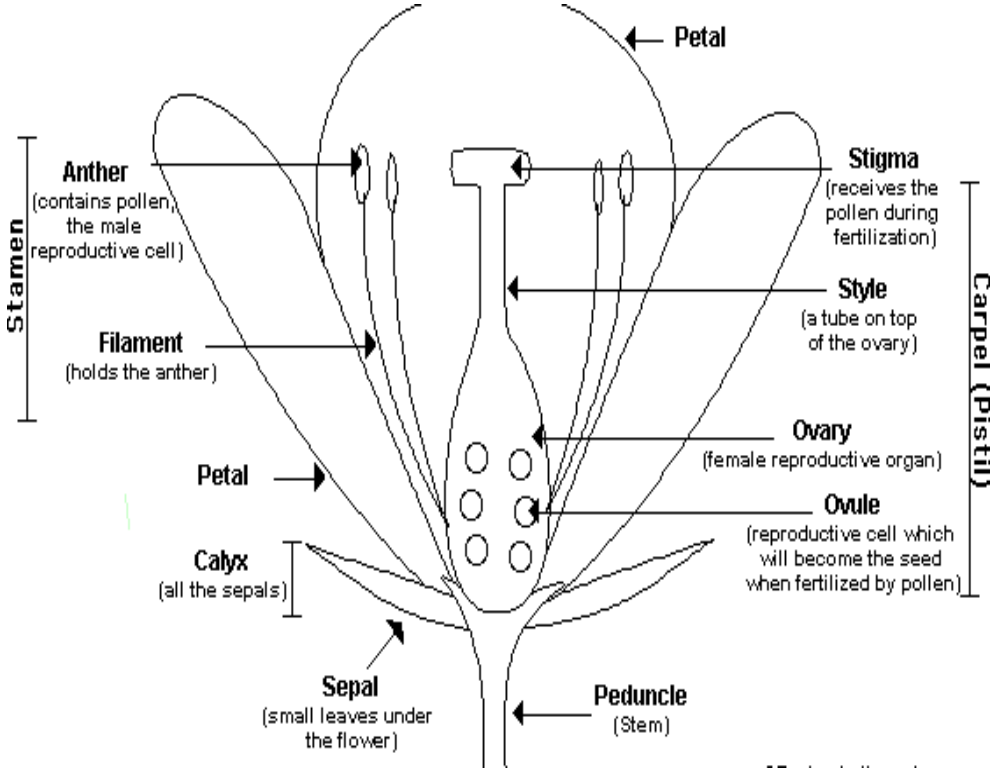

Fig. 3.1 Structure of flower

Flower parts are arranged in circles called **whorls**. They are attached at the enlarged base of the flower, the **receptacle**. As we know that flowers are made up of both sterile and fertile parts which are arranged in four whorls. These include the calyx, the corolla, the androecium and the gynoecium. Both the calyx (collective term of sepals) and corolla (collective term of petals) are the sterile parts, while the androecium and gynoecium are the fertile structures.

Basically, the androecium is referred to be the third set of floral organs composed of stamens or microsporophylls. Ordinarily, each stamen is composed of a slender stalk-like filament supporting a knob-like spore case or the anther. Each anther consists of two lobes (anther lobes) connected by a tissue (connective) which can be in some cases clearly seen on the dorsal side as an extension of the filament. Each anther lobe, again, has two pollen sacs or pollen chambers (microsporangia) placed longitudinally. Special cells within the pollen sacs undergo meiosis to form **pollen grains**. Each pollen grain contains two male gametes. When the pollen grains mature, the pollen sacs split open to release the dust-like **pollen**.

The **pistil** (carpel) is the female reproductive organ and consists of three parts: the stigma, style, and ovary. The **stigma** is an enlarged receptive part at the top of the pistil that becomes moist and sticky when mature. The **style** is the middle thin portion of the pistil. It can be long and slender, short, or even absent, depending upon the species. The **ovary** is the enlarged structure at the bottom of the pistil. The ovary contains one or more hollow compartments called **locules**. Each locule contains one or more **ovules**. Embryo sac (female gametophyte) is present in each ovule which has one **egg.**

Pollination occurs when pollen grains land on the sticky surface of the stigma and are trapped there. The pollen grain germinates and a **pollen tube** emerges due to the release of enzymes by stigmatic surface that digest the cell wall of pollen grain. The pollen tube grows down through the style to the ovary and enters the ovule, making a continuous passage for the two male gametes to enter the embryo sac inside the ovule. **Fertilization** occurs by fusion of male gamete with egg.

The fertilized egg ultimately develops into an **embryo**. The wall of the ovule thickens and forms a **seed**, thus enclosing and protecting the embryo. The ovary develops into a **fruit**.

After getting a concise knowledge about a flower, we will try to study the morphological features of the flower. For this purpose we will obtain a single flower and observe its parts carefully.

The **sepals** form the outermost whorl of the flower. The sepals are leaf-like structures that are usually green in color. Sometimes, the sepals are the same color as the petals, or appear to be another set of petals of a different color. The function of the sepals is to protect the inner part of the flower before it blossoms. Gently remove the sepals, tape them into position onto the paper, and label them. On the chart, observations should be recorded.

The **petals** are found directly under the sepals. The color and odor of the petals help to attract birds and insects to the flower for pollination. Gently remove the petals, tape them into position onto the paper, and label them. On the chart, observations are recorded.

The stalk-like structures inside the petals are the **stamens**, the male reproductive part. Depending on the species, the stamens may be attached to the receptacle, to the petals, or to the pistil. The enlarged portion at the top of the stamen is the **anther**. Inside the anther are **pollen sacs**, which produce pollen grains. When the **pollen grains** mature, the pollen

sacs split open, releasing the dust like pollen grains. The filament is the thin structure that supports the anther.

After examining flower morphologically let us try to know more about the stamen-

Stamens (Fig.3.2) are the male reproductive organs of a flower. Each stamen consists of an anther borne on a stalk-like filament. The anther dehisces at maturity in most of the angiosperms by a longitudinal slit to release the pollen grains. The pollen grains contain the highly reduced male gametophyte. These microgametophytes carry of male gametes that play a key role in plant reproduction during the process of double fertilization.

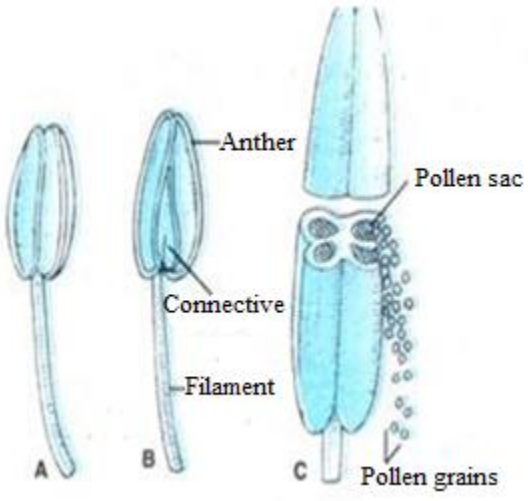

Fig.3.2 Stamen: A ventral view; B. Dorsal view; c. Three dimensional cut section of anther

3.2.1 Structure of Anther

As we can see in the Fig.3.2, a typical anther is a bilobed, dithecous structure with two microsporangia in each lobe. Therefore, an anther is a tetrasporangiate structure with four microsporangia. The non-sporangial tissue that joins the two anther lobes is known as the connective. A single vascular strand is embedded in the connective. In each lobe the two microsporangia are separated by a strip of sterile tissue. In a mature anther, the two sporangia in an anther lobe become confluent due to the enzymatic lysis of the sterile tissue to form a single locule. In some plants such as *Hibiscus rosa-sinensis,* the anther is one lobed consisting of two microsporangia which are fused at maturity to form a single locule (monothecous).

3.3 T. S. OF ANTHER

The transverse section of Anther (Fig.3.3) reveals that the mature anther wall is made up of the following four layers:-

Epidermis
Endothecium
Middle layers
Tapetum

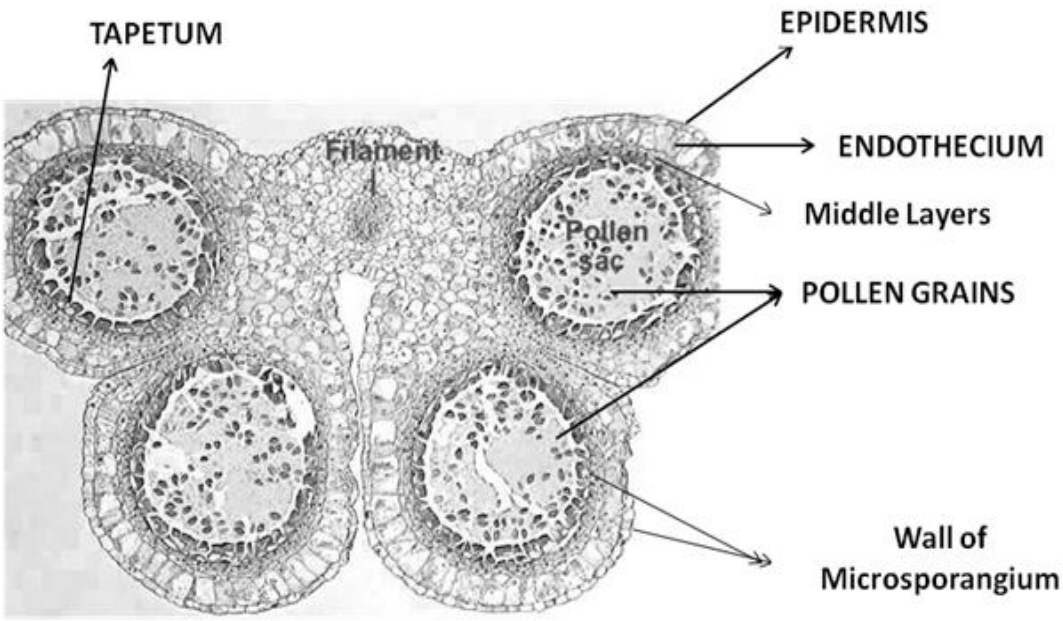

Fig.3.3 T.S. of Anther

3.3.1 Epidermis

The epidermis is the outermost layer of the anther and made up of tangentially stretched and flattened cells. Epidermis has a protective function. The epidermis prevents water loss from the anther, together with the endothecium provides structural support to the anther and plays a role in the anther dehiscence (Goldberg *et al*. 1993). In a mature anther, the epidermal cells are greatly stretched and flattened. In xerophytic plants, the epidermal cells are stretched to such an extent that these cell loose contact among themselves and appear as withering remains in a mature anther. The epidermal cells in the stomium region differentiate into small, specialized cells that split at maturity to facilitate dehiscence and release of pollen grains (Fig.3.4).

3.3.2 Endothecium

Endothecium also known as the subepidermal layer is the hypodermal layer that persists in the mature anther. Endothecium is usually single layered having radially elongated cells which attain maximum development when the anther is ready to dehisce for the discharge of mature pollens. The radial and inner tangential wall of endothecium cells are characterized by deposition of fibrous thickening bands. The outer tangential walls remain thin.

The endothecial cells at the junction of two pollen sacs (stomium) of anther lobe lack thickening in case of longitudinally dehiscing anthers. Thus the presence of fibrous bands, differential expansion of tangential wall layers and hygroscopic nature of endothecial cells play an important role in the dehiscence of anthers.

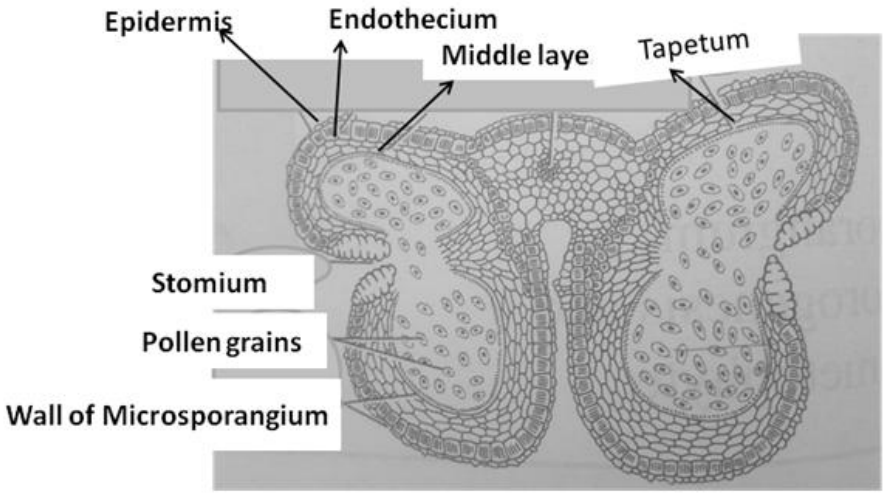

Fig. 3.4 T.S. of Anther showing the stomium region

3.3.3 Middle Layers

In general the cells of middle layers are ephemeral and, as a rule, become flattened and crushed during meiosis in the microspore mother cells. However, in some plants one or more middle layers may persist in anthers such as *Lilium*while in others, such as *Wolffia*and *Vallisneria*, middle layers are absent. The cells are flattened, thin-walled, uninucleate and vacuolated. In most angiospermic plants, the middle layers are referred to be the storage centers of reserve food material such as starch and other reserves which gets mobilized during the later development of pollen.

3.3.4 Tapetum

Tapetum is the innermost layer of the anther wall and is present in the form of a homogenous layer that completely surrounds the sporogenous tissue. It is usually single layered and has several nutritive and secretory functions related to pollen development and pollen germination. In many angiosperms, the tapetum is of dual origin. The outer portion of the tapetum (Fig.3.5), is contributed by the parietal layer (P-Tepetum) while the inner portion is derived from the connective tissue (C-Tapetum). The tapetum cells contain prominent nuclei and dense cytoplasm with an abundance of organelles such as mitochondria, plastids, endoplasmic reticulum, dictyosomes, vesicles and ribosomes.

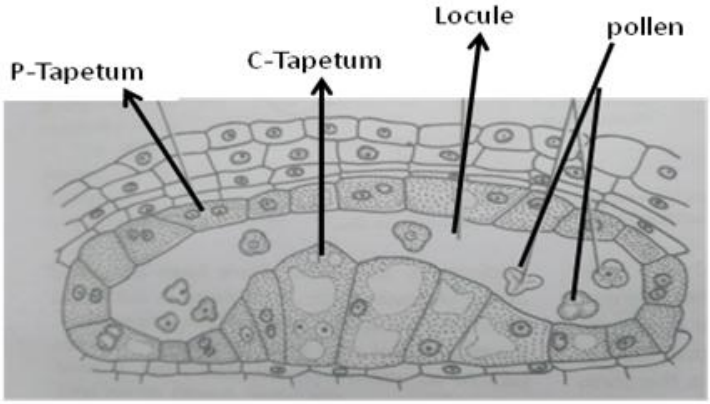

Fig.3.5 Tapetum showing dimorphic nature

The tapetum attains its maximum development at the tetrad stage of microsporogenesis. Later on it starts degenerating and is completely degenerated by the time the anther is ready to dehisce.

3.3.4.1 Function of Tapetum

The tapetum appears to play a significant role in the development of pollen. Its precocious degeneration during premeiotic and meiotic stages or its cellular persistence for unusually long period results in pollen sterility. Tapetum also helps in the transport of foodmaterial to the inside of the anther. The food material stored in tapetal cells is not utilised in the early stages of anther development. But after pollen mother cells have undergone meiosis the protoplast of tapetal cells enters into the pollen chamber to form periplasmodium which provides nutrition to the developing pollen grains and also helps in the exine formation.

3.4 PROCEDURE

For making neat and clean slide of Anther (T. S. of Anther), we should take few precautionary steps. These steps involve fine handling of plant material and a complex multi-stage process of staining to prepare microscope slides. This will depend on the amount and type of anther material available. Following are the major requirements that should be available with us-

- High power microscopes
- Suitable plant material as a source of anthers
- Plain slides
- Cover slips
- Staining solutions as safranin or fast green

3.4.1 Using the Microscope

- Lighting the slide is crucial, and students will need to use the condenser correctly, in order to achieve good contrast. Use of fine focus (and an oil immersion lens if available) will allow better viewing.
- It is important for us to obtain very young anthers to study, in which the pollen mother cells on the inner wall of the anther are still active. Look for white, translucent anthers.
- Some anthers do not take up the stain very readily, so it is wise to use a number of different sources of material. Possible sources of material are floral buds (in late summer or early autumn – before sprouting) of *Allium, Lilium,* or young buds of *Pelargonium* sp., *Tradescantia* sp. etc.

3.4.2 Location of Anthers in an Inflorescence

- Select the very smallest single bud on the inflorescence, which will also be the youngest.
- Carefully hold the base or stalk of the bud with forceps and pick the green sepals off with a mounted needle.

- Remove the small white petals that are around the outside of the flower and expose the ring of stamens in the middle of the flower. In some flowers, the stamens may themselves be attached to very small petals, do not discard these by mistake.

3.4.3 Preparation of Anthers for Further Investigation

- Anthers should appear white and translucent. If in doubt, try a smaller bud. Leave them attached to the flower throughout the staining process, as they are very easily lost.
- Stain anthers with safranin and sometimes with acetocarmine for five to six minutes and boil them upto three minutes in acetocarmine solution with the help of sprit lamp to see the cell division.
- Detach two or three anthers and transfer to a clean slide.
- Mature anthers can also be selected from the flower for section cutting.
- Then cut sections of anthers transversely in bulk quantity.
- Select the best sections and cover them with cover slips.

3.5 SUMMARY

The **angiosperms** are seed-bearing plants that produce flowers. The seeds, which contain the plant embryo, are produced in the flower. All the parts of a flower are actually modified leaves that are specialized for their roles in the reproductive process. Flower parts are arranged in circles called **whorls**. They are attached at the enlarged base of the flower, the **receptacle**.

As we know that flowers are made up of both sterile and fertile structures arranged in four whorls. Both the calyx and corolla are the sterile structures of a flower, while the androecium and gynoecium are the fertile structures.

The unitof the androecium is called the **stamen** and is the male structures in the flower. The stamen is made up of the filament and the anther. The **filaments** are the slender stalks and the **anther** is at the top of the stamen that contains pollen. The anthers often appear as yellowish because they contain pollen grains.

The well-differentiated anther wall comprises an epidermis, an endothecium, 1-3 middle layers and the tapetum. The epidermis is protective, The endothecium develops fibrous bands of lignocellulosic secondary thickening that provides the mechanical force for anther dehiscence. The middle layers are short-lived and get crushed during pollen development. The cells store nutrients for the developing pollen. Tapetum is the inner most nutritive layer which plays a crucial role in pollen development.

3.6 GLOSSARY

Angiosperm: A plant of a large group that comprises those that have flowers and produce seeds enclosed within a carpel, including herbaceous plants, shrubs, grasses, and most trees.
Receptacle: The modified or expanded portion of the stem or axis that bears the organs of a single flower or the florets of a flower head.

Filament: Part of a stamen, the male part of a flower
Pollination: A process by which pollen is transferred to the female reproductive organs of a plant, thereby enabling fertilization to take place.
Pollen tube: A hollow tube which develops from a pollen grain when deposited on the stigma of a flower. It penetrates the style and conveys the male gametes to the ovule.
Stamen: The male fertilizing organ of a flower, typically consisting of a pollen-containing anther and a filament.
Double fertilization: This process of forming a zygote and endosperm is called double fertilization, and it is unique to angiosperms.
Dithecous: The anther type, which contains two anther lobes connected to each other by connective.
Anther: An anther is the part of a stamen that produces and releases the pollen grains.
Anther dehiscence: Splitting of the anther at maturity along a built-in line of weakness.
Anther locule: A liquid filled cavity within the anthers in which the pollen grains develop and ripen.
Endothecium: The hypodermal layer of the anther wall characterized by the deposition of fibrous bands of lignocellulosic thickenings that provides the mechanical force for anther dehiscence.
Microgametogenesis: The process of formation of male/micro-gametes from the microspores.
Microsporogenesis: The series of events that lead to the development of haploid, uninucleate microspores from microspore mother cells within the microsporangium.
Pollenkitt: An oily, thick, viscous coating present over the pollen grain surface of many insect pollinated species that helps in adhering pollen grains together, adhering of pollen to insect pollinators and also to the stigma surface.
Tapetum: The innermost layer of the anther wall that plays an important secretory and transport function in pollen development, pollination and pollen germination.

3.7 SELF ASSESSMENT QUESTIONS

3.7.1 One Word Answer Type Questions:

1. Which substance is present in the fibrous thickenings of microsporangium?
2. Which is the innermost layer of microsporangial wall?
3. Which plant produce compound microspores?
4. Name the substance present in the exine of pollen grain.
5. Name the normal type of arrangement of microspore tetrad in angiosperms.
6. The pollen grains are liberated in angiosperm at what stage?
7. Which provides nutrition to the developing pollen grains and also helps in the exine formation?

3.7.2 Fill in the Blanks:

1. The branch of botany, which deals with the study of pollen, is……

2. The layer of cells present below the epidermis of a microsporangium is known as……
3. Each microspore mother cell undergoes **…….** and forms four haploid microspores.
4. The process of formation of microspors from the sporogenous tissue is known as **……**
5. The fertilized egg becomes an **……**
6. Special cells within the pollen sacs undergo meiosis to form **……...**
7. The outer portion of the tapetum is contributed by the parietal layer reffered as **……**
8. ……of endothecial cells play an important role in the dehiscence of anthers.

3.7.1 Answers Key:

1. Cellulose, 2. Tapetum, 3. *Acacia*, 4. Sporopollenin, 5. Tetrahedral type, 6. 2-celled stage, 7. Periplasmodium

3.7.2 Answers Key:

1. Palynology, 2. Endothecium, 3.Meiosis, 4. Microsporogenesis, 5. Embryo, 6. Pollen grains, 7. P- Tapetum, 8. Hygroscopic nature

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3.10 TERMINAL QUESTIONS

1. Draw a well-labeled sketch of Flower.
2. Draw three dimensional cut section of anther and describe the procedure.
3. Describe the procedure for the preparation of neat and clean slide of Anther.
4. Draw the T. S. of anther and describe it.
5. Draw the structure of a typical tetrasporangiate anther with well-differentiated wall layers and describe it.

UNIT-4 STUDY OF POLLEN GRAINS, PLACENTATIONS, AND OVULES USING TEMPORARY AND PERMANENT PREPARATIONS

4.1 OBJECTIVES

After reading this unit students will be able:

- To describe the temporary and permanent preparations of Pollen grains.
- To know the temporary and permanent preparations of Placentations.
- To understand the temporary and permanent preparations of various types of Ovules.
- To know the development of female gametophyte.

4.2 INTRODUCTION

When using a microscope, slides that are permanent can be examined and stored for a long time, while temporary slides are used for short time observations. Permanent slides must be properly made for successful long-term storage. Specimens must be finely sectioned and properly preserved for preparing a permanent slide.

Most permanent slides use the semi-solid form of mounting medium, which is the most stable. Liquid mounting mediums can also be used on permanent slides. This form suspends the specimen in liquid and uses nail polish to fix the cover slip to the slide. Nail polish makes the slide semi-permanent. It is permanent if left intact and temporary if the cover slip is removed, washed and dried for reuse. Slides using the liquid mounting method must be stored horizontally.

Preparation of a Temporary Slide

A temporary laboratory slide is in laboratories to view mounts under the microscope.
Apparatus: Material to be mounted, slide, watch glass, coverslip, petridish, filter paper, brush, microscope, stain as per the plant material.

Procedure:

1. Take a clean slide and put a drop of glycerine (10%) on the center of the slide. Excess amount of glycerine should be removed from the slide, if present.
2. Transfer material to be mounted on the drop of glycerine with the help of a small brush. Do this step very carefully. If the mount is not correctly placed observation under microscope becomes difficult.
3. Apply a thin coat of glycerine on the lower surface of cover slip to remove the air of this surface and now carefully place the coverslip over the glass slide covering the mount. Take extra care not to crush the mount much.
4. Remove extra fluid present on the slide with the help of filter paper. This is to obtain a clearer view on the microscope. Don't crush the coverslip, because it breaks easily.
5. Observe your mount under a microscope.

Once your slide is ready to be observed under a microscope place it in the viewing area of microscope and view it. Adjust light to obtaina clear view.

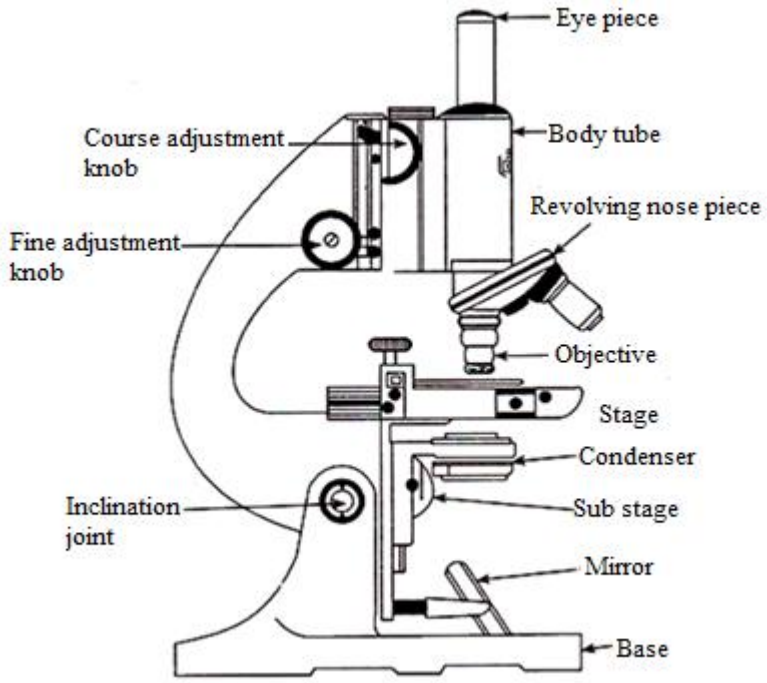


Fig.4.1 The compound microscope showing its various parts

Staining: Usually, material to be mounted is stained. Staining is generally done to get clear image under the microscope. For staining, different stains are commonly used. Iodine solution is commonly used for pollen studies. Take a little Stain in watch glass. Transfer material to be stained onto the watch glass. Wait for a few minutes. Now remove the material from the strain with the help of a brush and place it in watch glass containing clean water. Transfer stained material in drop of glycerine on glass slide. Put coverslip as described above and examine under microscope. When material to be stained is very small, place material in a drop off water. Add a drop off stain to it. After few seconds add drop of glycerine and put coverslip. Remove oozing fluid using filter paper.

Precautions:

1. Don't use excess amount of water.
2. Hold coverslip gently.
3. Use proper staining technique.
4. Don't crush the mount too much.
5. Use brush to transfer mount to slide from watch glass.

Prepare A Permanent Mount:

In certain casespreparations need to be stored permanently for future use. The method of preparation followed is described below.

1. The section is first stained with principal stain(aqueous hematoxylin, safranin or crystal violet).

2. The section is then washed with water till no more stain dissolves and water remains colourless.
3. Section is passed through a graded series of alcohol for dehydration. Different cavity blocks or petri dishes or watch glasses are filled with requisite amount of alcohol, (beginning with 30% alcohol) and the section is transferred to it. These cavity blocks or petri dishes or watch glasses should always be covered. The section is transferred to next higher series of alcohol after every 30 minutes which helps in gradual dehydration. In case if you don't want to disturb the section, used alcohol is removed by glass dropper. All the 30% alcohol is replaced with 50% alcohol. This procedure is repeated till 70% of alcohol grade is reached.
4. At this stage, counterstained is employed (e.g. safranin, fast green or erythrosine prepared in 80% or 90% alcohol).
5. This stain acts quickly and as such section is washed immediately after the requisite time is over.
6. De-staining is done by washing sections with90% or 100% alcohol.
7. The section is now transferred to absolute alcohol for complete dehydration.
8. Clearing now begins with 25% of xylol (25 cc of xylol and 75 cc of absolute alcohol). The sections are gradually passed through xylol series of 25%, 50%, 70%, 90% and finally transferred to pure xylol. If dehydration is not complete, pure xylol turns white or turbid. At this stage section should be passed through reverse series.
9. Pure xylol is the last stage of clearing. Section is now ready for mounting.
10. Mounting is done in Canada balsam or DPX mountant.

Mounting an Object

Mounting is necessary to properly position an object for clear view. Lactophenol, glycerine and glycerine jelly are used for temporary mounting while Canada balsam and DPX is used for permanent mounting.

(a) **Canada balsam:** It is a resin obtained from a conifer-*Abies balsamea*, most suitable for permanent slide preparation. The material to be mounted should come through alcohol (dehydration) and xylol (clearing) series.

(b) **Lactophenol:** It is a mixture of equal parts of phenol crystals, lactic acid, glycerine (sometimes two parts) and distilled water. Stains may be mixed with this medium (e.g. cotton blue in Lactophenol used to stain fungi) or copper acetate is added to preserve green colour of the pigment.

(c) **Glycerine:** Pure glycerine diluted to 10-25%is widely used. Semi-permanent and temporary preparations are mounted in glycerine.

(d) **Glycerine jelly:** Jelly is also used for mounting. It is made of gelatin 1: glycerine 7 water 6.Warm the gelatin for two hours by adding water. Phenol (1 %) is added later. Add crystals of safranin if desired. Allow the solution to cool and settle into jelly. Many other mounting media like cedar oil, dammar, balsam, venetian turpentine and synthetic resins are also used.

4.3 STUDY OF TEMPORARY AND PERMANENT SLIDE PREPARATIONS OF VARIOUS TYPES OF POLLEN GRAINS, PLACENTATION AND OVULE

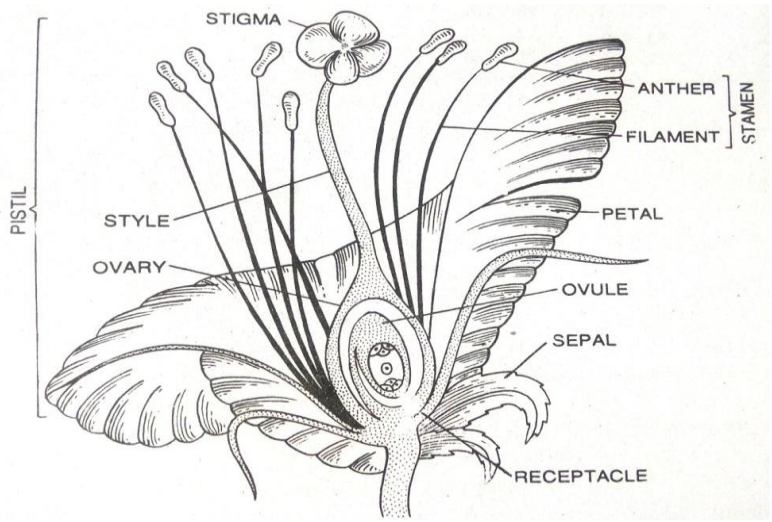


Fig.4.3. General Plan of a typical flower, Flower in section view (L.S.)

4.3.1- Pollen Grains

Pollen is a fine to coarse powdery substance comprising pollen grains which contain partial or fully developed male gametophytes Pollen grains have a hard coat made of sporopollenin that protects the gametophytes during the process of their movement from the stamens to the pistil of flowering plants. The study of pollen is called **palynology** and is highly useful in paleoecology, paleontology, archaeology, and forensics.

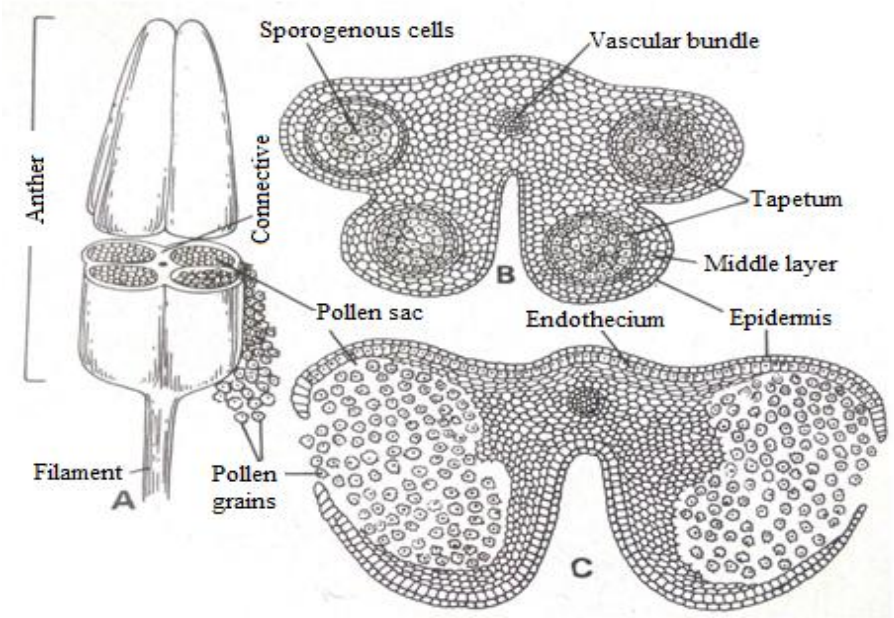


Fig. 4.4 Stamen. A- Pollen showing filament and anther lobe. The anther lobe have been cut transversely to show microsporangia and microspores (pollen grains). B- T.S. of young anther lobe showing four microsporangia .C- T.S. of anold anther.

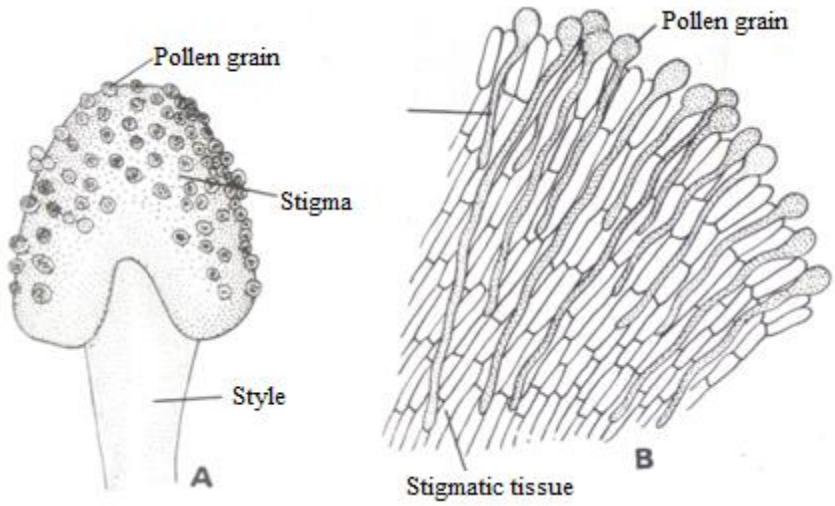
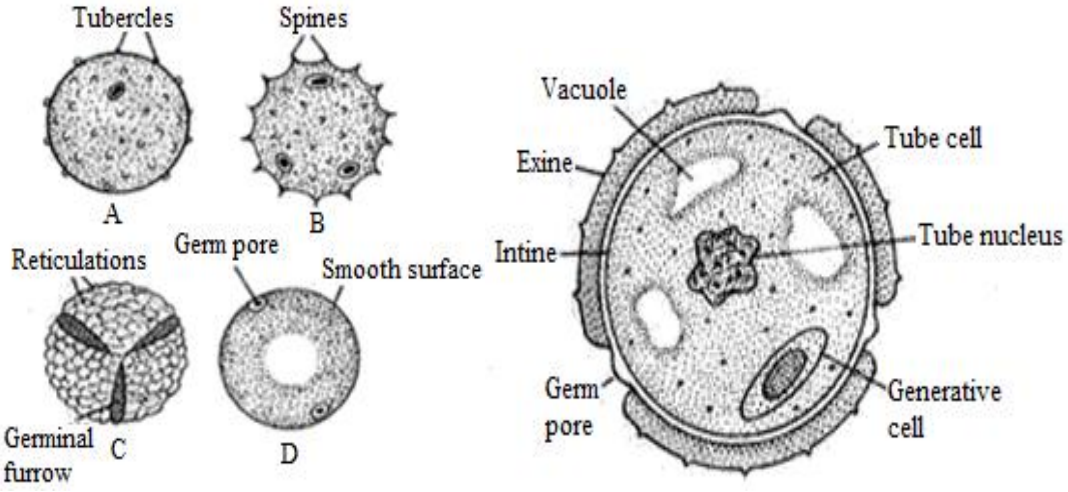


Fig.4.5. A- Pollen grains deposited on stigma; B- Pollen grains germinating through stigmatic tissue forming pollen tubes

The pollen grain of flowering plants is a haploid, uninucleate cell with double layered wall, an inner layer, the **intine**, and an outer layer, the **exine**. The intine is thin and consists of cellulose, while the outer layer, the exine is thick, sometimes spiny, very resistant to disintegration, and composed of sporopollenin.



(A) (B)

Fig. 4.6(A) Common pollen grain sculpturing (B) section of a mature 2 celled pollen grain of an angiosperm.

Characters of Pollen Grains for Study

1. Polarity: The pollen grains are often formed in tetrads. While in tetrad, one end of the individual grain is noted. Accordingly following are the axis: **Polar axis** (Hypothetical line connecting the two poles) and **Equatorial axis** (Hypothetical line that lies perpendicular to the polar axis).

(a) **Proximal pole**: The end of the pollen grain is directed towards the center of the tetrad.

(b) **Distal pole**: The end of the pollen grain is directed away from the center of the tetrad.

2. Symmetry: Pollen grains may be symmetrical (bilateral or radial) or asymmetrical (without any symmetry).

3. Apertures: The exine of pollen grains are often provided with apertures which are thin, more or less distinctly delimited areas formed only of a hyaline membrane.

4. Shape of pollen grain: It is determined by Px100/E formula, where P is the polar diameter and E the equatorial diameter.

5. **Exine stratification.** The wall is made of intine and exine. The intine is colourless and disappears during the slide preparation. Exine consists of two layers, the inner homogenous layer, the **endine** and the outer heterogeneous layer, the **ectine**. The ectine is composed of radial rods, the columellae, which are either free at their tips or are united to form a layer called **tegillum**.

6. **Exine ornamentation.** The following are some of the patterns.
(1) The columellae forming the ectine produce **Pilate** pattern with bright and dark areas.
(2) In some other cases columellae are arranged regularly and are fused to produce areas or **lumina**, the intervening areas between lumina being called **muri**.
(3) When a network is produced the pattern is **reticulate** which may be **retipilate** with incomplete fusion of columellae, **foveolate** with circular closely placed lumina, **scrobiculate** with circular but distantly placed lumina, and **fossulate** with elongated lumina.
(4) When lumina are parallel the pattern is called **striate** and when reticulate it is **rugulate**.
(5) A network with raised areas is called **areolate**.
(6) In some cases excrescences such as minute granules are present on the tegillum, the pattern is **granulose**, as **spinulose** (pointed or blunt ends), **gemmate (**rounded warts**)**, **verrucate** (base of the warts is not constricted), **tuberculate** (tubercles present), **spinose** (pointed), **baculate** (rod shaped) and **clavate** (club shaped).

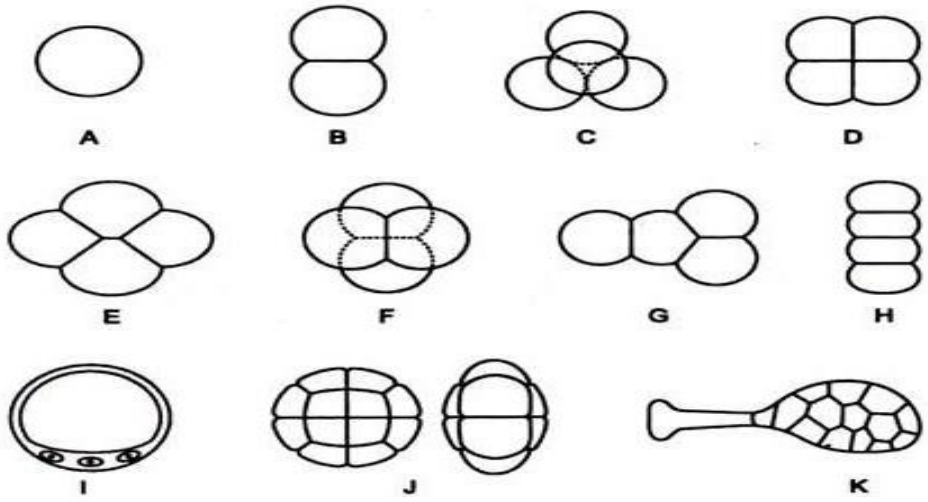


Fig. 4.7- Pollen units (A= Monad, B= Dyads, C= Tetrahedral tetrad, D= Tetragonal tetrad, E= Rhomboidal tetrad, F= Decussate tetrad, G= T-Shaped tetrad, H= Linear tetrad, I=Cryptotetrad, J = Polyads, K= Pollinia.

The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called **monad**. Sometimes rarer types like dyads (two pollen grains), Octads (eight pollen grains) and Polyads (many pollen grains) are also observed.

Dyads: Pollen grains which are united in pairs and shed from the anthers as doubles are called dyads. The dyads are formed due to the incomplete break up of individual grain or monad.

Tetrads: Four pollen grains are united to form tetrad. Tetrads are the unseparated product of meiosis. Tetrads maybe categorized into different types based on their arrangement.

Tetrahedral tetrad: Pollen grains are arranged in two different planes. Three grains are in one plane and one lies centrally over the other three. In some cases, the pollen grains are released from the anther in the tetrad condition. These types of tetrads are called obligate or permanent tetrads, viz., *Rhododendron* (Ericaceae).

Tetragonal tetrad: All the four pollen grains are arranged in one plane e.g., *Typha latifolia* (Typhaceae).

Rhomboidal tetrad: All pollen grains are arranged in one plane forming rhomboidal shape e.g., *Annona muricata* (Annonaceae).

Decussate tetrad: Pair-wise the pollen grains are at right angle to each other, e.g., *Magnolia grandiflora* (Magnoliaceae).

T-Shaped tetrad: The first division of pollen mother cell is transverse to form a dyad. The upper or lower cell of dyad undergoes a vertical or longitudinal division instead of transverse, yielding either straight or inverted T-shaped configuration, e.g., *Polyanthes*.

Linear tetrad: The first division of pollen mother cell is transverse and a dyad is formed. Each cell of the dyad again divides transversely to form a linear tetrad, e.g., *Mimosa pudica.*

Cryptotetrad: Here tetrads are formed without partition walls between the four compartments. One out of the four nuclei develops normally and the rest three obliterate. Thus an apparent monad but homologous to the tetrad is formed also called **Pseudomonad**, e.g., Cyperaceae.

Polyads: In most of the Mimosaceae members each of the tetrad cells divides once or twice or more, yielding a group of 8 to 64 cells which remain together after maturity. These compound grains are usually held together in small units and are called Polyadse g., *Acacia auriculiformis*.

Preparation of Pollen Grains for Study:

The following are the steps in the preparation of slides for pollen study.

(A) Collection of the material

1. The polliniferous material (anthers) is freshly collected. If possible use only freshly opened flowers so that contamination from other pollens is avoided.
2. The anthers are picked by a clean forceps.

(B) Preparation of the material

1. The anthers are tapped by needles or glass rod on a clean slide to obtain a mass of pollen grains.
2. This mass of pollen grains is picked up by the flat end of the forceps and transferred to the centre of another clean microscopic slide.

(C) Pre-treatment

1. Few drops of alcohol is added to the pollen grains to remove waxy surface from the pollen grain and to separate them from one another.
2. The ring developed by alcohol is wiped clean with cotton moistened with alcohol.

(D) Mounting

1. A small pellet of glycerin jelly pre-stained with methyl green is taken. It is placed over the mass of pollen grains. Coverslip is also placed over the pellet.
2. A small piece of paraffin wax (melting point 60-70°C) is placed close to the coverslip.
3. Both, jelly and wax are warmed over the flame of the spirit lamp in such a way that while the jelly spreads a little, the remaining vacant space below the coverslip is occupied by wax.

Germination of Pollen Grains

Requirements:

Anthers of *Antirrhinum* (snap dragon), *Catharanthus roseus* (Periwinkle; Sadabahar), *Papaver somniferum* (Poppy; Afim) or any other easily available plant, sugar, boron, cavity slides, coverslips, microscope, water, etc.

Procedure

1. Prepare 15% sugar solution by dissolving 1.5 gm sugar in 100 ml of distilled water.
2. Add a pinch of boron to sugar solution.
3. Clean the cavity slide and place a drop of this solution in the cavity.
4. Remove mature anthers from freshly opened flowers. Crush them on a slide. Collect the pollen grains with a brush from the crushed anthers. Dust the brush free of anthers in the cavity filled with solution.
5. Place a cover slip over the cavity.
6. Allow the slide to remain as such for a few hours or overnight.
7. Remove the coverslip slowly. Mount the coverslip on a fresh and clean slide in a drop of safranin. The lower side of the coverslip with germinated pollen grains should be in contact with safranin.
8. Observe the slide.

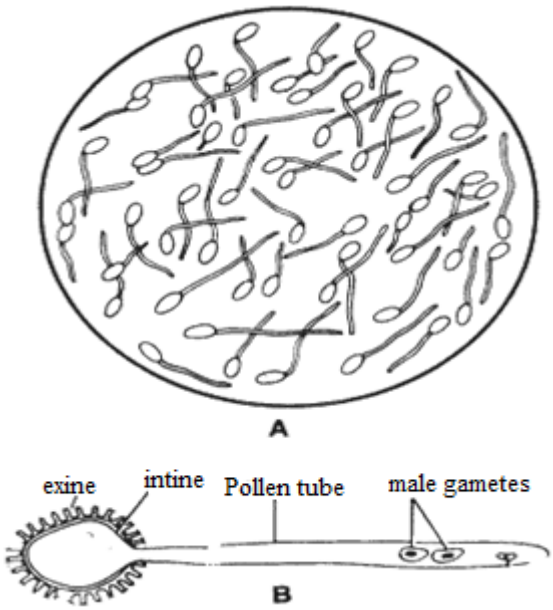


Fig. 4.8- Pollen grains: Germination of pollen grains A. seen under the light microscope, B. Details of the structure.

Observations

The following characters are observed.

1. Numerous germinated pollen grains are seen.
2. A pollen grain has a distinct ornamented exine with germ pores.
3. Intine lies internal to exine. It is thin and uniform.
4. Intine forms a pollen tube that comes out through one of the germ pores.
5. Pollen tube shows a vegetative nucleus and two small male gametes.

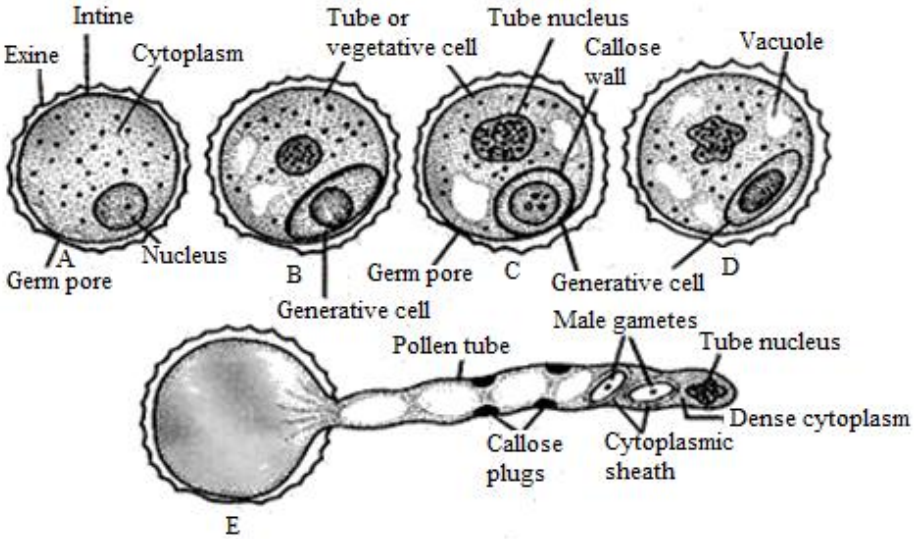


Fig.4.9. Germination of pollen grain and formation of male gametophyte in an angiosperm

Preparing a Permanent Mount for Pollen Grains

Permanent slides of pollen grains can be used as a reference for identifying unknown pollen samples. It is therefore important, that the pollen grains remain in an authentic, natural shape.

The preparation and mounting of the pollen can introduce artifacts: the pollen may lose some of its pigment, start to shrink and shrivel or absorb water and swell. A careful preparation is therefore necessary. There are several methods of preparing pollen grains, each one offers advantages and disadvantages.

Mounting Techniques

(i) Glycerol wet mount: Place a small drop of glycerol on a clean slide and tap the anthers of the plant so that the pollen falls into the glycerol. If necessary, carefully separate large chunks of pollen grains by stirring. Place a cover slip on top and seal the sides of the cover slip with nail polish. Use a very small amount of glycerol to make sure that the nail polish has enough area to stick the coverslip to the slide. Glycerol wet mounted slides can be stored for months if properly sealed with nail polish. The glycerol will withdraw water from the pollen. If the pollen is not dry, then there is a possibility of the pollen to shrink.

(ii) Air mounts (dry mounts): In this case, no liquid mounting medium is used. A cover slip is placed on top of the pollen grains and sealed on the side, either with nail polish or with tape. Nail polish may flow very quickly between cover slip and slide, so it may be best to use a nail polish of high viscosity (by letting some solvent evaporate first).

(iii) Glycerol jelly: This is a very popular mounting medium for pollen. It is phenol-free (antiseptic additive) and therefore non-hazardous. It contains 10g of gelatin, 35ml distilled water and 30ml of glycerol (glycerin). After mounting, the sides of the cover slip need to be sealed. Due to the lack of an antiseptic, it is also necessary to work in a sterile manner, otherwise there is the risk of fungal growth in the medium. It is a good to treat the pollen grains first in alcohol to reduce the chance of fungal contamination by spores.

(iv) Non-water-based mounting media: Euparal is a mounting medium which is not water based. Specimens which are present in alcohol can be directly transferred to Euparal. Place a pollen suspension on the slide and let the alcohol evaporate. Before mounting pollen in Euparalthe pollen are first washed in alcohol and then compared to the original shape. Washing in alcohol may result in an unacceptable shrinking of the pollen or unacceptable loss of pigments, if not, then mounting the pollen in Euparal may be an alternative.

4.3.2- Placentations

In biology, placentation refers to the formation, type and structure, arrangement or position of the placenta. The function of placenta is to transfer nutrients, respiratory gases, and water from maternal tissue to a growing embryo, and in some instances to remove waste from the embryo.

In botany, the term placentation most commonly refers to the arrangement of placenta inside the ovary. In flowering plants, placentation occurs where the ovules are attached inside the ovary. The ovule inside an ovary is attached via funicle. The part of the ovary where the funicle is attached is referred to as the placenta.

Types of Placentations:

1. Marginal: The ovary in which the placenta forms a ridge along the ventral suture of the ovary and the ovules develop on two separate rows is known to have marginal placentation. The ovules are borne along the junction of the two margins of the carpel. It occurs in monocarpellary and unilocular ovary, e.g., Leguminosae (pea, beans).

2. Parietal: The placenta is formed by the swelling up of cohering margins, where later on develop the ovules in rows. Here ovary is one-chambered but it becomes two- chambered due to the formation of the false septum. The ovules develop on the inner wall of the ovary or on peripheral part. It occurs in bicarpellary or multicarpellary but unilocular ovary, e.g., mustard, cucurbita and Argemone.

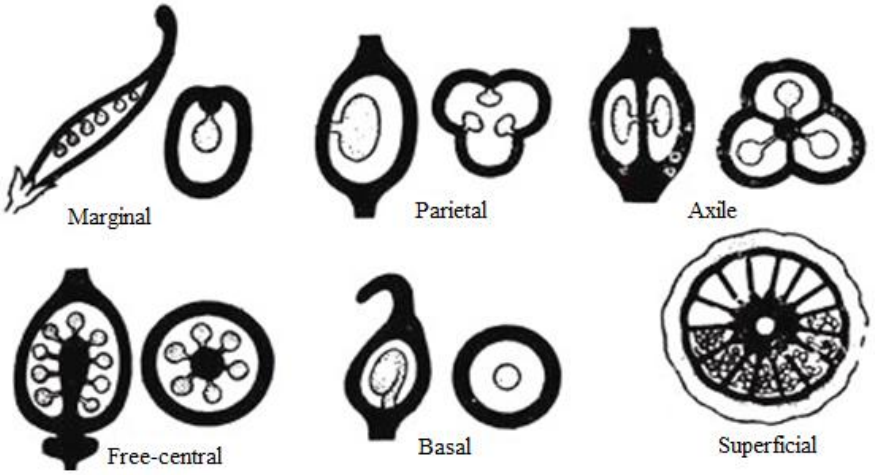


Fig. 4.10- Types of Placentations

3. Axile: The placentae develop from the central axis which corresponds to the confluent margins of carpels. The ovary is sectioned by radial spokes with placentas in separate locules. It occurs in bi-to multilocular ovary, e.g., Solanaceae (apple, hibiscus) and Malvaceae.

4. Free-central: The placenta develops in the centre of the ovary as a prolongation of floral axis and the ovules are attached on this axis. The ovules are borne on central axis and septa are absent. It occurs in multicarpellary but unilocular ovary, e.g., Dianthus, Primula and Sandalwood

5. Superficial or Laminar: The ovules develop over the entire inner surface of the carpels. It occurs in multicarpellary ovary. Ovary is multilocular and syncarpous e.g., Nymphaea.

6. Basal: The placenta is at the base (bottom) of the ovary. The placenta develops directly on the thalamus and bears a single ovule at the base of the unilocular ovary, e.g., Compositae (sunflower).

7. Apical: where one or few ovules develop at the top of a simple or compound ovary.

Preparation of Placentation for Study

Requirement: Commonly available flowers, forceps, razor/scalpel blade, brush, slides, cover slip, filter paper, dissecting microscope, compound microscope, etc.

Preparation of material: Dissect the ovary of freshly collected flower. Cut the T.S. of ovary, mount it on a slide and observe the type of placentation.

Procedure:

1. Take a clean slide and put a drop of water on the middle of the slide. The drop of water is where the mount is to be transferred to.
2. Transfer material to be mounted on the drop of water with the help of a small brush. Do this step very carefully. If the mount is not correctly placed observation under microscope becomes difficult.
3. Add glycerine to the mount. Now carefully place the coverslip over the glass slide covering the mount.
4. Remove extra fluid present on the slide with the help of filter paper.
5. Observe your mount under the microscope.

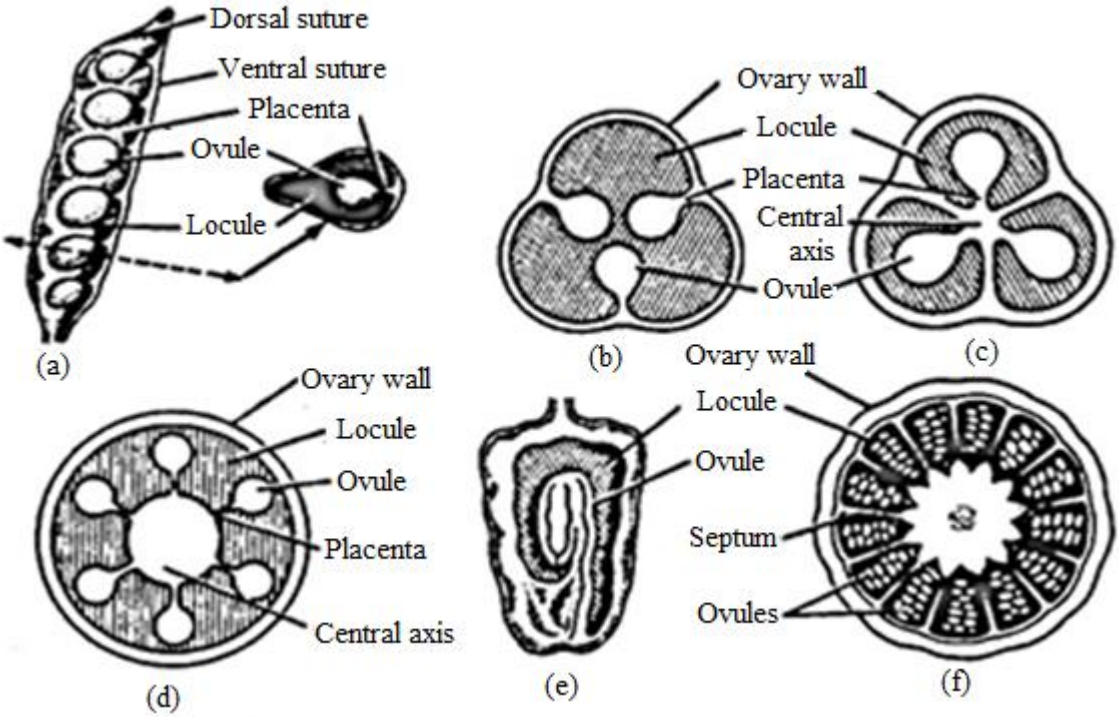


Fig.4.11 Detailed Diagram showing placentations: (a) Marginal, (b) Parietal, (c)Axile, (d) Free-central, (e) Basal, (f) Superficial.

Observations study:

1. The Ovary consists of the ovary wall, the locule or locules, and in a multilocular ovary, the partitions.
2. The ovules are found to be situated on the inner or adaxial (ventral) side of the ovary wall.
3. The ovule-bearing region forms the placenta.
4. In a carpel the placenta occurs close to the margin (Marginal).

5. The ovules develop on the inner wall of the ovary or on peripheral part (Parietal).
6. The ovary is sectioned by radial spokes with placentas in separate locules (Axile).
7. The ovules are borne on central axis and septa are absent (Free-central).
8. The ovules develop over the entire inner surface of the carpels (Superficial).

Placentation can also be seen (generally more easily) from the observation of older ovaries, and fruits (while stamens have to be observed on fresh or even non-open flower). But sometimes, septa tend to secondarily disappear in ovaries with axile placentation. Thus when a cross-section of ovary or a fruit show only one locule, it is necessary to observe a longitudinal section of the ovary to say whether the placentation is free-central or axile.

4.3.3- Ovule

Components of ovule:

In seed plants, the ovule ("small egg") is the structure that gives rise to and contains the female reproductive cells. It consists of three parts: The **integument** forming its outer layer, the **nucellus**, and **embryo sac** or **female gametophyte** formed from haploid megaspore at its center.

The Nucellus

The nucellus is the largest part of the ovule. It houses the embryo sac as well as nutritive tissue and actually remains present in some flowering plants after fertilization as a source of nutrients for the embryo.

The Integuments

The integument is the tough outer protective layer of the ovule. In the diagrams below we can see that gymnosperms, such as pine trees and spruce trees, usually have one integument in an ovule, so we call them unitegmic. On the other hand, angiosperms, like maples and daisies, typically have two integuments, and we call them bitegmic. The integument encloses the nucellus except for a small gap, which is called the *micropyle*.

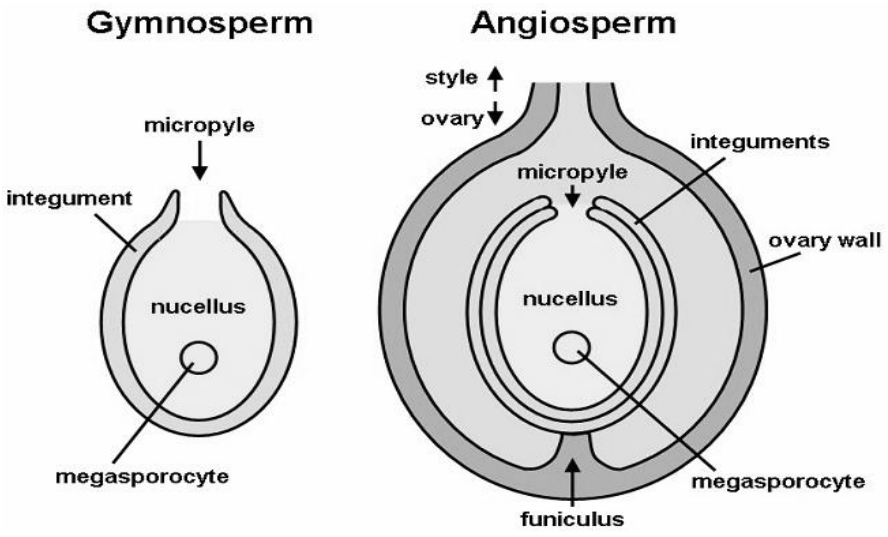


Fig.4.12 Ovules showing integument of gymnosperms and angiosperms

Types of Ovule:

1. Orthotropous (Atropous)-This is where the body of ovule is straight, so that the micropyle lie on the same vertical axis with the funicle and chalaza, e.g. **Polygonium.**

2. Anatropous-In this type the body of the ovule becomes completely inverted, so that the micropyle and hilum come to lie very close to each other. The hilum is a scar that marks the point where the ovule remains attached to the funicle.The micropyle and chalaza lie on the same vertical axis but not funicle, **e.g., Helianthus, Castor.**

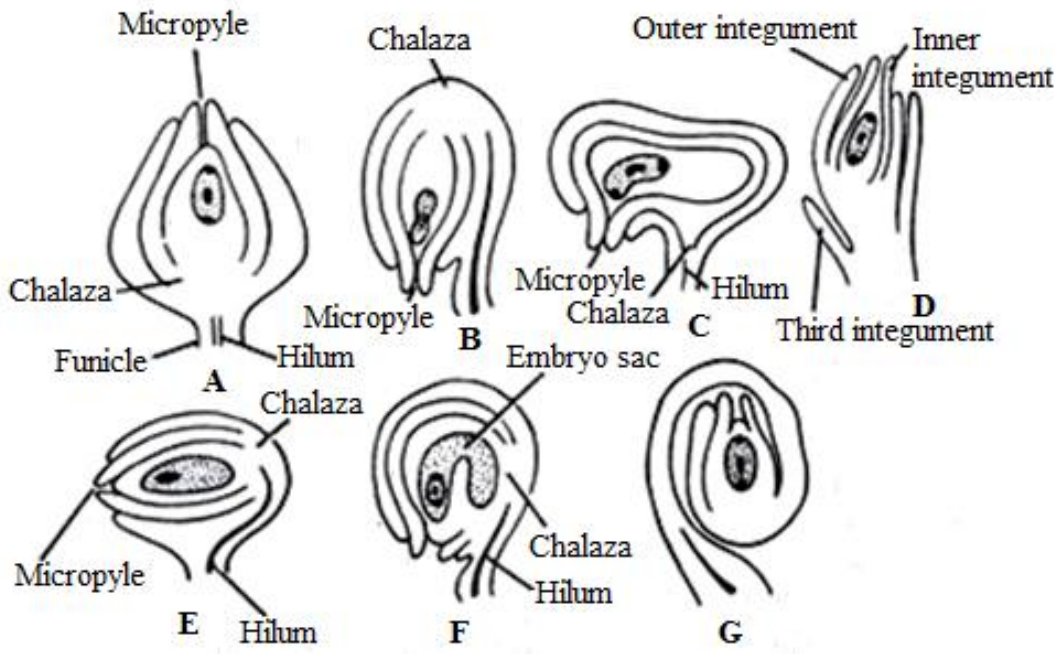


Fig.4.13 Types of ovules: (A) Orthotropous, (B) Anatropous, (C) Campylotropous, (D) Ovule with three integuments, (E) Hemi-anatropous, (F) Amphitropous, (G) Circinotropous

3. Campylotropous-When the micropylar end of the ovule is bend downwards hence the micropyle and chalaza do not lie on the same straight line, it is called campylotropous, **e.g. Pea, Mustard.**

4. Hemi-anatropous-In this type, the nucellus and integuments lie more or less at right angles to the funicle. The micropyle and chalaza lie in one straight line e.g**. Ranunculus**.

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

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

Study the Slide Showing L.S. Of Anatropous Ovule

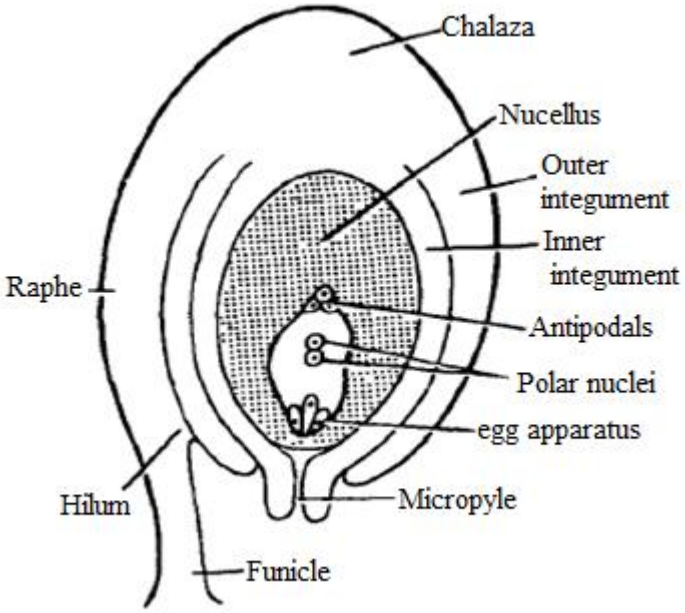


Fig.4.14 The Ovule, L.S. of anatropous ovule

Observations:

The following characters are observed.

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

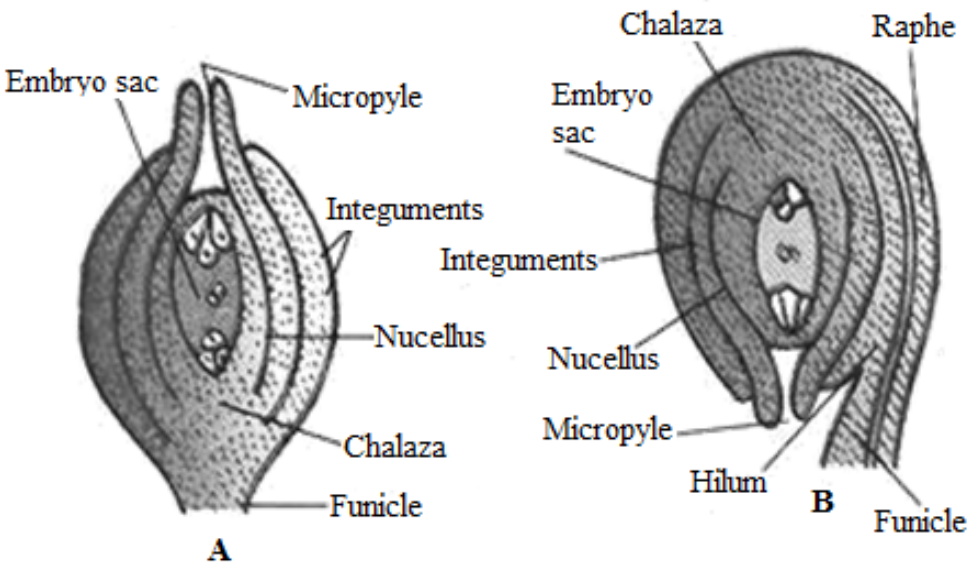


Fig.4.15 Comparing ovules, (A) L.S. of Orthotropous, straight ovule, (B) L.S. of curved anatropous ovule

The Female Gametophyte

The female gametophyte also called the **embryo sac** in angiosperms develops from free nuclear divisions in megaspore nucleus.

Embryo sac: The embryo sac has the egg-apparatus towards the micropylar end. The egg-apparatus has one egg cell (female gamete) and two **synergids.** The egg cell, which is enlarged lies below the synergids. At the chalazal end of the embryo sac there are three **antipodal** cells. These antipodal cells have no definite function and soon gets disorganized. At the center of the embryo sac there are two polar nuclei or their fusion product known as **secondary nucleus.**

Development of female gametophyte:

1. The functional megaspore forms female gametophyte or embryo sac.
2. The nucleus of megaspore divides into two, four and finally eight daughter nuclei. Four of which are located at each pole.
3. One nucleus from each pole migrates to the center to form two polar nuclei which later on fuse to form a diploid fusion or secondary nucleus.
4. Three nuclei at the base of embryo sac form antipodal cells. The remaining three nuclei at the micropylar end get surrounded by cytoplasm to form pyriform cells.
5. These three cells together constitute egg apparatus, which consists of an egg and two other cells known as synergids. The synergids bear special cellular thickenings at the micropylar tip called filiform apparatus, which play an important role in guiding pollen tubes into synergids.
6. The egg cell fuses with one male gamete during fertilization and gives rise to zygote which ultimately develops into embryo, while synergids get disorganized soon after fertilization. The antipodal cells sooner or later also get disorganized.

7. Another male gamete fuses with two polar nuclei or secondary nucleus to form a triploid (3X) primary endosperm nucleus which later on develops into endosperm.

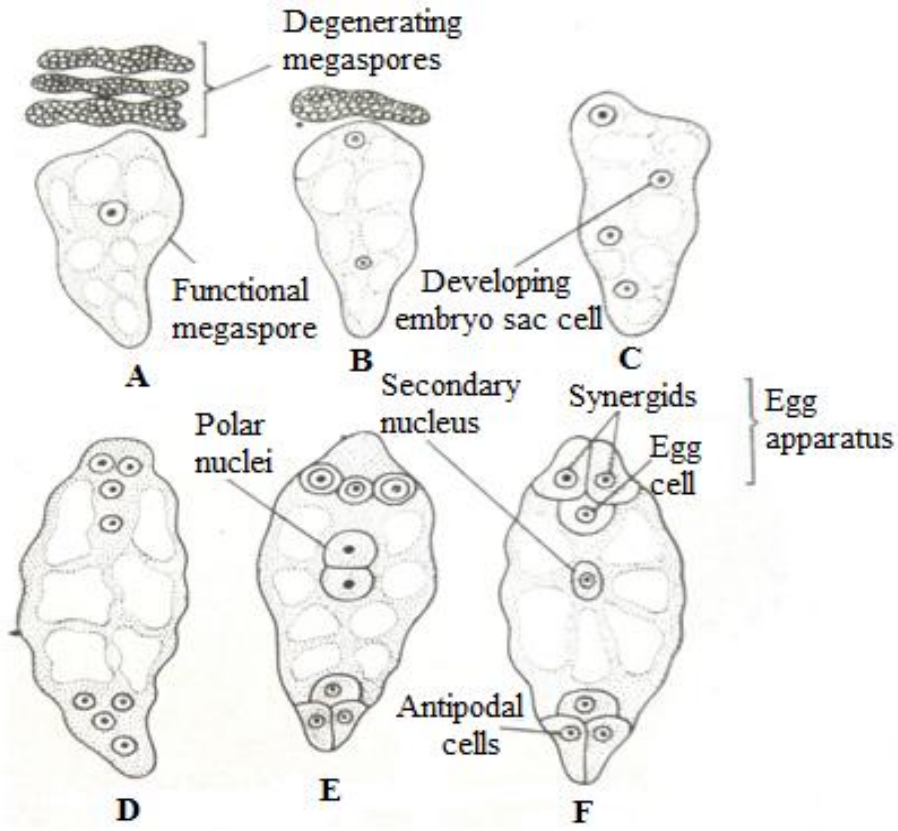


Fig. 4.17 Female gametophyte. A-F, Development of embryo sac of normal type (*Polygonium* type)

To Dissect Out Heart-Shaped Embryo:

Take a small seed of mustard. Locate the micropyle under the dissecting microscope. Remove the seed coat starting from this point. A small white to yellowish embryo can be seen under the microscope.

Observations:

It shows following characters.

1. Heart-shaped embryo consists of a suspensor and a heart -shaped mass of cells.
2. The suspensor is a row of cells arranged in a single line.
3. The uppermost cell of suspensor lies closer to micropyle. It is swollen and is known as vesicular cell.
4. The lowermost cell of suspensor lies close to the embryo proper. It is known as hypophysis.
5. Heart-shaped embryo is formed as a result of cell divisions in globular embryo at places where cotyledons develop.
6. Heart-shaped embryo is differentiated into outer dermatogen, middle periblem and innermost plerome.

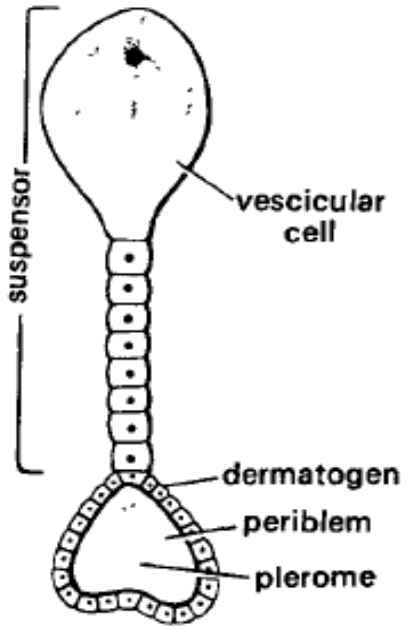


Fig.4.18-Embryo, a heart shaped embryo

4.4 SUMMARY

The mounting of specimens on microscope slides is often critical for successful viewing. The problem has been given much attention in the last two centuries and is a well-developed area with many specialized and sometimes quite sophisticated techniques. In a dry mount, the simplest kind of mounting, the object is merely placed on the slide. A cover slip may be placed on top to protect the specimen and the microscope's objective and to keep the specimen still and pressed flat. This mounting can be successfully used for viewing specimens like pollen. In a wet mount, the specimen is placed in a drop of water or other liquid held between the slide and the cover slip by surface tension. This method is commonly used, for example, to view microscopic organisms that grow in pond water or other liquid media, especially when studying their movement and behavior. The mounting medium is the solution in which the specimen is embedded, generally under a cover glass. Simple liquids like water or glycerol can be considered mounting media, though the term generally refers to compounds that harden into a permanent mount.

The pollen grain of flowering plants is a haploid, uninucleate cell having two layers, the outer layer **exine** and inner layer **exine**. Permanent slides of pollen grains can be used as a reference for identifying unknown pollen samples. It is therefore important, that the pollen grains remain in an authentic, natural shape. The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called **monad**. Sometimes rarer types like dyads, Octads and Polyads also occur. The pollen grain is uninucleate in the beginning. At the time of liberation, it becomes 2 celled, with a small generative cell and a vegetative cell. In the nutrient medium, the pollen grain germinates. The tube cell enlarges and comes out of the pollen grain through one of the germ pores to form a pollen tube. The tube nucleus

descends to the tip of the pollen tube. The generative cell also passes into it. It soon divides into two male gametes.

In botany, the term placentation most commonly refers to the arrangement of placentae inside an ovary. The part of the ovary where the funicle attaches is referred to as the placenta. The types of placenta are, Marginal, Axile, Superficial, Basal, Parietal, Free-central and Apical.

Mature ovule contains an embryo sac also known as female gametophyte. Female gamete (egg) fuses with male gamete to form zygote which later on develops into embryo.

4.5 GLOSSARY

Abiotic: of or characterized by the absence of life or living organisms.
Adaxial: situated on the side toward the axis or stem.
Amphitropous: (of an ovule) inverted so that the funicle is in the middle of one side.
Antipodal: Cells present towards the chalazal end
Aperture: an opening, as a hole, slit, crack, gap, etc.
Authentic: having an origin supported by unquestionable evidence.
Bicarpellary: (of an ovary) having two carpels.
Biotic: pertaining to life.
Campylotropous:(of an ovule) curved so that the micropyle and funiculus almost touch.
Chalaza: the basal part of an ovule, where the integuments and nucellus are joined.
Clavate: shaped like a club with the thicker end uppermost.
Cohering: to stick or hold together in a mass that resists separation.
Columellae: any of various small, column-like structures of animals or plants; rod or axis.
Compound ovary: an ovary composed of more than one carpel.
Confluent: flowing or running together; blending into one.
Congruent: If one thing is congruent with another thing, they are similar or fittogether well. constituents.
Cross-pollination: the transfer of pollen from the flower of one plant to the flower of a plant having a different genetic constitution
Curvature: the act of curving or the state of being curved.
Dermatogen: a thin outer layer of the meristem in embryos and growing points of roots and stems, which gives rise to the epidermis.
Distal: situated away from the point of origin or attachment.
Dyad: a secondary morphological unit, consisting of two monads.
Embryo sac: the structure within a plant ovule that contains the egg cell; develops from the megaspore
Embryo: the rudimentary plant usually contained in the seed**.**
Equatorial: of, like, or existing at or near the equator.
Exine: the outermost coat of a pollen grain or a spore.
Fertilization: the union of male and female gametes, during sexual reproduction, to form a zygote

Filiform: having the form of a thread, filamentous.
Fossulate: hollowed; grooved.
Foveolate: having foveolae, or very small pits.
Funicle: the stalk of an ovule or seed.
Gemmate: having buds; increasing by budding.
Gynoecium: the pistil or pistils of a flower; the female parts.
Haploid: pertaining to a single set of chromosomes.
Heterogeneous: composed of parts of different kinds; having widely dissimilarelements or
Hilum: the mark or scar on a seed produced by separation from its funicleor placenta.
Homogeneous: composed of parts or elements that are all of the same kind.
HyalineMembrane: The thin, clear basement membrane between the inner fibrous layer of a hair follicle and its outer root sheath.
Integument: the protective layer around an ovule that becomes the seed coat.
Intine: the inner coat of a spore, especially a pollen grain.
Locule: a small compartment or chamber, as the pollen-containing cavitywithin an anther.
Lumina: (of a cell) the cavity that the cell walls enclose.
Megasporangium:a sporangium containing megaspores.
Micropyle: the minute orifice or opening in the integuments of an ovule.
Monad: any simple, single-celled organism.
Multicarpellary: (of a plant gynoecium) having or consisting of many carpels.
Multilocular: having or comprising several small cavities or compartments.
Nucellus: the central part of a plant ovule containing the embryo sac.
Oblate: having an equatorial diameter of greater length than the polar diameter.
Obliterate: to remove or destroy all traces of; do away with; destroy completely.
or spores, sometimes in masses.
Ornamented: if something is ornamented with attractive objects or patterns, is decorated with them.
Orthotropous: (of an ovule) straight and symmetrical, with the chalaza at the evident base and
Paleoecology: the branch of ecology dealing with the relations and interactions between ancient life forms and their environment.
Paleontology: the science of the forms of life existing in former geologic periods, as represented by their fossils.
Palynology: the study of live and fossil spores, pollen grains, and similar plantstructures.
Peripheral: pertaining to, situated in, or constituting the periphery.
Placenta: the part of the ovary of flowering plants that bears the ovules.
Placentation: the disposition or arrangement of a placenta or placentas.
Pollen grain: a single granule of pollen.
Pollen: the fertilizing element of flowering plants, consisting of fine, powdery, yellowish grains
Pollentube:the protoplasmic tube that is extruded from a germinating pollengrain and grows.
Pollination: the transfer of pollen from the anther to the stigma.
Polliniferous: producing or bearing pollen.

Prolongation: the state of being prolonged.
Proximal: situated close to the centre, median line, or point of attachment or origin.
Raphe:(in certain ovules) a ridge connecting the hilum with the chalaza.
Ridge: a ridge is a raised line on a flat surface.
Scar: a mark indicating a former point of attachment, as where aleaf has fallen from a stem.
Scrobiculate: furrowed or pitted.
Self pollination: the transfer of pollen from the anther to the stigma of the sameflower, another
Shrivel: to contract and wrinkle, as from great heat, cold, or dryness.
Spinose: full of spines; spiniferous; spinous.
Suture: a line of junction between two parts.
Syncarpous: of the nature of or pertaining to a syncarp.
Synergids: one of two small cells that lie inside the embryo sac of a flowering plant and nourish
Tetrad: a group of four cells formed by meiosis from one diploid cell.
Tuberculate: having tubercles.
Unilocular: having or consisting of only one loculus, chamber, or cell.
Ventral: of or designating the lower or inner surface of a structure.
Vesicular: characterized by or consisting of vesicles.

4.6 SELF ASSESSMENT QUESTIONS

4.6.1 One wordAnswers type questions:

1. The arrangement of ovules within the ovary wall known as?
2. Fertilization in which pollen tube enter the ovule through micropyle is called?
3. An ovule which becomes curved so that the nucellus and embryo sac lie at right angles to the funicle is?
4. The deposition of pollen on stigma of another flower of the same plant is known as?
5. The ovary in which the placenta form a ridge along the ventral suture of the ovary and ovules develop on two separate rows is known as?
6. The placenta develops from the central axis which correspond to the confluent margins of carpels, known as?
7. The ovule is located inside the portion of the flower, which is called?
8. The integument encloses the nucellus except for a small gap, which is called?
9. Ovules are attached to the placenta in the ovary through a stalk-like structure known as?
10. When ovule is curved in such a way, so that the micropyle and chalaza do not lie on the same straight line, called?

4.6.2 Fill in the blanks:

1. Study of pollen grains is known as_____
2. A stamen consists of_____ and _____
3. The anther that consists of only one anther lobe is called_____

4. Phenomenon of the formation of more than one embryo per ovule is called_____
5. A small pore in the ovule through which the pollen tube enters is called_____
6. Inside ovary, ovule develop from a special tissue called_____
7. For staining material_____ solution is commonly used.
8. Canada balsam is a resin obtained from a conifer_____
9. Semi-permanent and temporary preparations are mounted in _____
10. Pollen grains have hard coat made of _____
11. The outer and inner layer of pollen are_____ and_____
12. Intine of pollen grains is composed of_____ and_____

4.6.3 Multiple choice questions:

1. When pollen from one flower are deposited on the stigma of another flower borne on the same plant the pollination is known as

(a) Self pollination (autogamy) (b)Self pollination (geitonogamy)
(c) Cross pollination (allogamy) (d) Anemophily

2. Marginal placentation is found in

(a) Poppy (b) Mustard
(c) Sunflower (d) Pea

3. Double fertilization is a characteristic of

(a) Gymnosperm (b) Angiosperm
(c) Pteridophytes (d) Bryophytes

4. How many male cells are there in the pollen tube of angiosperm

(a) One (b) Two
(c) Three (d) Four

5. Type of placenta in which ovary is syncarpous unilocular and ovules on sutures is called

(a) Apical placentation (b) Parietal placentation
(c) Marginal placentation (d) Superficial placentation

6. Abiotic means of pollination is carried by

(a) Animals (b) Insects
(c) Water (d) Birds

7. After fertilization the seed coat develops from

(a) Chalaza (b) Ovule
(c) Embryo sac (d) Integuments

8. Which of the following statements is true for the pollen tube

(a) It shows only tip growth
(b) It is composed of three non cellular zones

(c) It shows chemostatic movements
(d) It shows radial cytoplasmic streaming

9. Pollen grains are shed at which stage
(a) Two celled (b) Three celled
(c) Single celled (d) Usually at two celled, but sometimes three celled

10. Filiform apparatus is characteristic of
(a) Egg (b) Synergids
(c) Antipodal cells (d) Anther wall

11. Pollen grains of flower pollinated by insects or wind are not
(a) Large and showy (b) Rough and sticky
(c) Smooth and dry (d) Rough and dry

12. Pollinia are sac like structures
(a) In which anther lobes are present
(b) Which are present in megasporangia
(c) In which pollen grains are present in mass
(d) Which secret yellow substance called pollenkit material

13. The ovule develop over the entire inner surface of the carpels
(a) Basal (b) Free-central
(c) Marginal (d) Superficial

4.6.1 Answer key: 1-Placentation, 2- Porogamy, 3- Hemi-anatropous, 4-Geitonogamy, 5-Marginal Placentation, 6-Axile placentation, 7- Gynoecium, 8-Micropyle, 9- Funicle, 10-Anatropous ovule.

4.6.2 Answers Key: (1) Palynology, **(2**) Filament and Anther, **(3)** Monothecous, **(4)** Polyembryony,**(5)** Micropyle, **(6)** Placenta, **(7)** Iodine, **(8)** *Abies balsamea*, **(9)** Glycerine, **(10)** Sporopollenin, **(11)** Exine and Intine, **(12)** Lipid and Protein

4.6.3 Answers key:1-(a), **2-**(d), **3-**(b), **4-**(b), **5-**(b), **6-**(c), **7-**(d), **8-**(a), **9-**(d), **10-**(b), **11-**(a), **12-**(c), **13-**(d)

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4.9 TERMINAL QUESTIONS

1. Describe the procedure of temporary mount preparation. How it is different from permanent mount?
2. What are the common media used for mounting an object. Describe any four of them.
3. Write a short essay note on pollen grains. What are the various characteristics of pollen grains for study?
4. Illustrate the entire procedure for the germination of pollen grains. Also draw the schematic diagram showing germination of pollen grains.
5. Discuss the different mounting techniques to prepare a permanent mount for pollen grains.
6. What is placentation? Write about the different types of Placentations with example.
7. Write a short essay note on ovule. Explain different components and functions of ovule.
8. Draw a labelled diagram of the longitudinal section of an anatropous ovule. Also write the observation characteristics.
9. Write about the different stages of ovule development and female gametophyte.
10. Draw a detailed diagram showing longitudinal section of ovule of a typical angiosperm.

BLOCK-2 EMBRYOLOGY, ANATOMY AND MORPHOGENESIS

UNIT-5 DEMONSTRATION OF USUAL TECHNIQUES OF PLANT ANATOMY, SECTION CUTTING, T.S., L.S. OF LEAF, STEM AND ROOT

5.1 OBJECTIVES

This unit is written to explain the following points:

- The student will be familiar with the general features of microscopy and different parts of compound microscopes.
- Students would be able to learn different techniques of anatomy like sectioning and staining along with mounting media and mounting techniques.
- What are the common stains for plant cells.
- Section cutting techniques for leaf, stem and roots
- Anatomical features of root, stem and leaves

5.2 INTRODUCTION

The practical knowledge develops the scientific outlook of the subject and while working in the laboratory a rational approach develops in the students. Here a clear cut differentiation develops in the mind of the student regarding the theory of the subject. As in all experimental sciences, research in plant anatomy depends on the laboratory methods that can be used to study cell structure and function. Many important advances in understanding cells have directly followed the development of new methods that have opened novel avenues of investigation. The basic method used in plant anatomy, or the study of internal plant structure, is the preparation of thin slices which are studied microscopically. From this the science "derives its name (in Greek, *anatome* means "dissection"). The emergence of the field of plant anatomy is closely related to the invention and perfection of the microscope. The English physicist R. Hooke observed in 1665 the cellular structure of thin slices of cork, elder pith, and wood from various plants, using a microscope of his own improved design.
The objectives of undertaking practical studies in botany are to:

- develop practical skill for better understanding through firsthand experience;
- demonstrate the principles covered in the theory;
- develop observational skill in the form of identifying and locating desired parts in specimen;
- develop manipulative skills in arranging and handling the apparatus and instruments
- collect material and to mount it and to develop skill in preserving biological material and specimens;
- draw, label and record experimental results and interpret them.

Through practical work, not only the theoretical concepts are tested but also it trains you in the scientific method.

Some Common Instruments for Anatomy

There are some instruments, which you will use frequently while working in the laboratory. One of these is the compound microscope.

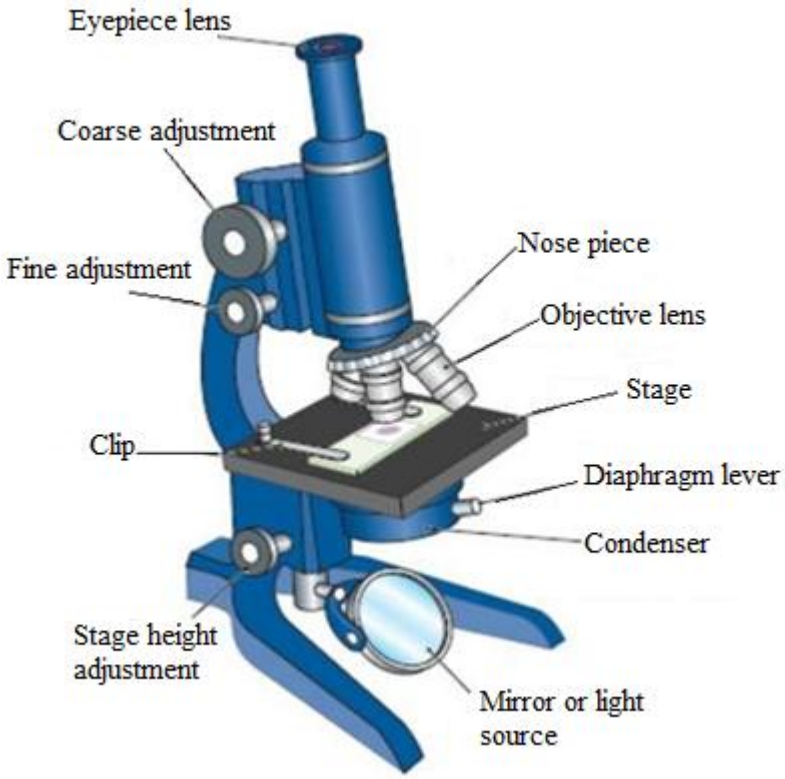

Fig.5.1: Compound Microscope

(1) Compound Microscope: It is an indispensable instrument in a Biology laboratory. Study the diagram of the microscope and compare it with an actual one in the laboratory.
Eye-Piece: Contains lenses to increase magnification.
Body Tube: Holds lenses of eyepiece and objectives at proper working distance from each other.
Arm: Supports body tube and coarse adjustment.
Nose Piece: Permits interchange of low and high powered objectives.
Coarse Adjustment: Moves body tube up and down to the correct distance from the specimen for focusing the object.
Objective lense: Lenses of different magnification as 10X, 40X etc.
Stage: Supports slide over hole that permits light from mirror below.
Diaphragm: Regulates amount of light passing through the specimen.
Stage Clips: Hold slide firmly in place.
Base: Firm support bearing weight of microscope.
Mirror: Reflects light upward through diaphragm and hole in stage.
Fine Adjustment: Meant for the exact focusing by moving stage or body tube up or down very slightly.
Inclination Joint: Permits tilting to adjust the eye level.

Using the Microscope:

- Always use both hands when carrying the microscope, one hand beneath the base and the other holding the arm of the microscope in an upright position.
- Walk, holding the microscope close to your body.

- Set the microscope at least 5 inches from the edge of the table to avoid its knocking off accidently.
- Always clean the lenses and mirror of the microscope with the lens paper/ cloth. Otherwise there might be scratches on them.
- Adjust the mirror by slightly tilting it and by seeing through the eye piece so that sufficient light enters the microscope when you view under low magnification objectives.
- Place the prepared slide directly over the hole in the stage.
- Secure the slide on the stage with the stage clips to prevent accidental movement of the slide.
- Look through the eye piece and slowly bring the low magnification objective towards the material by using the coarse adjustment until the specimen comes into view.
- To change to high power, rotate the nose-piece to bring the high power objective in position (taking precaution that the body tube does not move up or down).
- Look through the eye piece, if the light is insufficient, open out the diaphragm slightly.
- Gently raise the objective by using fine adjustment. If the image worsens without improving, start lowering the objective by the same fine adjustment.
- Do not use coarse adjustment while viewing under high power. By gently moving up and down you will be able to get a clear focus.
- While removing the slide from the stage release the spring clips. Do not allow the stage clips to extend out of the stage.
- When work gets over, rotate nose piece such that the objective lens is not over the hole in the stage.
- When not in use keep it covered by a polythene cover and/or lock it in its box.

(2) A simple hand lens: Contains a single double convex lens mounted on a handle. Can magnify things four to five times and used for smaller magnification.

(3) Scalpel: Works like a knife, used to cut out thin slices and peel.

(4) Fine scissors: Used for cutting.

(5) A pair of forceps: Used for picking up very thin slices or material.

(6) Fine needles: Used for (i) adjusting sample/teasing any biological material on a glass slide without touching it, placing the cover slip on the slide.

(7) Fine hair brush: Mainly used for transferring material for mounting on the slides.

(8) Spatula: Used to pick up solid chemicals.

(9) Glassware:

(i) **A dropper:** Used for putting a drop of liquid on the slide.

(ii)**Plain glass slides:** Used for preparing temporary or permanent mounts.

(iii) **Cover slips (Very thin glass cover):** Used for covering the material placed on glass slide to be observed under the microscope. This protects the objective lens.
(iv) **Petridish:** Is a shallow dish often with a cover. Used for soaking specimen for the purpose of preservation, staining etc. Also used to keep a medium on which bacteria or small organisms may be cultured.
(v) **Beaker:** Available in various sizes from 100 ml to 1000ml. Used for preparing and storing chemicals and performing experiments.
(vi) **Flask:** A bottle with a narrow neck used in the laboratory for performing experiments (keeping solution, for heating solution etc).
(vii) **Funnel:** Available in various sizes i.e. in different diameter of the mouth of the funnel. Used during filtration of solutions.
(viii) **Pipette**: A slender graduated glass tube for measuring and transferring known volume of liquid.
(ix) **Spirit lamp or Bunsen burner**: Used for heating. It should be extinguished immediately after use.

5.3 DEMONSTRATION OF USUAL TECHNIQUES

5.3.1-Plant anatomy

Plant anatomy is a basic core subject in the study of Botany. The cells of plants are quite minute and microscopic in size, so cannot be observed by naked eyes. Such objects are visible only under microscopes. Our eye has limited magnification or resolution power so unable to distinguish the objects smaller than 0.1 mm. Moreover the living cells are transparent in ordinary light and cannot be distinguished among various cellular components. The microscopes are the most important tools in the plant anatomy and their magnification power is achieved by lenses of various types.

Solid material should be sectioned in several planes in order to discover the distribution of the various tissues within it. The complete investigation of axial structures, such as stem or root, normally requires a transverse (cross) section at one or more levels and radial longitudinal, and tangential longitudinal sections at different depths from the surface to the center. Foliar structures generally require transverse and vertical longitudinal sections may occasionally be necessary. Hand sections of plant organs can be obtained readily and this, together with the use of simple staining schedules, allows the visualization of the structure using a light microscope. The exercise can be performed by students at all levels after having demonstrated the techniques to them. Students will need help initially in identifying cell and tissue types. Color photographs will be useful to serve as a guide for identification purposes. Some of the techniques are given below.

5.3.2-Section cutting

Free Hand Sectioning Methods

Most plant parts are too thick to be mounted intact and viewed with a microscope. In order to study the structural organization of the plant body, sections have to be made so that enough

light can be transmitted through the specimen to resolve cell structures under the microscope. A free hand section is the simplest method of preparing specimens for microscopic viewing. This method allows one to examine the specimen in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs. The fixation of materials is generally not required for temporary preparations.

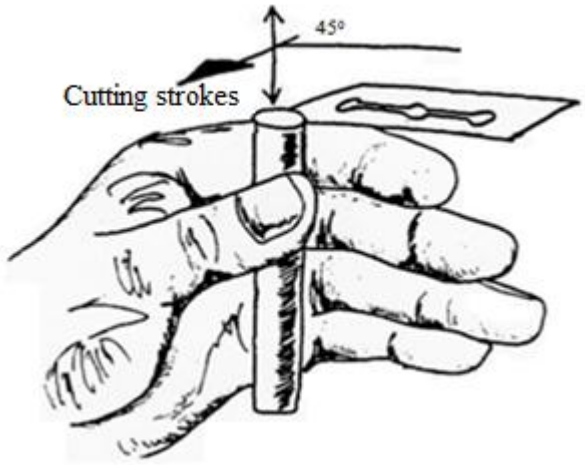

Fig.5.2: One method of holding a specimen for free hand sectioning

In order to reveal the cellular organisation, the plant material is usually cut into following types of sections:

1. **Cross Section**: Here the section is cut at the right angle to the plant material and it is of two type:

a) **Transverse section**: Section at right angle to vertical axis of the material such as in root and stem as in figure given below.

Fig.5.3: T.S. of Tomato and Banana

b) **Vertical Section**: Section at right angle to the transverse axis such as in leaves and thallus.

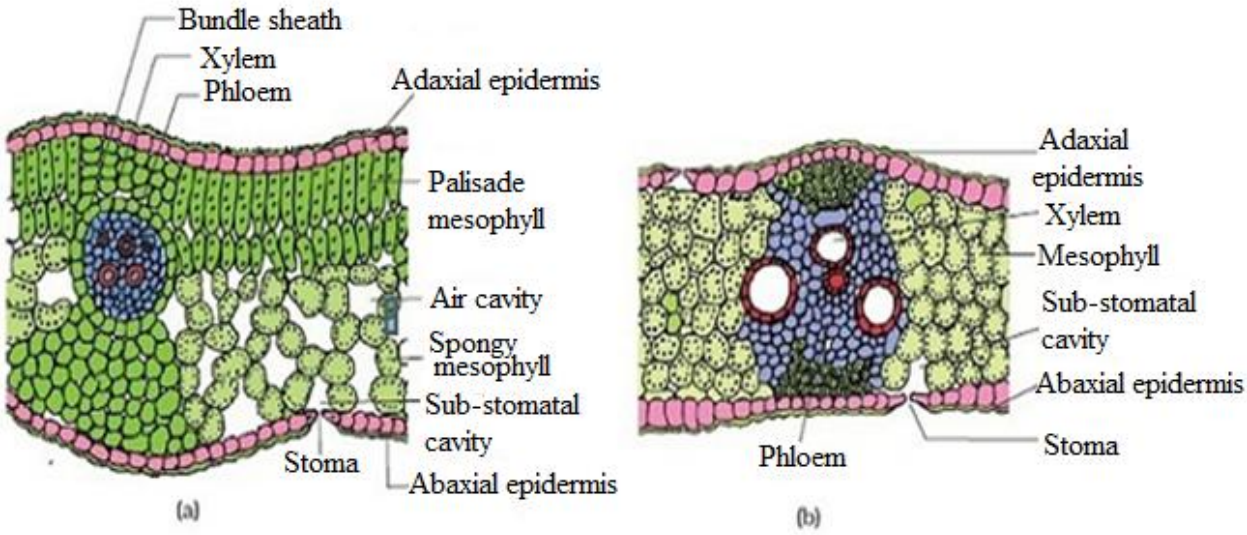

Fig.5.4: V.S. of Dicot and Monocot Leaf

2. Longitudinal Section: The section is cut at right angle to the transverse axis and is also of two types:

a) **Radial Longitudinal Section (RLS):** In this the section is cut through the radius

b) **Tangential Longitudinal Section (TLS):** In this the section is cut through the tangent and do not pass through the central part.

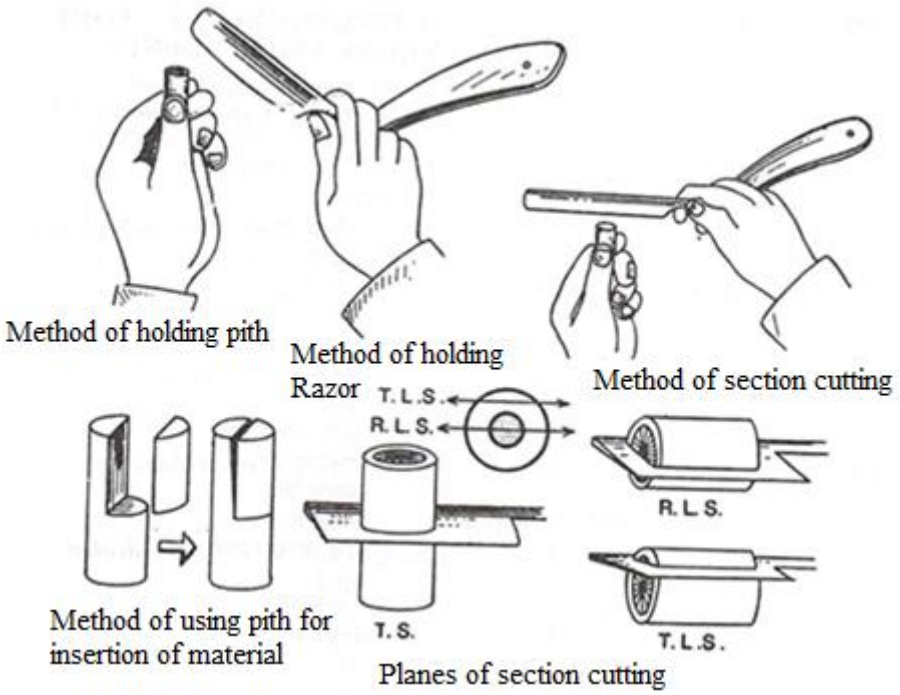

Fig.5.5: Sectioning procedure for TLS and RLS

Procedures of Section cutting:

1. Obtain a new double edge razor blade. To minimize the risk of cutting oneself, cover one edge of the razor blade with masking tape. Rinse the blade with warm tap water to remove traces of grease from the surface of the blade if necessary.
2. Hold the plant material firmly. The material should be held against the side of the first finger of the left hand (or right hand) by means of the thumb. The first finger should be kept as straight as possible, while the thumb is kept well below the surface of the material out of the way of the razor edge.
3. Flood the razor with water. This will reduce the friction during cutting as sections can float onto the surface of the blade. Take the razor blade in the right hand (or left hand) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen.
4. Draw the razor across the top of the material in such a way as to give the material a drawing cut. This results in less friction as the razor blade passes through the specimen. Cut several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the "thick" sections!
5. Transfer sections to water, always using a brush, not a forceps or needle.
6. Select and transfer the thinnest sections (the more transparent ones) onto a glass slide and stain (see next section).

Note: For cross sections, special care should be taken during sectioning to see that the material is not cut obliquely. In our experience, as long as the sections are not obliquely sectioned, even "thick" sections are usable. During sectioning, a number of sections should be cut at the same time and one should not worry about the section thickness at this time. By slightly and progressively increasing the pressure with the razor blade on the first finger, and simultaneously exerting increasing pressure onto the specimen by the thumb, a number of sections can be cut without moving the material or the thumb.

For delicate and hard to hold specimens such as thin leaves and tiny roots, additional support can be used to facilitate hand sectioning. The following methods will allow for the sectioning of thin leaves and small, soft specimens such as roots. Tissue pieces can be inserted into a small piece of pith such as a carrot root. Once the tissue is firmly in place, the hand sectioning technique can be applied.Longitudinal sections are also difficult to obtain by hand without supporting material as small stem and root pieces are difficult to hold with one's finger. However, by cutting a v-shaped notch into the pith support, it is possible to hold the tissue firmly for free hand sections.

Preparation Techniques: Dry Mounts, Wet Mount, Squash, Staining

Permanent Preparation: There are several ways to prepare slides for histological analysis. To notice or detect physiological changes, it is needed to embed the samples in resin or paraffin. First of all, the section fixing should be as soon as possible and we can fix it in several reagents. We can use FAA (Formaldehyde, acetic acid and Alcohol 70% or 50%. After that dehydration in an ethanol series (10, 30, 50, 70, 90, and 100%) for 20-30 minutes

in each ethanol grade is done. After finishing the dehydration you need to make a transition of ethanol to resin (3:1, 1:1, 1:3,) the time for each step is variable depending upon the size of your sample. After this we can mount the section in Canada balsam or DPX and cover it with cover slip.

Mounting Media and Mounting

For temporary preparations: Glycerine medium with 10 to 20% pure glycerine + 90 to 80ml water or lacto phenol medium is used for temporary preparation. It is prepared by adding equal amount of phenol, lactic acid, glycerine and distilled water.

For permanent preparations: Canada balsam and DPX mountant are used for permanent slide as mounting media.

Mounting is done at the center of the slide. For this put a drop of mounting media at the center of the slide and the material is transferred in the drop of medium with help of a brush. With the help of a needle we put cover slip over it gently in such a way that air bubble should not be there. Extra amount of fluid can be removed by blotting paper.

Maceration Technique

A maceration method has been very useful in studying the features of intact cells. In this maceration procedure, the middle lamella, which normally cements adjacent cells together, is dissolved by acid which allows the cells to separate from one another.

Maceration fluid preparation: The maceration fluid is prepared by combining 1 part of a 30% solution of hydrogen peroxide, 4 parts of distilled water, and 5 parts of glacial acetic acid. Be sure to use a clean bottle and prepare this solution in the fume hood. Avoid contact with the solution, wear gloves if necessary.

Procedures: Temporary preparations

1. A variety of plant tissues such as soft pith tissues and woody xylem samples can be studied using this technique. Cut plant tissues into small pieces and place these into a vial containing the maceration fluid. The volume of fluid required is approximately 10X the volume of the tissue.
2. Cap tightly. Place the vials in an oven at about 56^0C for 1-4 days. The duration of maceration depends on the nature of the material. For soft tissues, such as the sunflower stem, 12-24 hours is sufficient.
3. If the maceration has been completed, the fluid will be clear and the tissues appear whitish to translucent. Often, the tissue remains intact after this treatment. If the material is not as described, add fresh maceration fluid and leave it for an additional one to two days.
4. When the maceration is complete, gently rinse tissue in three changes of water (several hours between each change) and leave the tissue in water overnight. Give the material a final rinse in water and store in water or 30% glycerol solution.

5. If necessary, transfer a small mass of cells into a vial containing water; otherwise simply process the tissue using the original vial. Be sure to cap the vial tightly, and shake vigorously until the water becomes clouded with cells.
6. Apply a small drop of the mixture to a glass slide, cover it with a cover glass and examine.

Staining - Application of stain to a sample to color cells, tissues, components, or metabolic processes. This process may involve immersing the sample in a dye solution and then rinsing and observing the sample under a microscope. Some dyes require the use of a mordant, which is a chemical compound that reacts with the stain to form an insoluble, colored precipitate.

Botany specimens from differing divisions (based on their taxonomy) respond to stains in a unique way. Stains that Bryophytes require might not be the same for Algae or Fungi, or even Pteridophytes. The most commons stains used in laboratory work are Aniline blue, Fast green, Safranin, Cotton blue, Methylene blue or Crystal violet. Media used for mounting may vary between Glycerine 10%, Glycerine jelly, Lactophenol, Erythrosine or Canada balsam (or D.P.X. Mountant) depending on whether they are for temporary or permanent preparations.

Algae: Temporary preparations

- Single staining: Iodine solution, Aniline blue - 0.1% aqueous, Fast green - 0.5% aqueous.
- Mounting media: Glycerine 10 % or glycerine jelly

Fungi: Temporary preparations

- Single staining: Cotton blue, Aniline blue
- Mounting media: Lactophenol or glycerine 10%

Bryophytes: Temporary preparations

- Single staining: Safranin or Fast green
- Mounting media: glycerine 10% or glycerine jelly

Pteridophytes: Temporary and permanent preparations
Double staining

- Primary stains: Safranin and Crystal violet
- Secondary stains: Fast green, Aniline blue and Erythrosine
- Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. Mountant for permanent preparations.

Gymnosperms: Temporary and permanent preparations
Double staining

- Primary stains: Safranin and Crystal violet
- Secondary stains: Fast green , Aniline blue and Erythrosine

Mounting media: Glycerine 10% for temporary preparations and Canada balsam or D.P.X. Mountant for permanent preparations

Mixtures of Some Common Stains

Crystal violet:

- Crystal violet: 3 g
- Distilled water: 80 ml
- Ethyl alcohol (95%): 20 ml, dissolved and mixed with 0.8 g of ammonium oxalate.
  It is a violet dye and is used to stain the lignified tissues.

The color of stain by gentian violet depends on the acidity. At pH 1.0, the dye is green, but in an alkaline solution it is colorless.

Methylene blue:

- Methylene blue: 0.3 g
- (0.01%) distilled water - 100 ml
- Ethyl alcohol (95%): 30 ml, dissolved and mixed with potassium hydroxide
  It is used to stain cellulose walls.

Safranin:

- Safranin: 0.25 g
- Alcohol (95%): 10 ml
- Distilled water: 100 ml
  This is mainly used to stain lignified tissues.

Fast Green:

- Fast Green: 0.5 g
- Alcohol (95%): 100c.c.
  Fast green is the green dye used to stain thin walled tissue.

Common Biological Stains: Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas. Some of the most common biological stains are listed below. Unless otherwise marked, all of these dyes may be used with fixed cells and tissues; vital dyes (suitable for use with living organisms) are noted.

Carmine: Carmine is an intensely red dye used to stain glycogen, while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually aluminum.

Crystal violet: Crystal violet, when combined with a suitable mordant, stains cell walls purple. Crystal violet is the stain used in Gram staining. Crystal violet stains the acidic components of the neuronal cytoplasm a violet colour, specifically nissl bodies.

Eosin: Eosin is most often used as a counter stain to haematoxylene, imparting a pink or red colour to cytoplasmic material, cell membranes, and some extracellular structures. It also

imparts a strong red colour to red blood cells. Eosin is a red dye that stains cytoplasm. It is water-soluble and thus can be used to follow water movement through plants.

Acid fuchsine: Acid fuchsine may be used to stain collagen, smooth muscle, or mitochondria. Acid fuchsine is used as the nuclear and cytoplasmic stain. Acid fuchsine is also a traditional stain for mitochondria. The dye fuchsine is a biological stain that is produced by oxidation of a mixture of aniline and toluidine, producing a brilliant bluish red.

Haematoxylene: Haematoxylene is a nuclear stain. Used with a mordant, haematoxylene stains nuclei blue-violet or brown. It is most often used with eosin in H&E (haematoxylene and eosin) staining—one of the most common procedures in histology.

Iodine: Iodine is used in chemistry as an indicator for starch. When starch is mixed with iodine in solution, an intensely dark blue colour develops, representing a starch/iodine complex. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as Gram staining, used in microbiology.

Methyl green: Methyl green is used commonly with bright-field microscopes to dye the chromatin of cells so that they are more easily viewed.

Methylene blue: Methylene blue is used to stain animal cells, such as human cheek cells, to make their nuclei more observable. Also used to stain the blood film and used in cytology.
Safranin: Safranin (or Safranin O) is a nuclear stain. It produces red nuclei, and is used primarily as a counter stain. Safranin may also be used to give a yellow colour to collagen.

5.3.3- T.S.of leaf, stem, root

Leaf, stem and root of plants are made up of different types of tissues. These tissues form different layers in the composition of leaves, stems and roots. To study the structural details of the leaf, stem or root of a monocot or dicot plant, it is essential to be familiarized with the sectioning and staining techniques used with plant materials. It is also necessary to take the sections with uniform thickness so that the light passes through them equally and the different tissues found in the material are clearly visible under the microscope. To examine the tissues clearly, it is desirable to stain the section with suitable stains, as different stains colour the tissues differently.So till now we have studied all these aspects of practical. Now we are going to read the anatomy of leaf, stem and root.

Leaf Anatomy

T.S. Leaf: Leaf comprises following cells or tissue:

- **Epidermal layer-**barrel shaped cells on the dorsal and ventral surfaces.
- **Palisade mesophyll** – tightly packed cells that absorb light
- **Spongy mesophyll** – loosely packed cells with air spaces
- **Stomata** – pore-like openings for taking in CO_2 and releasing O_2

- **Guard cells** – cells that open and close the stomata

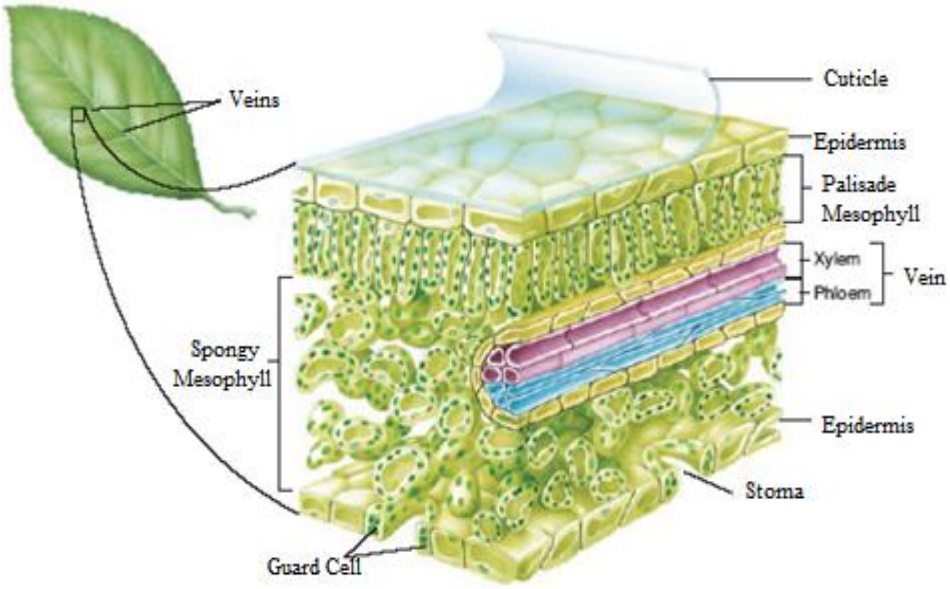

Fig.5.6: Leaf Anatomy

Leaves are of two types; dorsiventral common in Dicots and isobilateral common in Monocots. These are clearly distinguished on the basis of their venation, mesophyll cells, and distribution of stomata and even on the basis of their colour.

Leaf of Banyan (Dicot):

A transverse section through the leaf of Banyan (*Ficus benghalensis* of family Moraceae) would reveal the anatomical characters more or less similar to the previous one.

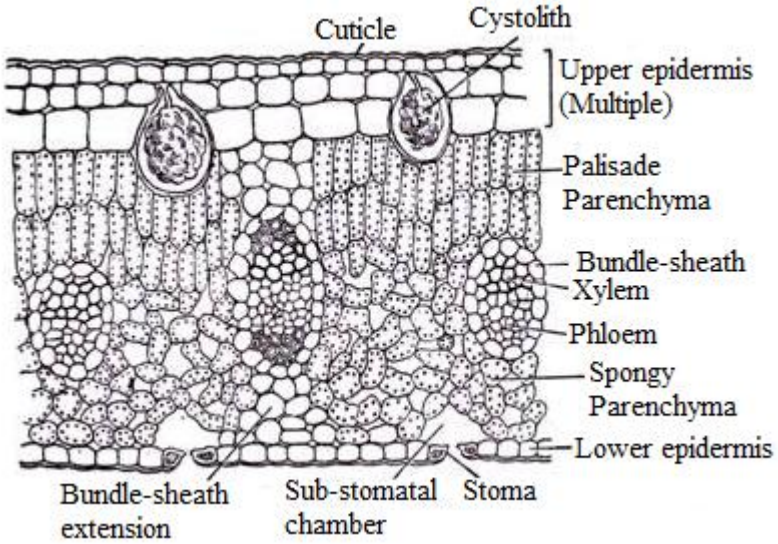

Fig.5. 7: T.S. *Ficus benghalensis* leaf

I. Epidermis:

The upper epidermis is multiseriate, being made of a few layers of cells. Lithocysts are frequently present and well-developed calcium carbonate crystals, the cystoliths, occur here and there. The lower epidermis is uniseriate. The outer layer of upper epidermis and the lower epidermis as a whole are made of compactly-arranged tubular cells with cutinised outer walls having cuticle. The degree of cutinisation is more pronounced on the upper side. Stomata occur on the lower epidermis.

II. Mesophyll:

It is differentiated into palisade and spongy cells. Two or three layers of columnar cells with abundant chloroplasts remain arranged more or less at right angles to the upper epidermis. These are palisade cells. This is the principal photosynthetic tissue. The spongy cells occurring towards lower epidermis are isodiametric, and often irregular in shape, and have profuse intercellular spaces. The number of chloroplasts is naturally much smaller here in comparison to palisade cells.

III. Vascular bundles:

The bundles are as usual collateral and closed ones, with xylem lying on the upper and phloem on the lower sides. They remain surrounded by parenchymatous bundle sheath. In case of bigger bundles bundle sheath extensions are present.

2. Leaf of Maize (Monocot):

A section through a leaf of maize (*Zea mays* of family Graminaceae) shows the following structure:

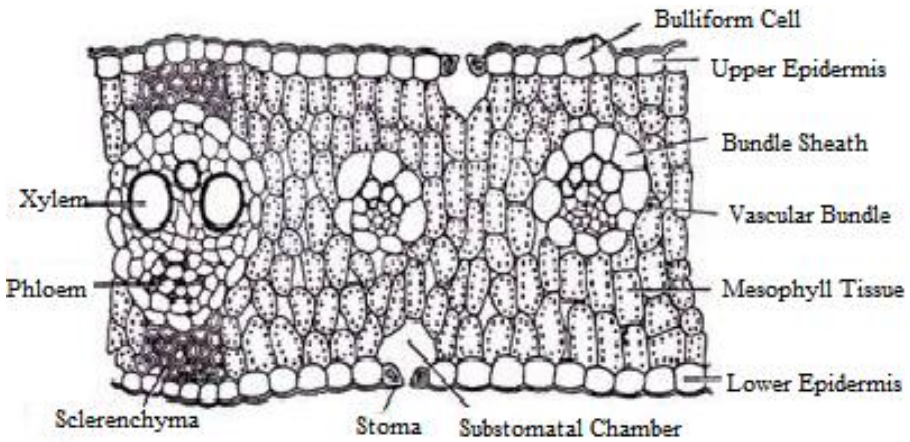

Fig.5.9: *Zeamays* leaf T.S.

I. Epidermis:

Both upper and lower epidermal layers are uniseriate and composed of more or less oval cells with cuticularised outer walls. Upper epidermis may be easily identified due to presence of large and empty bulliform cells. Stomata occur on both the epidermal layers.

II. Mesophyll:

The mesophyll does not show differentiation into palisade and spongy cells, but is made of rather compactly-arranged isodiametric cells.

III. Vascular bundles:

The bundles are collateral and closed ones which remain arranged in parallel series. Majority of the bundles are small, but fairly large bundles occur at regular intervals. Small bundles have xylem on the upper and phloem on the lower sides surrounded by large parenchyma cells forming the bundle sheath.The cells of the sheath contain plastids, often with starch grains. It is assumed that this layer serves as a temporary storage tissue, apart fromconducting the products of photosynthesis to the phloem. Xylem, as usual, consists of tracheary elements, and phloem of sieve tubes and companion cells.

Floating Leaf of Water-lily:

A section through the leaf of water lily (*Nymphaea stellata* of family Nyphaeaceae) would reveal the following anatomical structure. As an aquatic plant it has extremely reduced vascular and supporting tissues and well-formed air chambers.

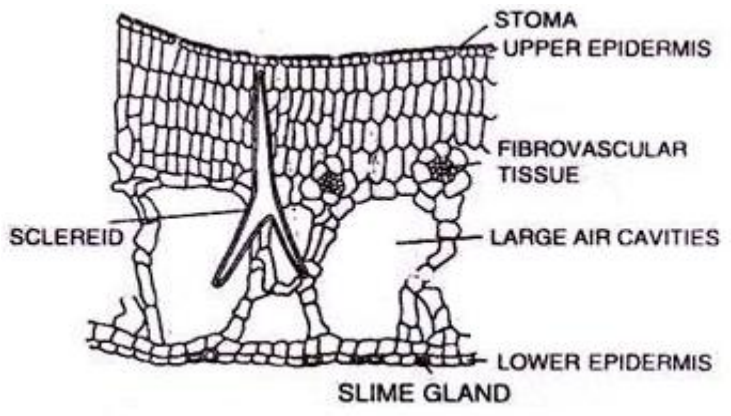

Fig. 5.10: T.S. Nymphaea Leaf

I. Epidermis:

Epidermal layers are uniseriate both on the adaxial and abaxial sides. They are composed of closely-set cells. Stomata occur on the upper side. Moreover, there is deposition of waxy matters which prevents wetting and clogging of the stomata.

II. Mesophyll:

It is differentiated into palisade and spongy cells. A few layers of columnar cells occur towards the adaxial side forming the palisade. The spongy cells present towards lower epidermis and irregular in outline. Large air chambers are present in the mesophyll. Elongated sclerotic cells—the trichosclereids commonly called ‘internal hairs’, often with branched ends are frequently present.

III. Vascular Bundles:

These are very much reduced. As usual they are composed of xylem and phloem, and remain surrounded by parenchymatous bundle sheath.

Stem Anatomy

The stem and other plant organs are primarily made from three simple cell types: parenchyma, collenchyma, and sclerenchyma cells. Parenchyma cells are the most common plant cells. They are found in the stem, the root, the inside of the leaf, and the pulp of the fruit. Parenchyma cells are responsible for metabolic functions, such as photosynthesis. They also help repair and heal wounds. In addition, some parenchyma cells store starch.
Collenchyma cells are elongated cells with unevenly-thickened walls. They provide structural support, mainly to the stem and leaves. These cells are alive at maturity and are usually found below the epidermis.

Dicot stems

Dicot stems with primary growth have pith in the center.
Vascular bundles forming a distinct ring visible when the stem is viewed in cross section.

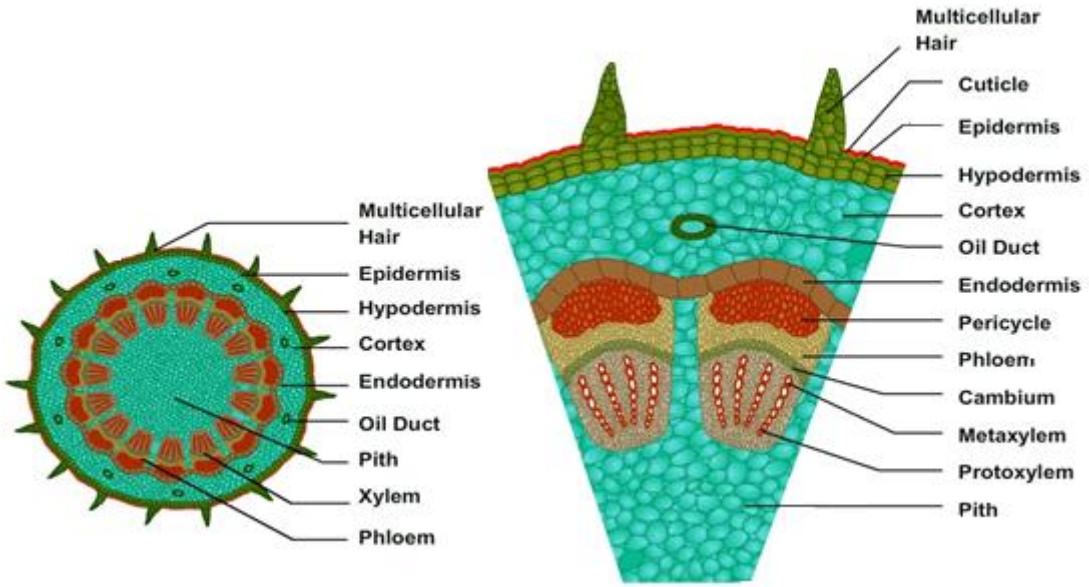

Fig. 5.11 Detailed Structure of Dicot Stem T.S.

- The outside of the stem is covered with an epidermis, which is covered by a waterproof cuticle.
- The epidermis may also contain stomata for gas exchange and multicellular stem hairs called trichomes.
- Cortex comprises the cells of collenchyma, and parenchyma.
- A cortex is flanked by hypodermis (collenchyma cells) outside and endodermis (starch containing cells) inside.
- Collenchyma cells lie under the epidermis and constitute three to four layers of cells with cell walls thickened at the corners. The collenchyma cells contain chloroplasts.

- The parenchyma cells make up the bulk of the cortex. They synthesized organic food (mainly starch) is stored here. The intercellular air spaces are responsible for gaseous exchange.
- Endodermis is starch sheath which forms the innermost layer of the cortex.
- This is a single layer of tightly-packed rectangular cells bordering the stele of the stem.
- The cells of this tissue store starch. It allows solutions to pass from the vascular bundles to the cortex.
- The vascular cambium cells divide to produce secondary xylem to the inside and secondary phloem to the outside.
- As the stem increases in diameter due to production of secondary xylem and secondary phloem, the cortex and epidermis are eventually destroyed.
- Before the cortex is destroyed, a cork cambium develops there. The cork cambium divides to produce waterproof cork cells externally and phelloderm cells internally.
- These three tissues form the periderm, which replaces the epidermis in function. Areas of loosely packed cells in the periderm that function in gas exchange are called lenticels.

Monocot stems

- Vascular bundles are present throughout the monocot stem, although concentrated towards the outside and not in a ring as found in dicots.
- The shoot apex in monocot stems is more elongated.
- Monocots rarely produce secondary growth and are therefore seldom woody, with Palms and Bamboo being notable exceptions.
- However, many monocot stems increase in diameter via anomalous secondary growth occur.
- Monocot stems, such as corn, palms and bamboos, do not have a vascular cambium and do not exhibit secondary growth by the production of concentric annual rings.
- They have scattered vascular bundles composed of xylem and phloem tissue.
- Each bundle is surrounded by a ring of cells called a bundle sheath.
- The structural strength and hardness of woody monocots is due to clusters of heavily lignified tracheids and fibers associated with the vascular bundles.
- The following illustrations and photos show scattered vascular bundles in the stem cross sections of corn (**Zea mays**):

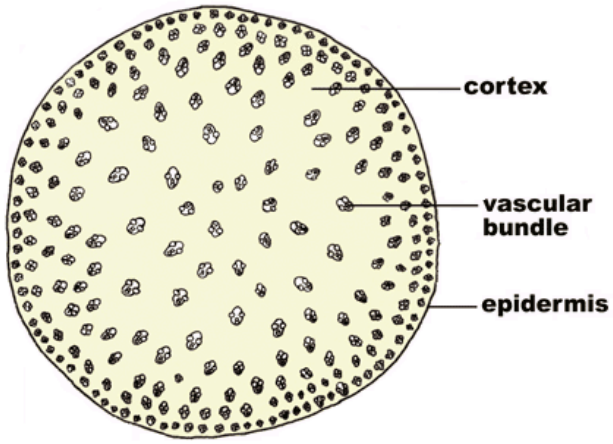

Fig. 5.12. Zea mays Stem T

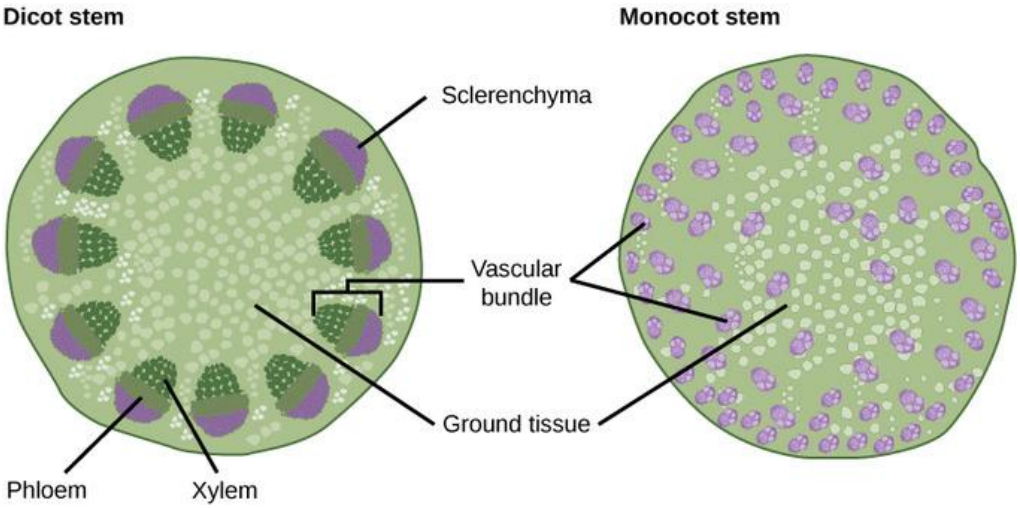

Fig.5.13: Comparative Anatomy of Dicot and Monocot Stem

Root Anatomy

In vascular plants, the **root** is the organ of a plant that typically lies below the surface of the soil. Roots can also be aerial or aerating, that is growing up above the ground or especially above water. The root is best defined as the non-leaf, non-node bearing parts of the plant's body. However, important internal structural differences between stems and roots exist.

When dissected, the arrangement of the cells in a root is root hair, epidermis (epiblema), cortex, endodermis, pericycle and, lastly, the vascular tissue in the centre of a root to transport the water absorbed by the root to other places of the plant.

Monocot Root: The typical monocot roots show following features:

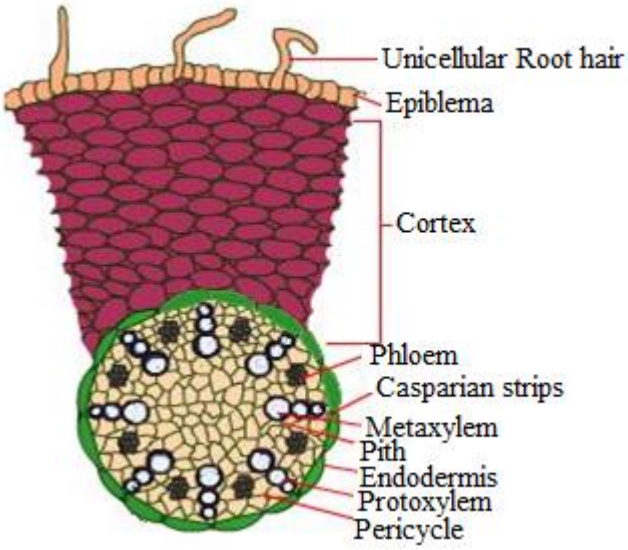

Fig. 5.14: Monocot Root T.S. (*Zeamays*)

1. **Epiblema** is the outermost single layer made from compactly arranged parenchymatous cells without intercellular space. Usually Epiblema has no stomata but bears unicellular epidermal root hairs and less amount of cutin. It contains more cuticle than dicot roots. The root hairs and thin walled epidermal cells take part in the absorption of water and minerals from the soil. The epiphytes have several layered hygroscopic epidermis, called **velamen tissues**. It is made from spongy dead cells which help in absorption of water from atmosphere.
2. **Cortex** is a multi-layered well developed and made from oval parenchymatous cells with intercellular spaces. The intercellular spaces usually help in gaseous exchanges, storage of starch, etc. Cortex helps in mechanical support to the roots (like hypodermis to stem).
3. **Endodermis** is innermost layer of cortex made from barrel shaped parenchyma. It forms a definite ring around the stele. These cells are characterized by the presence of casparian strips. It is deposition of suberin and lignin, on their radial and tangential walls. Usually passage cells are absent in monocot roots. Due to presence of **casparian strips**, endodermis forms water tight jacket around the vascular tissues, hence it is also called biological barrier.
4. **Pericycle** is uniseriate (multiseriate in Smilax) and made from thin walled parenchymatous cells. It is outermost layer of stellar system. Usually it is made from parenchymatous cells but it may become sclerenchymatous in older roots. Several lateral roots arise from this layer.
5. **Vascular bundle** is radial, arranged in a ring (except mangrove, which also contains lenticels), **polyarch** (presence of many alternating xylem and phloem bundles). Xylem and phloem are found at different radii alternating with each other (radial). The number of xylem and phloem vary from, 8 to 46 (100 in *pandanus*). The xylem is exarch, i.e. the protoxylem lies towards periphery and metaxylem toward center.

The phloem is also exarch (protophloem towards the periphery and metaphloem towards the center). Secondary growth is absent in monocot roots due to lack of vascular and cork cambium. **Conjunctive tissue** is parenchymatous tissues which separates xylem and phloem bundles.

6. **Pith** is large, well developed portion of monocot root. It occupies the central portion and made from thin walled parenchymatous tissue with intercellular spaces. It contains abundant amount of starch grains.

Primary Structure of Dicot root

The transverse section of the dicot root shows the following plan of arrangement of tissues from the periphery to the centre.

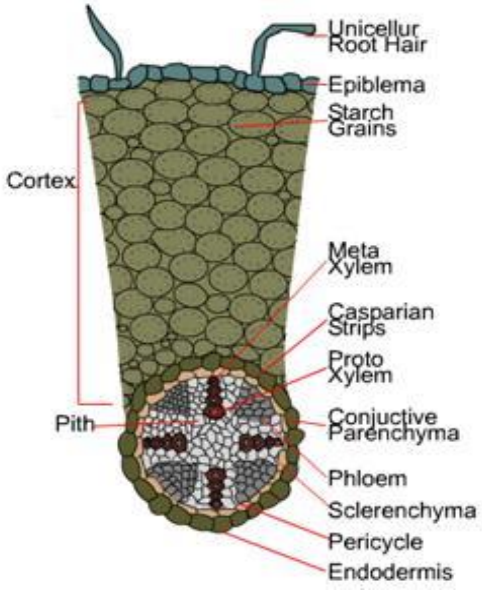

Fig. 5.15- T.S. of Dicot Root of Gram

1. Epiblema or Epidermis - It is the outermost unilayered with several unicellular root hairs. It consists of thin walled, compactly arranged living parenchymatous cells. Usually epiblema is characterised by absence of stomata and cuticle.

2. Cortex - It is thin walled, multi-layered region made from circular or polygonal parenchymatous cells usually with intercellular spaces. The cortical cells have no chloroplast but may contain leucoplast for storage of starch grains. The cortex is responsible for transportation of *water and salts* from the root hairs to the center of the root.

3. Endodermis - It is the innermost layer of cortex and covers the stele. It consists of compactly arranged barrel shaped parenchyma without intercellular spaces. Most of the cells are characterised by the presence of special thickening of suberin and lignin on their radial and tangential walls called **casparian strips**. Some endodermal cell near protoxylem has no casparian strips and called **passage cells** or transfusion cells. These cells allow radial diffusion of water and minerals through the endodermis.

4. Pericycle - It is the outermost layer of stele and composed of uniseriate layer of parenchymatous cells without intercellular spaces. Some dicots and hydrophytes do not bear pericycle. Several lateral roots and lateral meristem arise from pericycle region (hence lateral roots are endogenous in origin). At the time of secondary growth, it produces secondary cambium or phellogens.

5. Vascular bundles - They are 2-8 in number, radial and arranged in ring. Xylem and phloem bundles are separated from each other by parenchymatous cells called conjuctive or **complementary tissue**.

- **Xylem** is exarch (i.e. protoxylem towards the periphery and metaxylem towards the centre) and consists of tracheids, vessels, xylem parenchyma and xylem fibres.

- The **phloem** forms oval masses beneath the pericycle, alternating with xylem bundles. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Usually phloem fibers are absent or reduced.

6. Pith - it is feebly developed and centrally located. It consists of thin walled, polygonal parenchyma cells with intercellular spaces. In dicots roots, it may be reduced or absent. It helps in storage of food materials.

5.3.4-L.S. of leaf, stem and root

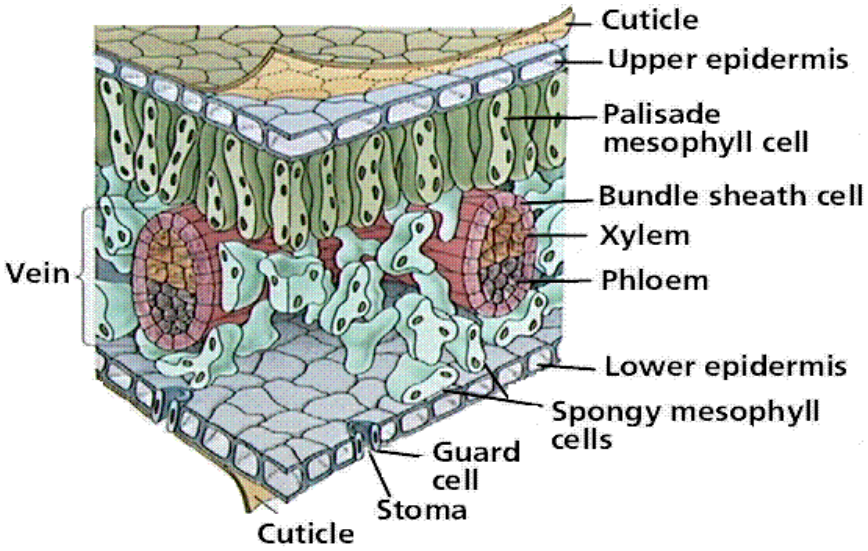

Fig. 5.16: L.S. of Leaf

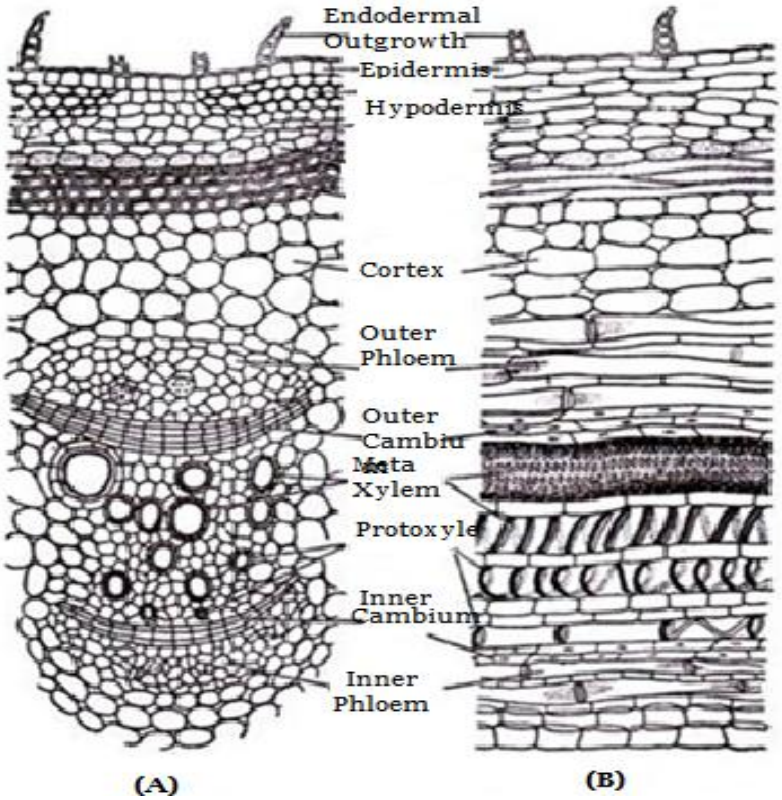

Fig. 5.17. Cucurbita Stem (A) T.S. and (B) L.S

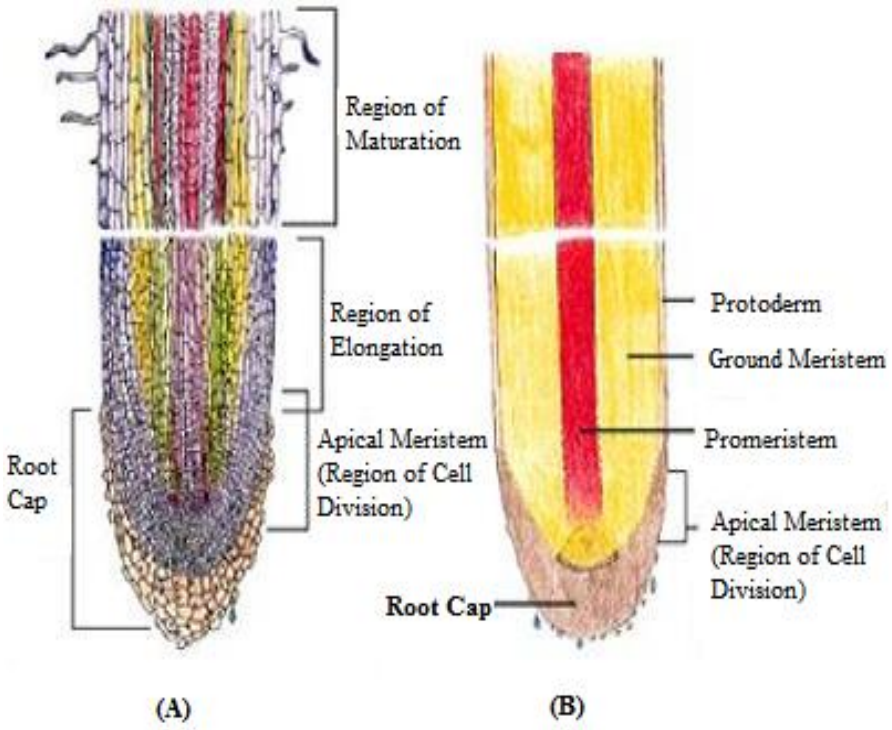

Fig.5.18. Dicot Root Tip L.S.

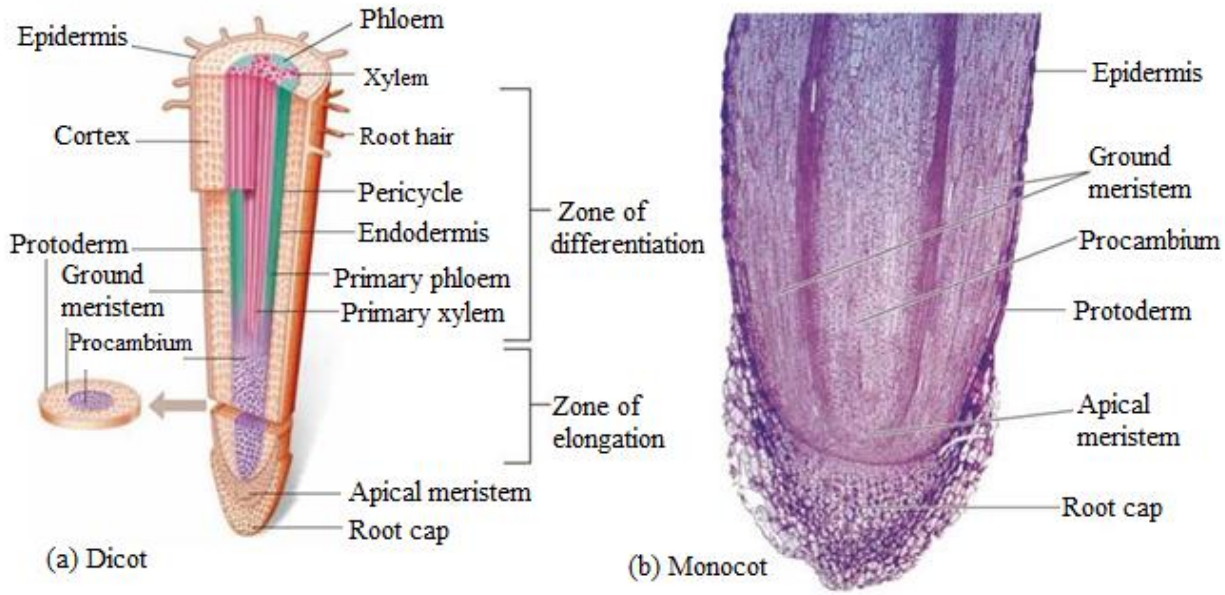

Fig. 5.19. Dicot and Monocot Root Tip L.S.

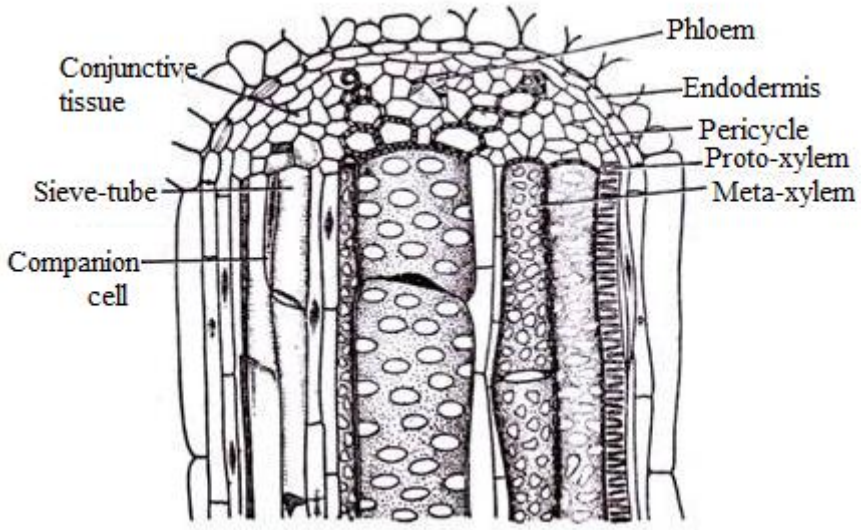

Fig. 5.20 Longitudinal Section of a Dicot Root

5.4 SUMMARY

The unit comprising different practical tools, sectioning procedure, process of staining the section. Microscopes are important tools for observation due to immense resolution power and the magnification of the microscope is determined by multiplying the magnification of the eyepiece by the magnification of the objective lens. In order to reveal the cellular structure, plant materials are being cut in various planes. Normally cross and longitudinal sections are taken for the study. These sections are stained through chemical stains and then after mounting we put them under microscope for the study. Unit has detailed idea regarding all the glasswares and the sectioning procedure along with staining. Root, stem and leaves anatomical features have been described in detail with the differentiating features between dicot and monocot plants.

5.5 GLOSSARY

Microscopy: Technique to see or visualize the microscopic objects though lenses.
Cross Section: Here the section passes at the right angle to the material.
Cyanin: Blue pigment.
Double Staining: Use of two dyes for coloring the different tissues of plants.
Longitudinal Section: Section is cut at the right angle to the transverse axis.
Maceration: Process of separation of cells from the surrounding cells.
Mounting: Keeping a section on the slide.
Sectioning: Process of cutting plant material into thin slices.
Staining: Technique to colour the tissues by different chemicals.
Squash: Technique for studying cell by crushing them over a slide.

Cuticle - The waxy, water-repelling layer on the outer surface of a leaf that protects it from dying out.
Epidermis - The protective, outer layer of cells on the surface of a leaf. The guard cells (and stoma) are part of the epidermis.
Guard Cell - One of a pair of sausage-shaped cells that surround a stoma (a pore in a leaf).
Lamina - The blade of a leaf.
Mesophyll - The chlorophyll-containing leaf tissue located between the upper and lower epidermis. These cells convert sunlight into usable chemical energy for the plant.
Palisade Mesophyll - A layer of elongated cells located under the upper epidermis. These cells contain most of the leaf's chlorophyll, converting sunlight into usable chemical energy for the plant.
Sclerenchyma- Tissue composed of thick-walled cells containing lignin for strength and support.
Spongy mesophyll - The layer below the palisade mesophyll; it has irregularly-shaped cells with many air spaces between the cells. These cells contain some chlorophyll. The spongy mesophyll cells communicate with the guard cells (stomata), causing them to open or close, depending on the concentration of gases.
Stoma - (plural stomata) a pore (or opening) in a plant's leaves where water vapor and other gases leave and enter the plant. Stomata are formed by two guard cells that regulate the opening and closing of the pore. Generally, many more stomata are on the bottom of a leaf than on the top.

5.6 SELF ASSESSMENT QUESTION

5.6.1 Objective type questions:

(i) Casparian strips have compound:
(a) Cellulose (b) Lignin
(c) Suberin (d) Cutin

(ii) A parenchymatous sheet of tissues separates the phloem strands from xylem and it becomes:
(a) Pericycle (b) Endodermis
(c) Stele (d) Cambium

(iii) Which is incorrect for monocot root?
(a) The pericycle gives rise to lateral roots (b) Cambium is absent
(c) The pith is present (d) Secondary growth does not occur

(iv) Pith is composed of:
(a) Collenchyma (b) Parenchyma
(c) Sclerenchyma (d) None

(v) Which is correct for dicot root?

(a) Vascular bundles are scattered irregularly in ground tissues
(b) The vascular bundles are open
(c) Cambium is absent
(d) There is no hard bast

(vi) Which meristem helps in increasing girth?

(a) lateral meristem (b) intercalary meristem
(c) primary meristem (d) apical meristem

(vii) Pith and cortex do not differentiate in
(a) monocot stem (b) dicot stem
(c) monocot root (d) dicot root

(viii) Where do the casparian bands occur
(a) epidermis (b) endodermis
(c) pericycle (d) phloem

(ix) Bordered pits are found in
(a) sieve cells (b) vessel wall
(c) companion cells (d) sieve tube wall

(x) Vertical section such as in leaves and thallus.
(a) Section at right angle to the transverse axis (b) Section at 180^0
(c) Section at 90^0 (d) Section 120^0

5.6.2 Short answer question.

i. What is sectioning?
ii. What is staining?
iii. What is a transverse section?
iv. Define single staining in plants.
vi. What is mounting?
vii. What is a chlorenchyma?

5.6.3Answer the following questions in about 100 words.

i) What are the different parts of compound microscope?
ii) Name different types of stains used for algae, fungi, bryophytes, pteridophytes and gymnosperms.
iii) Define sectioning technique.
iv) What is double staining?
v) Name the glassware used during anatomical study.

5.6.1 Answer Key:

i. (b) ii. (d) iii. (a) iv. (b) v. (b), vi. (b), vii. (a), viii.(b), ix. (d), x. (b)

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5.9 TERMINAL QUESTIONS

5.9.1. Answer the following questions in about 100 words.

i) Bring out the characters of transverse section of a dicot leaf.
ii) Explain the anatomical feature of monocot leaf.
iii) Draw a labeled diagram of dicot stem T.S.
iv) Compare the anatomical features of dicot and monocot root.
v) Cut a transverse section of young stem of a plant from your garden and observe it under the microscope. How would you ascertain whether it is a monocot stem or a dicot stem? Give reasons.

5.9.2 Answer the following questions in about 200 words.

i. Write an essay on the structure and function of microscope.
ii. Describe in detail with diagram about the anatomy of monocot stem.
iii. Define the anatomy of dicot root in detail.
iv. Define the transverse section of dicot stem.

UNIT-6 NORMAL AND ABNORMAL SECONDARY GROWTH IN *BOERHAVIA, BOUGAINVELLIA, NYCTANTHES, SALVADORA, DRACAENA* AND *TINOSPORA*

6.1 OBJECTIVES

In this section student will be able to understand:

- What is normal secondary growth and how does it take place?
- What is anomalous secondary growth?
- What are the reasons of anomalous secondary growth?
- Explanation of different types of anomalous secondary growth in different species?
- Significance of anomalous secondary growth?

6.2 INTRODUCTION

In botany, **secondary growth** is the growth that results from cell division in the cambia or lateral meristems and that causes the stems and roots to thicken, while **primary growth** is growth that occurs as a result of cell division at the tips of stems and roots, causing them to elongate, and gives rise to primary tissue. Secondary growth occurs in most seed plants, but monocots usually lack secondary growth. If they do have secondary growth, it differs from the typical pattern of other seed plants.

In many vascular plants, secondary growth is the result of the activity of the two lateral meristems, the cork cambium and vascular cambium. Arising from *lateral* meristems, secondary growth increases the girth of the plant root or stem, rather than its length. As long as the lateral meristems continue to produce new cells, the stem or root will continue to grow in diameter. In woody plants, this process produces wood, and shapes the plant into a tree with a thickened trunk.
Because this growth usually ruptures the epidermis of the stem or roots, plants with secondary growth usually also develop a cork cambium. The cork cambium gives rise to thickened cork cells to protect the surface of the plant and reduce water loss. If this continues for many years, this may produce a layer of cork. In case of oak it will yield harvestable cork.

Anomalous secondary growth refers to the deviation of the secondary growth from the normal type of growth. It is also known as abnormal or more appropriately unusual secondary growth, as the term encompasses some less common type of secondary growth patterns. Though secondary growth is an exclusive feature of dicotyledonous plants, but there are some monocotyledons that also show secondary growth.

6.3 NORMAL AND ABNORMAL SECONDARY GROWTH IN PLANTS

Normal Secondary Growth

It is the result of the activity of the vascular cambium, which occurs in between xylem, and phloem of each vascular bundle. Hence, it is known as intra-fascicular cambium or fascicular cambium. In addition, towards the beginning of secondary growth there is a process of

dedifferentiation in some of the parenchyma cells of the medullary rays which are at the level of cambium cells and adjoining the vascular cambium. As a result, these cells now become meristematic and represent the inter-fascicular cambium. The meristematic cells of the intra-fascicular cambium and inter-fascicular cambium join and result in the formation of a continuous strip of meristem called cambium ring. The cambium ring at this stage has primary xylem on its inner surface and primary phloem on its outer surface.
The cambium ring exhibits mitotic activity on both the sides. The mitotic activity on the inner surface results in the formation of cells, which differentiate into xylem. It represents the secondary xylem. Similarly, the mitotic activity on the outer surface results in the formation of cells, which differentiate into phloem. It represents the secondary phloem. Due to the formation of secondary xylem, the primary xylem becomes pushed more towards the pith and the pith gets slightly reduced. However, the secondary phloem grows and completely masks the primary phloem. Hence, it is not visible.

The mitotic activity of the cambial ring is purely seasonal. It occurs only twice during every year, once in the spring and once in the autumn season. Thus, every year two sets of secondary xylem and two sets of secondary phloem are formed. Each year, the mitotic division of the cambial ring usually begins in the spring season. The secondary xylem that is formed in the spring season is therefore known as springwood or early wood, while the secondary xylem formed in the autumn is known as autumn wood or late wood. The springwood is generally characterized by the presence of xylem vessels having wider lumen. This is because, spring is the ideal season for growth and the water requirement of the plant is more in the spring. The autumn wood has xylem vessels with narrow lumen, since water requirement in the winter is less.

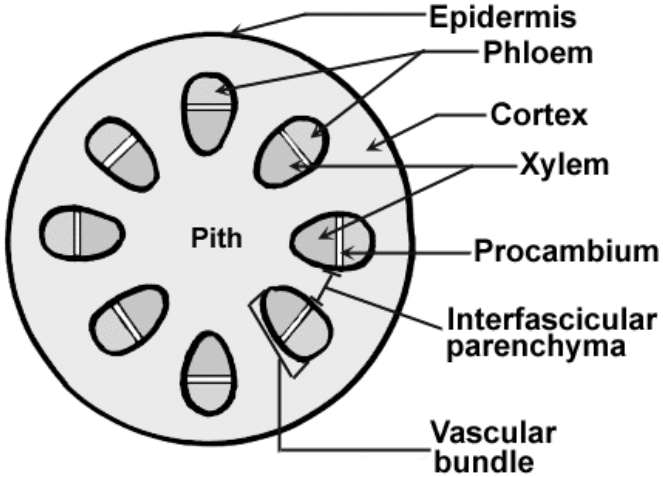


Fig.6.1. T.S. Dicot Stem before Secondary Growth

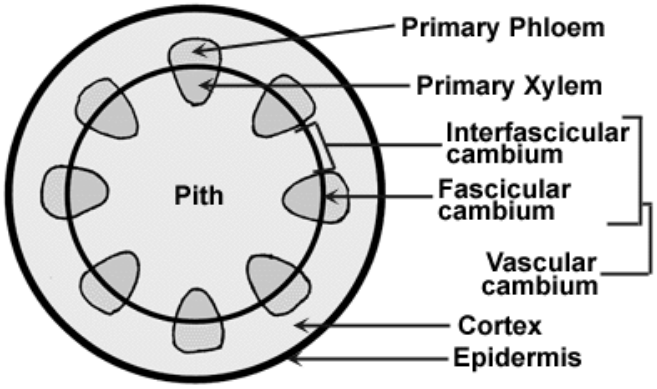


Fig.6.2. T.S. Dicot Stem after Initiation of Secondary Growth

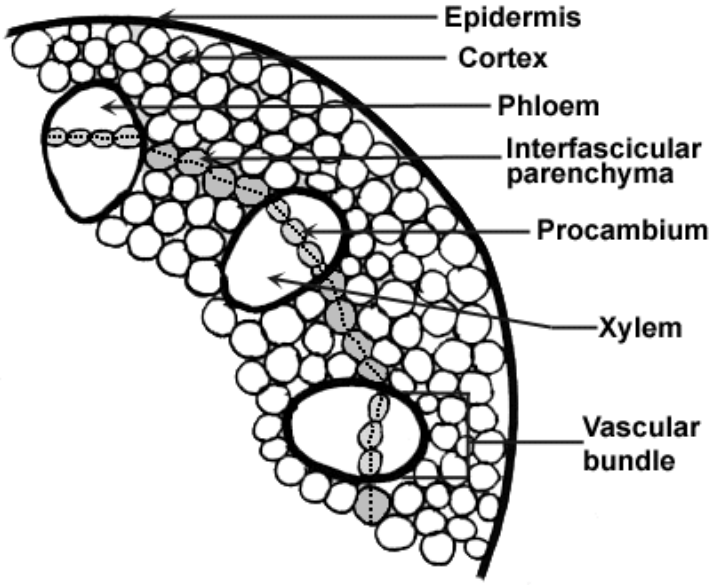


Fig.6.3. T.S. Dicot Stem Showing Formation of Cambium Ring

Activity of Cork Cambium

In dicot plants due to secondary growth a pressure is put on the epidermal layer and then the parenchymatous cells of the cortex become meristematic behaving like cambium and known as cork cambium or phellogen. It produces cork cells or phellem (bark) towards outside containing a waxy substance known as suberin. The bark protects the plant against physical damage, insects, termites, microbes and helps reduce water loss. The cork cambium also produces a layer of cells known as phelloderm, which grows inwards. The cork cambium, cork cells, and phelloderm are collectively termed as periderm. The periderm substitutes for the epidermis in mature plants. In some plants, the periderm has many openings, known as lenticels, which allow the interior cells to exchange gases with the outside atmosphere. This supplies oxygen to the living- and metabolically-active cells of the cortex, xylem, and phloem.

Mechanism of Secondary Growth

1. The mature dicot stem shows secondary growth and increases in girth.
2. This results by the activity of two lateral meristems--- vascular cambium and cork cambium or phellogen.
3. Secondary growth occurs in two parts—
   A) In stelar region by the vascular cambium
   B) In extrastelar region by the phellogen (cork cambium)

A) Sec. Growth in Stelar Region by Vascular Cambium

1. Interfascicular cambium develops outside the vascular bundles joining intrafascicular cambium and forming complete ring.

2. Now this complete ring produces cells towards inside as secondary xylem and towards outside as secondary phloem. Thus new cells produced and the girth increases slowly.

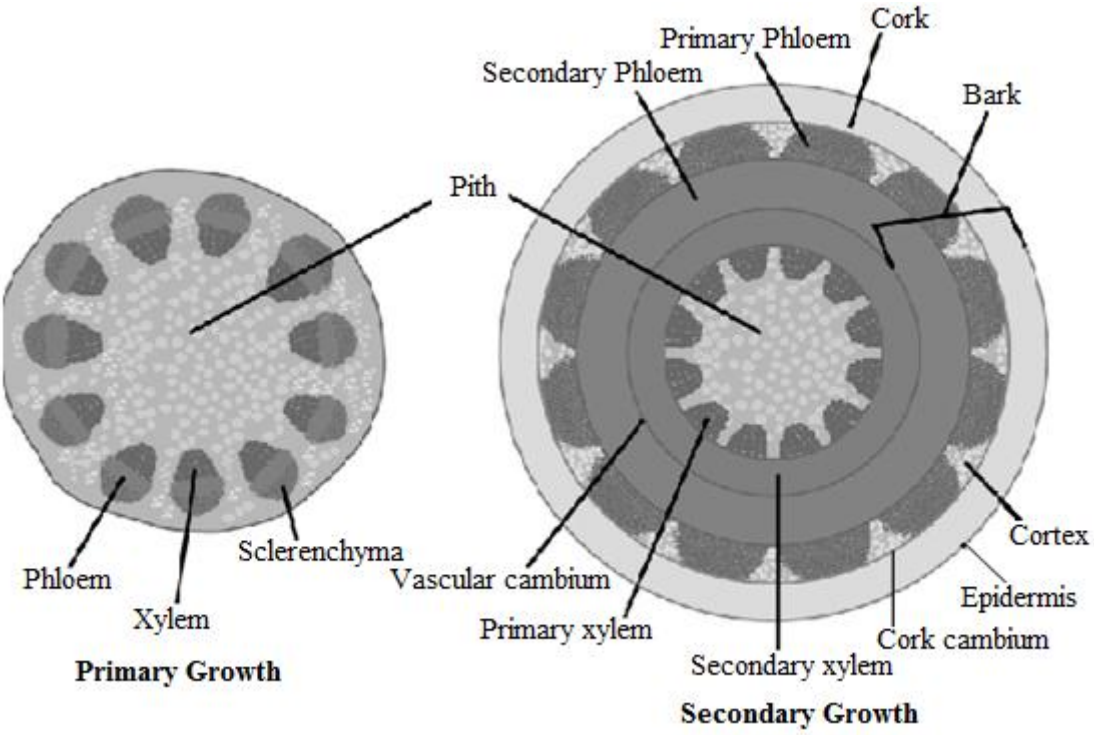


Fig.6.4. Primary and Secondary Growth in Cross Section

Formation of Annual Rings

- The vascular cambium remains active throughout life.
- Activity of vascular cambium is affected by seasonal variations.
- Variation in activity is seen during different seasons, resulting in shape and structure of wood. Because the activity of the cambium varies so the production of new cell also varies resulting in a clear cut colour variation in different seasons.
- This results in the formation of annual rings, which is used to determine the age of the tree.

B) Sec. Growth in Extra Stelar Region by Cork Cambium

- As long as the lateral meristems continue to produce new cells, the stem or root will continue to grow in diameter.
- In woody plants, this process produces wood, and shapes the plant into a tree with a thickened trunk.
- This growth usually ruptures the epidermis of the stem or roots, plants with secondary growth usually also develop a cork cambium.
- For this any outer layer of cortex becomes meristematic and begins to divide. This is known as Phellogen or cork cambium.
- The cork cambium gives rise to thickened cork cells to protect the surface of the plant and reduce water loss.
- Phellogen or cork cambium divides to produce outer cork (Phellem) and inner secondary cortex (phelloderm).
- All the three tissues phellem, phellogen and phelloderm are together known as periderm.

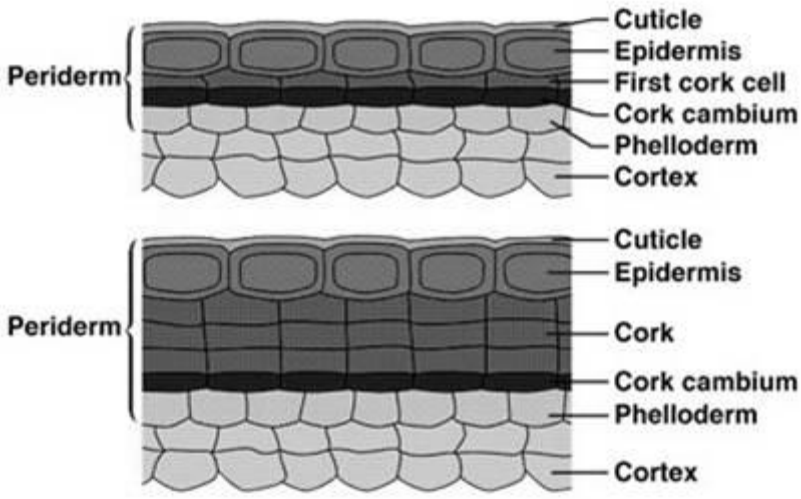


Fig.6.5. Activity of Cork Cambium

Anomalous Secondary Growth in Dicot Stem

Anomalous or unusual secondary growth may occur due to:

1. Unusual position of the vascular cambium
2. Unusual activity of the vascular cambium
3. No development of usual cambium or if so happens, its replacement by other accessory cambium formation and its activity
4. Formation of included or interxylary phloem
5. Development of interxylary cork

For better understanding, the anomalous secondary growth may be studied under the above stated categories in some representative plant species:

Unusual Position of the Vascular Cambium

The vascular cambium normally lies in between primary xylem and primary phloem. But sometimes, in plants such as *Thinouia sp., Serjania sp., Paullinia sp., Bauhinia langsdorffiana,* etc. cambium may be present elsewhere and its location may not be well differentiated. Cambium in such unusual position shows unusual activity resulting in anomalous secondary structure.

Unusual Activity of the Vascular Cambium

The cambium is normal in position but it shows an abnormal activity leading to irregular arrangement of secondary tissues. Such as formation of unusually large amount of secondary vascular tissue and formation of increased size of vascular bundles. This may happen when only intrafascicular cambium is active and it forms secondary vascular tissues in the region of vascular bundles only, e.g. stem of *Cucurbita* sp.

One more thing can happen i.e. formation of wide medullary rays. A normal cambium ring is formed by the union of intra- and interfascicular cambium, but it shows unusual activity where interfascicular cambium forms parenchyma only resulting in the formation of wide medullary rays.

Cambium in the Form of Folds or Ridges

In the young stem of climbers e.g. *Thinouia scandens*, the cambium is thrown into folds or ridges. At the time of secondary growth, the cambium separates at the folds and gives rise to separate groups of vascular tissues, resulting in a lobed stem

Cambium in the form of Separate Strips

This is commonly found in climbers, *Serjania ichthyoctona* and *Paullinia* of family Sapindaceae. The cambium here originally appears in many separate strips, each of which surrounds small parts or may be some strands of primary xylem and phloem. As the secondary growth starts, each cambial strip forms a separate entire ring of its own secondary tissue and behaves normally by cutting secondary xylem inside and secondary phloem outside. The mature stem thus has many distinct vascular bundles. Such a stem seems to be made up of many fused stems. In older stems, the discrete vascular bundles develop their own periderm and may progressively get separated from each other. The stem thus, seems to be made up of a number of strands of smaller stems closely suppressed to each other, resembling strands in a rope.

Accessory Cambium formation and its Activity

In many genera, a new cambium ring or accessory cambial rings originate(s) in the cortex or pericycle where either the normal cambium ring is altogether absent, e.g. *Amaranthus,* or there is cessation of its activity, e.g. *Boerhaavia*. The unusually positioned cambium (referred to as extrastelar in origin and accessory cambium) behaves unusually resulting in the formation of successive rings of vascular bundles embedded in parenchyma or conjunctive tissue. Here, the first ring of cambium arises in the pericycle region and it shows unusual activity by cutting off secondary xylem in patches alternating with parenchyma cells (or conjunctive tissue) on the inner side whereas externally, initially forming parenchymatous

layers and afterwards forming secondary phloem. It forms a complete ring of vascular bundles and then it stops functioning. A new ring of cambium called accessory cambium is formed from the parenchyma cut off externally by the earlier cambium. This newly formed cambium also behaves unusually in a similar manner forming another ring of vascular bundles embedded in parenchyma and then becomes inactive. Likewise, more accessory cambia are formed giving rise to successive rings of vascular bundles.

Formation of Included or Interxylary Phloem

The groups of secondary phloem cells embedded in the secondary xylem is referred to as included or interxylary phloem. By definition, it is 'the phloem that develops within secondary xylem'. These are formed due to unusual activity of the cambium.

Sometimes small segments of the cambium at different intervals starts cutting secondary phloem towards inside instead of secondary xylem (abnormal behavior). After sometimes these segments behave normally and as usual cut secondary xylem towards inside. Thus inwardly formed secondary phloem gets embedded in secondary xylem.

Sometimes small segments of cambium cease to function and their cells converted into secondary phloem. New cambial strips develop outside. Later these newly formed cambial strips unite with the edges of general cambium and the normal activity of cambium is resumed and thus the phloem cells get embedded in secondary xylem.

Consequently, included phloem is observed in several dicot families such as Asclepiadaceae, Nyctaginaceae, Onagraceae, Salvadoraceae, and Amaranthaceae. Included phloem is a characteristic feature of some xerophytic plants and has a physiological significance. Being embedded in the xylem tissue, they are retained and they continue to function even in the unfavourable conditions. They serve to assimilate food for the developing buds on the restoration of favorable conditions.

6.3.1-*Boerhaavia* (Family: Nyctaginaceae)

Boerhaavia shows anomalous secondary growth due to anomaly in its primary structure as well as accessory cambium formation and its activity at the time of secondary growth. The young stem is typically dicotyledonous in structure with a few unusual features:

Epidermis

- Single layered epidermis consists of small, radially elongated cells.
- Multicellular epidermal hairs arise from some cells.
- A thick cuticle is present on the epidermis.
- Some stomata are also present.

Cortex

- It is well differentiated and consists of few layered collenchymatous hypodermis followed by chlorenchyma.
- Collenchyma is 3 to 4 cells deep, but generally near stomata it is only one layered.
- Chlorenchyma is present inner to collenchyma in the form of 3 to 7 layers.

- Chlorenchymatous cells are thin walled, oval, full of chloroplasts and enclose many intercellular spaces.
- Endodermis is clearly developed and made up of many, tubular, thick-walled cells.

Pericycle

- Inner to the endodermis is present parenchymatous pericycle but at some places it is represented by isolated patches of sclerenchyma.

Vascular System

- Vascular bundles are present in three rings. In the innermost ring are present two large bundles; in the middle ring the number ranges from 6 to 14 while the outermost ring consists of 15 to 20 vascular bundles.

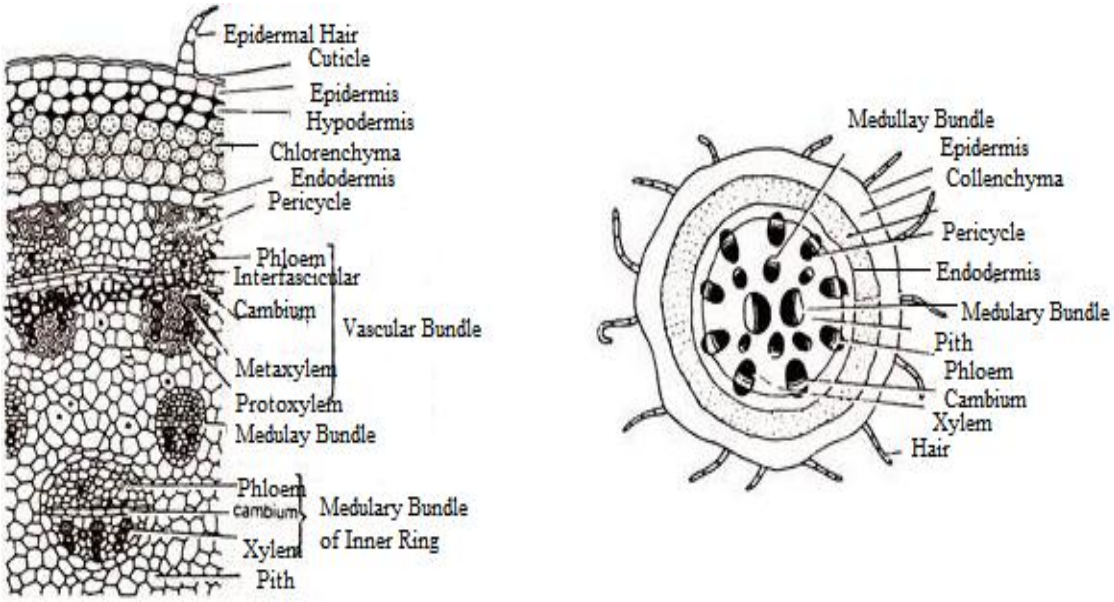


Fig.6.6. Anomalous Secondary Growth in *Boerhaavia*

- Vascular bundles are conjoint, collateral, open and endarch.
- There are three rings of vascular bundles which are primary in origin – the innermost two large medullary bundles, middle ring composed of 6-14 loosely arranged bundles and the outermost ring of 15-20 small bundles.
- Two vascular bundles of the innermost ring are large, oval and lie opposite to each other with their xylem facing towards center and phloem outwards.
- Middle ring consists of 6-14 small vascular bundles.
- Vascular bundles of inner and middle rings may show a little, restricted secondary growth with only small increase in size, and the intrafascicular cambium in these bundles behave normally.
- It forms secondary xylem towards inside and secondary phloem towards outside with primary phloem pushed to lie only as a cap like structure towards outside.

- The cambium of the outermost ring of the vascular bundles forms a complete ring at the time of secondary growth by the union of inter- and intrafascicular cambium.
- The intrafascicular cambium forms secondary xylem on the inside and secondary phloem on the outside, whereas the interfascicular cambium forms conjunctive tissue on the inside and parenchymatous tissue on the outside.
- The interfascicular cambium functions for some time, and then it ceases its activity.
- Soon after, a new accessory cambium ring arises by the union of the secondary parenchyma cells lying above and the cells of pericycle positioned outside the phloem.
- This first accessory cambium ring behaves in a similar manner as of the vascular cambium, forming secondary xylem alternating with conjunctive tissue on the inner side and secondary phloem above parenchyma tissue on the outside.
- As a result, another ring of vascular bundles is formed which are of secondary origin. This process may be repeated to form four or more successive rings of vascular bundle.

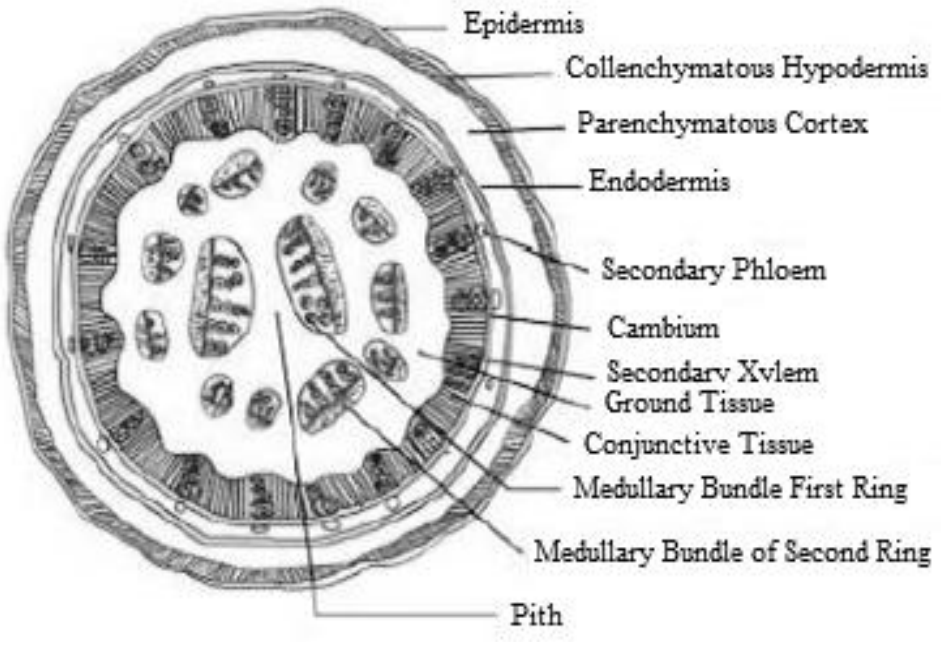
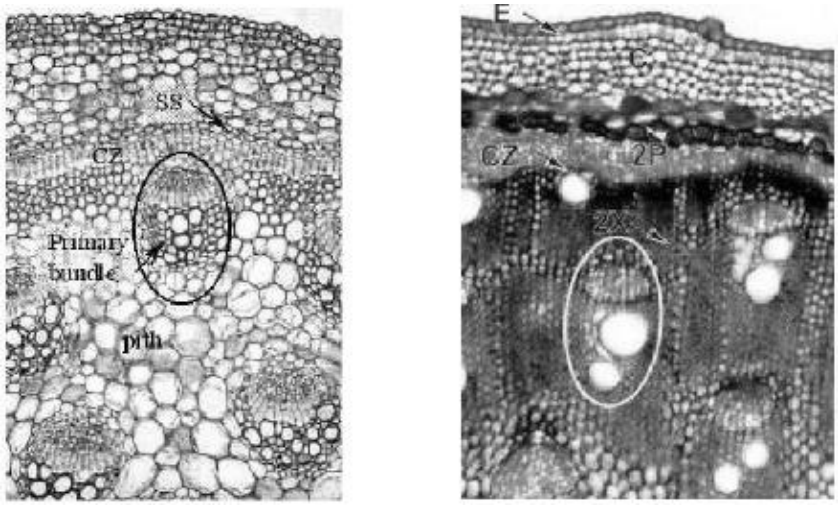


Fig.6.7. T.S. *Boerhaavia* Stem

- The stem in *Boerhaavia* contains well-defined anomalous secondary growth, which is characterized by the presence of successive rings of xylem and phloem.
- Alternate bands of lignified and parenchymatous bands are distinct in the stem.

6.3.2-*Bougainvellia* (Family: Nyctaginaceae)

Bougainvillea is a member of the Nyctaginaceae and is an example of a dicotyledonous stem which displays **anomalous secondary growth**.



(A) Bougainvillea Young Stem (B) Bougainvillea Old Stem

Fig.6.8.T.S.*Bougainvillea* stem

- The stem is circular in outline when young.
- It has a uniseriate epidermis covered by a thick cuticle, collenchymatous hypodermis and a well-developed parenchymatous cortex.
- The ill-defined endodermis is followed by a pericycle made of parenchyma with intermittent sclerenchymatous patches.
- In the TS, near the center of the stem, we can see some **primary vascular bundles** embedded in lignified pith parenchyma.
- The primary vascular bundles are seemingly scattered in the ground tissue and are not arranged in a ring.
- The first ring of cambium arises from the pericycle thus is extrastelar in origin. This is followed by formation of successive rings of cambia, though it was also believed that all the secondary tissue derivatives arise from a single cambium.
- Each cambial ring cuts off xylem alternating with parenchyma internally and, phloem and alternating patches of parenchyma externally.
- The parenchyma so formed usually gets lignified, which is then referred to as conjunctive tissue.
- Thus, concentric rings of vascular bundles are formed embedded in conjunctive tissue.
- In some species, the conjunctive tissue is sclerenchymatous only and is hardly distinguishable from the tracheary elements of the embedded vascular bundles.
- The phloem appears as an isolated patch actually surrounded by the conjunctive tissue, which is often mistaken to be included phloem.
- Secondary phloem and secondary xylem lie on either side of it. The secondary xylem is composed of **tracheids, fibres** and **narrow-diameter vessels**.
- Interspersed with the secondary xylem you will be able to see small pockets of phloem and what vessels look like large-diameter metaxylem.
- These are reminiscent of the primary bundles towards the center of the stem. These are in fact primary vascular bundles embedded within the secondary xylem, hence the use of the term, **anomalous growth** in this instance.

- The phloem is described as being **included phloem**, which by definition is phloem tissue which lies between regions of secondary xylem.
- The **anomalous growth** results as a result of differential cambial activity. Newly-produced vascular cambia result in the outer lateral meristem becoming quiescent and this cambium returns to activity only when the internal vascular cambium becomes less active.
- Vascular cambia do not produce rays in Nyctaginaceae but do produce vessels, axial parenchyma and sometimes fibers to the inside and variable secondary phloem to the outside.

6.3.3-*Nyctanthes* (Family–Oleaceae)

The outline of T.S. appears quadrangular and reveals the following tissues from outside within:

Epidermis

- Single-layered epidermis consists of rectangular cells.
- A thick uninterrupted cuticle is present on the epidermis.
- Many multicellular hairs are present.

Cortex

- It is differentiated into collenchyma and parenchyma.
- Collenchyma is several cells deep below the four protruded comers while only few layers deep at the other places just beneath the epidermis.
- Parenchyma is present below the collenchyma. Many intercellular spaces are present. The region extends up to the vascular tissue.

Cortical bundles

- Four vascular bundles are present in the cortex, situated one in each protruded bulge.
- Each conical bundle faces its pointed xylem end towards outer side, i.e., epidermis, and is conjoint, collateral, open and exarch.
- These bundles may show secondary growth at maturity.

Endodermis

- Not well-developed.

Pericycle

- It is in the form of sclerenchymatous patches.

Vascular System

- It consists of primary phloem, secondary phloem, cambium, secondary xylem and primary xylem.
- Primary phloem is crushed and irregularly present in patches below pericycle.

- Secondary phloem is present in the form of a continuous ring and consists of sieve tubes, companion cells and phloem parenchyma
- Cambium is one to three cells thick continuous layer present in between phloem and xylem.
- Secondary xylem is present just inner to the cambial ring and consists mainly of thick walled wood parenchyma and fibres. Tracheids and vessels are also present
- Primary xylem is situated just near the pith facing its protoxylem towards the centre.

Pith

- It is thin walled and parenchymatous.

Abnormality

- Abnormality in Nyctanthes is the presence of cortical bundles, which are inversely oriented, 4 in number and never directly connected with the main axial ring of the vascular cylinder. These are leaf trace bundles.
- Cortical bundles have also been reported in some other families such as Casuarinaceae (Casuarina), Umbelliferae (Eryngium), Papilionaccae (*Latkyrusmarytimus*). Mclastomaccac, Rutaccae, etc.

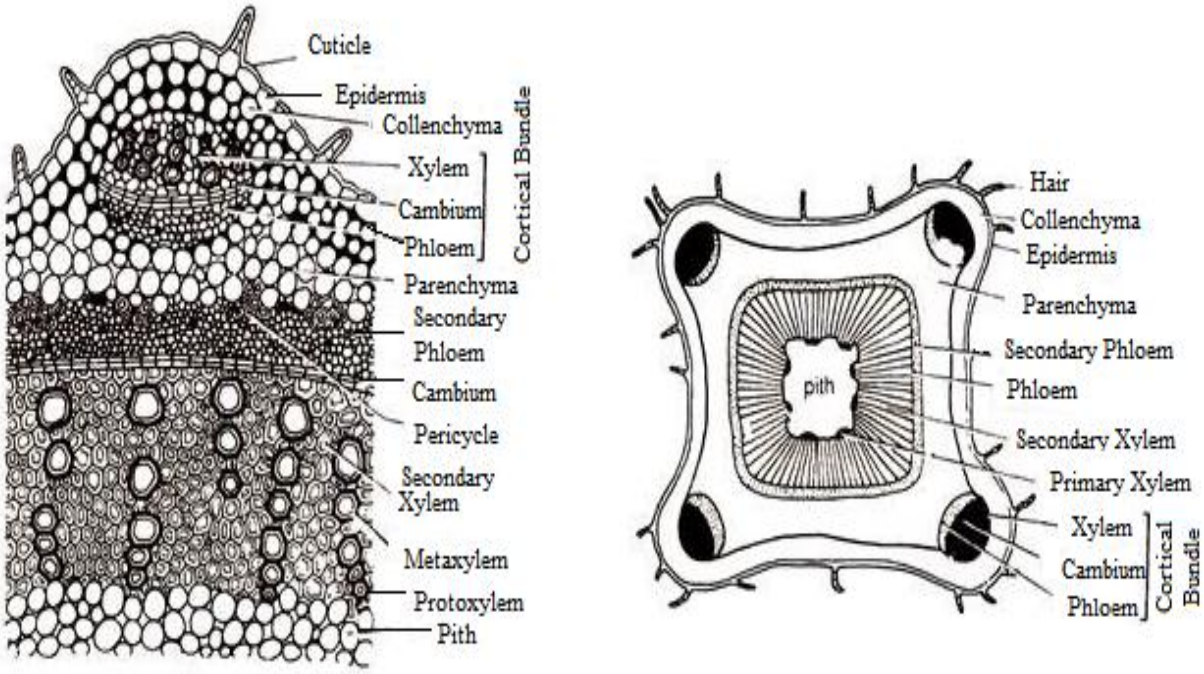


Fig.6.9. *Nyctanthes* T.S.

6.3.4-*Salvadora* (Family: Salvadoraceae)

T.S. reveals the following tissues from outside within:

- Epidermis isthe outermost layer with barrel shaped cells. Cells are covered by a thick cuticle.
- Cortex consists of parenchymatous hypodermis, few layers of chlorenchyma and an innermost layer of endodermis.

- Hypodermis is generally thin walled, parenchymatous, but sometimes 2-3 layers of collenchyma are seen.
- Chlorenchyma is present inner to the hypodermis. It is 3-5 cells deep and cells are filled with chloroplasts.
- Few layers of parenchyma are also present below the chlorenchyma. The cells contain intercellular spaces.
- Endodermis is the innermost layer of cortex made up of barrel shaped cells which contain starch grains.
- Pericycle is a discontinuous layer present in the form of patches consisting of many widely spaced strands of thick walled fibres.
- Vascular bundles are conjoint, collateral, open and endarch.
- Vascular system composed of primary phloem, secondary phloem, cambium, secondary xylem, primary xylem and included phloem.
- Primary phloem is crushed and found in the form of patches.
- Secondary phloem is present just outside the cambium in the form of a ring.
- Cambium strip consists of rectangular cells arranged in radial rows.
- Secondary xylem forms a complete cylinder. It is represented by wide vessels and xylem parenchyma.
- Many medullary rays traverse the secondary xylem.
- Wide vessels of metaxylem and narrow protoxylem vessels can be observed in the primary xylem present near the pith.
- Secondary xylem is interrupted by many groups of thin walled phloem representing included or interxylary phloem or phloem islands.

Pith

It is well developed, thin-walled, parenchymatous and present at the center.

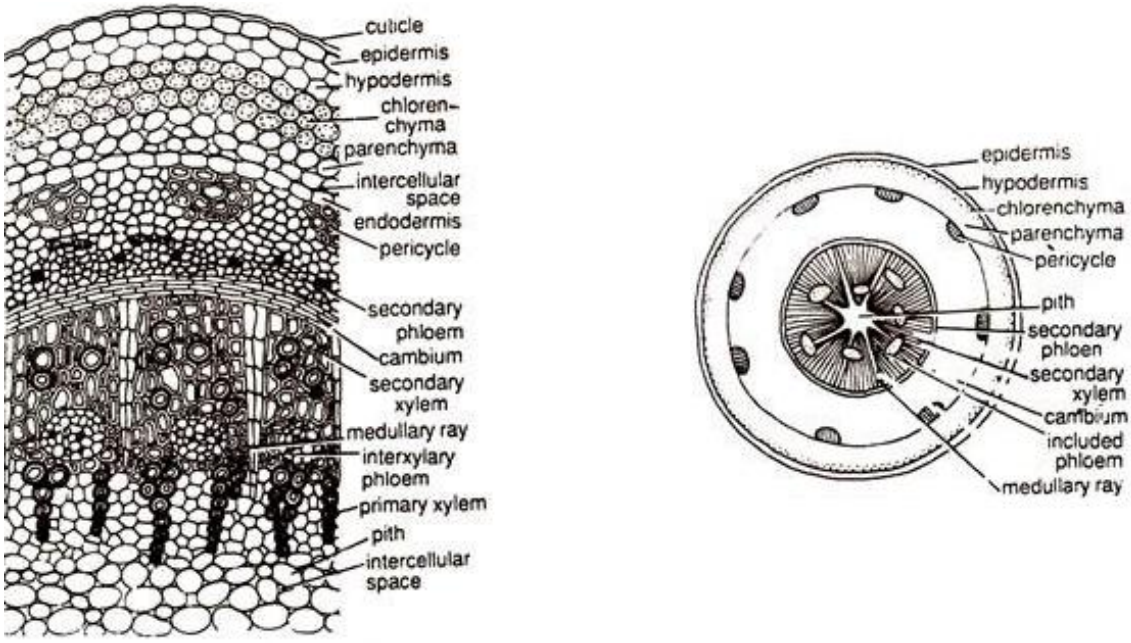


Fig. 6.10.T.S. Salvadora Stem

6.3.5-*Dracaena* (Family: Asparagaceae)

Normally the vascular bundles of the monocotyledonous stems are closed ones. Thus due to absence of the cambium" they lack secondary growth in thickness and the vascular system is wholly composed of primary tissues.The bundles remain irregularly scattered in the ground tissues, where the limits of cortex and other ground tissues can be hardly seen.Some monocotyledons belonging to the family Liliaceae, mainly the arborescent ones like *Dracaena*, *Yucca*, *Cordyline, Agave, Aloe* and others exhibit a peculiar type of secondary increase in thickness, an account of which is given here.

Dracaena (the Dragon's blood tree) is the only monocot which have secondary growth in roots. *Dracaena* is a monocot. The stems undergo a specialized secondary growth, which manifests itself in the production of additional parenchymatous elements. Their later growth pattern is termed diffuse secondary growth, and consists mostly of a proliferation of ground parenchyma cells and additional vascular bundles near the periphery.

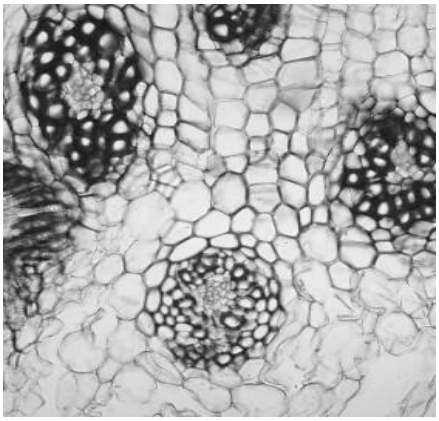


Fig. 6.11 T.S. Dracaena Stem

Young stem has a typical monocot structure having single epidermis, sclerenchymatous hypodermis and numerous closed, collateral vascular bundles scattered in the parenchymatous ground tissue.

In Dracaena secondary growth is due to:

A) Extrastelar cambium ring in monocot stem at the cortex
B) Abnormal activity of the cambium

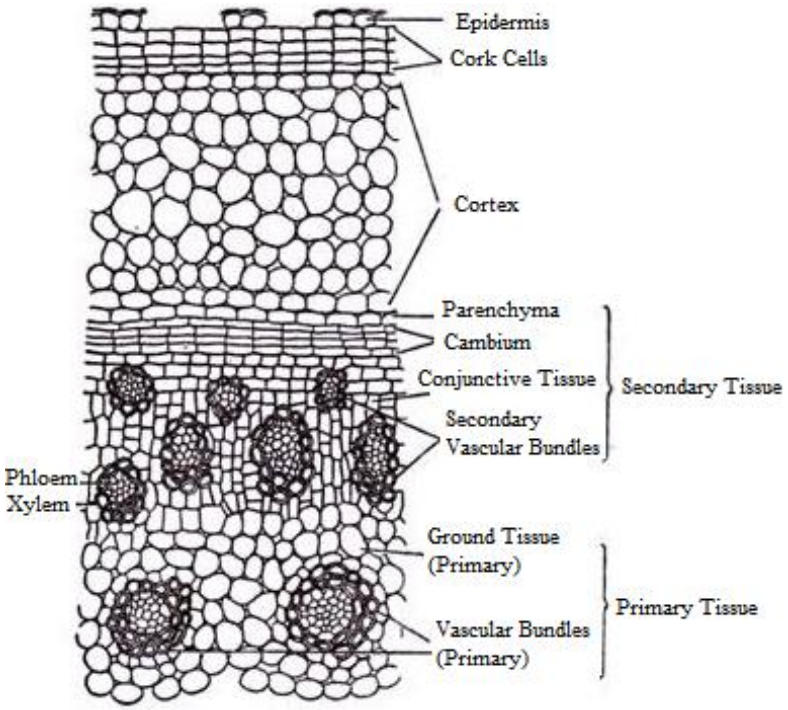


Fig. 6.12 T.S. *Dracaena* Stem Showing Special Type of Secondary Growth

When secondary growth starts:

- Formation of secondary meristem or secondary cambium occurs in the inner region of parenchymatous cortex.
- The activity of cambium is abnormal.
- It produces secondary vascular bundles on its inner side only and parenchymatous cells on the outer side.
- Secondary vascular bundles are amphivasal where phloem is surrounded by xylem.
- The secondary ring of vascular bundles are alternating in position with the first ring.
- The vascular bundle in the last inner ring is embedded in a mass of lignified conjunctive tissue.
- Cork cambium activity is normal and produces cork and secondary cortex in the outer region.

6.3.6-*Amaranthus* Stem (Family: Amaranthaceae)

The primary structure of the stem shows a number of shallow ridges and furrows with a thickly cuticularised single layer of epidermis.

Epidermis

- It consists of single layer of barrel shaped cells covered externally by thick cuticle.
- Lateral and inner walls are thin.

Cortex

- It is well differentiated into collenchyma and chlorenchyma.

- Collenchyma is present just below the epidermis. It is more prominent below ridges. Comers of the cells are thick and the cells are-oval or polygonal in shape.
- Chlorenchyma is present inner to collenchyma. Thin walled cells are spherical to oval in shape, filled with chloroplasts and contain many intercellular spaces.
- Endodermis is poorly developed and sometimes absent. The cells are elongated and lack casparian strips.

Pericycle

- It consists of few layers of thin walled, compactly arranged cells. It becomes sclerenchymatous in older stems.

Vascular System

- The normal ring of vascular bundles is absent. Instead there are two rings of medullary bundles formed by the activity of accessory cambia.
- The first accessory cambium differentiates in the pericycle. It behaves unusually by first forming small amount of parenchyma on the outside, and then cutting xylem alternating with parenchyma on the inner side and consequently forming phloem alternating with parenchyma on the outside.
- As a result, a ring of conjoint, collateral, endarch and open type of vascular bundles is formed which gets embedded in parenchymatous tissue.
- After sometime, this cambium ceases to function and becomes passive. A second accessory cambium arises from the parenchyma cut off by the previous one on the outside.
- It also behaves in a similar fashion producing a second ring of vascular bundles again included in the parenchyma, but alternating to the first one.
- Similarly, numerous accessory cambia develop consecutively producing consecutive rings of vascular bundles, giving a scattered appearance in the ground tissue of the stem.
- The final accessory cambium ring forms sclerenchyma alternating with xylem internally thus the last ring of vascular bundles seems to be embedded in sclerenchyma.
- On maturity, sometimes the medullary bundles along with some adjoining parenchyma may degenerate creating cavity.
- Primary phloem is crushed and present in patches.
- Secondary phloem is present in the form of a complete ring which consists of sieve tubes, companion cells and phloem parenchyma.
- Cambium is distinct and present in one to many layers located in between phloem and xylem.
- Secondary xylem remains embedded in conjunctive tissue and consists of proto-and metaxylem vessels and abundant parenchyma.
- Conjunctive tissue is present in abundance and consists of thick walled and lignified cells.
- Primary xylem is present near the pith facing its protoxylem towards centre.

- Though the normal vascular cambium is not formed but the cork cambium is formed and functions normally.

Medullary Bundles

Many scattered medullary bundles are present in the pith.
Each medullary bundle is conjoint, collateral and endarch, with the cambium either feebly developed or functionless or absent.

Pith

It is parenchymatous and cells show some intercellular spaces.

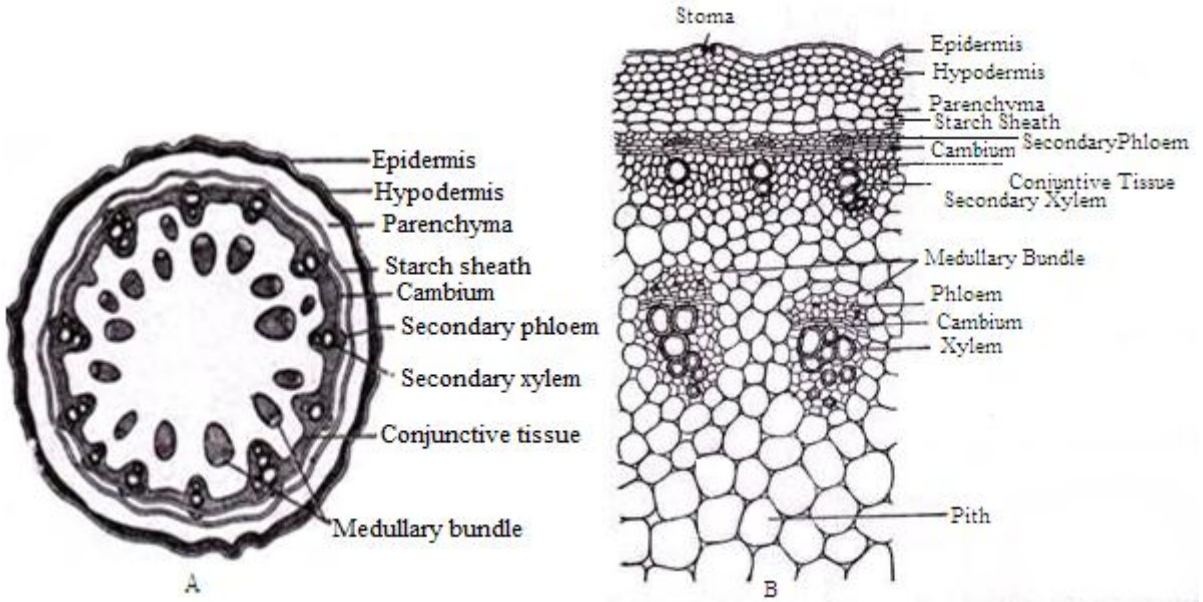


Fig.6.13. Stem of *Amaranthus* Showing Secondary Growth (A) T.S. Stem (Diagrammatic) (B) Magnified View of Stem T.S.

Secondary Growth in *Amaranthus* Stem

The vascular bundles are medullary ones. They are large in number and remain scattered in the pith. The bundles are collateral and open. Cambial activity is confined to the individual bundles, and it ceases soon. Secondary growth occurs due to development of a new meristem— the secondary cambium outside the stele. The cambium cuts off similar bundles with xylem on the inner side and phloem on the outer side. The secondary bundles remain embedded in thin-walled conjunctive tissue, which is wavy in outline on the inner side.

In the pericycle region the outer primary bundles become meristematic and develop few layered cambium. This cambium cuts collateral vascular bundles towards inner side consisting of secondary phloem and secondary xylem. Cambium also cuts many layered parenchymatous conjunctive tissue which becomes lignified and thick walled. The vascular bundle lies completely embedded in conjunctive tissue.

Significance of Anomalous Secondary Growth

It is believed that plants show anomalous secondary growth primarily because of two reasons:

i) As an adaptation to the environment – Some anomalies in the plant structure arise in response to the environment to cope with it. Such forms are termed as adaptive type. This includes the plants with:

a) Climbing habits – This is found in climbers and lianas. The climbers should have soft tissue like parenchyma or secondary phloem in abundance to promote their flexibility and twining or twisting habit. These tissues may also split the solid woody cylinder into strands helping the plant to climb, e.g. *Aristolochia* and *Tinospora* show fluted vascular bundles; Bignonia show phloem wedges; *Leptadenia*and *Thunbergia* show presence of interxylary phloem.

A flattened stem is sometimes encountered in climbers which helps the plant to hold onto the support while climbing, e.g. as seen in Bauhinia.

In some other climbers, such as *Serjania, Thnouia,Ichthyoctona*and*Paullinia*, cambium develops in the form of separate strips and the mature stem has many distinct vascular bundles which develop their own periderm and may progressively get separated from each other. The stem thus, seems to be made up of a number of strands of smaller stems closely oppressed to each other, resembling strands in a rope. This provides strength to the stem against extension and breakage facilitating twisting and twining.

b) Storage roots - Many plants have storage roots where the reserve food material is stored in the parenchymatous tissue. A considerable amount of storage parenchymatous tissue is formed as a result of anomalous secondary growth in them which is considered to be an adaptation to their storage function, e.g. *Beta vulgaris, Raphanus sativus, Ipomoea batatas*and*Daucuscarota*.

c) Floating habits – The parenchymatous tissue when encloses a lot of air space (referred as aerenchyma) can provide buoyancy to the aquatic plant, e.g. in *Jussiaea*, cork cambium produced at the time of secondary growth gives rise to parenchyma only that help in buoyancy.

ii) Variation in the cambial activity – In nature there is variation in the position, development, behaviour and/or nature of cambium found in some plants leading to varied structural organizations. Such forms, with structural anomalies which are not because of the environment, are referred to as non-adaptive type. This is found in many plants such as, *Boerhaavia, Mirabilis, Amaranthus, Chenopodium, Bougainvillea, Dracaena*etc.

6.4 SUMMARY

Most monocots either have no secondary growth or else anomalous secondary growth of some type. For example, palm trees increase their trunk diameter due to division and

enlargement of parenchyma cells, which is termed *diffuse secondary growth.* In some other monocot stems with anomalous secondary growth, a cambium forms, but it produces vascular bundles and parenchyma internally and just parenchyma externally.The word anomalous means deviating from the general or common order or type. Thus, the term, anomalous growth reflects a growth condition which is not commonly seen and which is present in a limited number of families or genera. This exercise explores a few examples of anomalous growth; bear in mind, there are many to choose from! The examples here illustrate aspects that are common - and include multiple cambia, included vascular bundles, and multiple vascular cylinders. Whereas the development, arrangement, activity of the vascular cambium in most woody dicotyledonous and Gymnospermous plants tends to be very similar, there are some alternatives which produce new secondary tissues that do not follow a normal pattern. As a result, the secondary plant structures that are formed are termed anomalous. Most anomalous growth is associated with the formation of multiple cambia.

6.5 GLOSSARY

Cell membrane: A slim layer of fat and protein that surrounds a cell though still located inside the cell wall. It is semi-permeable, which means it allows for some substances to pass through it while keeping others out.

Cell wall: A tough, rigid layer that surrounds a plant cell. The cell wall is located outside of the cell membrane and acts to support, filter incoming substances, and protect the cell from over-expansion due to water intake. Cell walls can also attach to other cell walls to help form the structure of a plant.

Chlorophyll: A green molecule vital for photosynthesis. Chlorophyll captures light energy from the sun in order to convert carbon dioxide and water into oxygen and sugar for plant consumption.

Chloroplast: Disc-shaped organelle containing chlorophyll and the location where photosynthesis occurs.

Collenchyma: Tissue composed of cells with unevenly thickened walls.

Cotyledon: One of the first leaves of the embryo of a seed plant; seed leaf.

Cristae: The folded membranes inside the mitochondria. The walls of the cristae contain proteins and are the site where cell energy production occurs (ATP is produced).

Cytoplasm: A gooey substance that contains all the cell's organelles outside of the nucleus. Most cellular activity occurs within the cytoplasm.

Dicotyledon: Flowering plants that have two seed leaves that emerge after germination.

Monocotyledon: Flowering plants that have one seed leaf that emerges after germination.

Parenchyma: Thin-walled cells, varying in shape, size, and function.

Plasmodesmata: The site where communication and transport of materials between plant cells occurs.

Phloem: The food-conducting tissue of a vascular plant.

Schlerenchyma: Tissue composed of thick-walled cells containing lignin for strength and support.

Sieve element: Cell in the phloem tissue concerned with longitudinal conduction of food materials. In flowering plants, it is called a sieve-tube element.
Sieve tube: A series of sieve-tube elements arranged end to end and interconnected through sieve plates.
Vacuole: Membrane-lined area within a plant cell that is filled with water. This organelle takes up much of the space inside a cell and help maintains its shape and size.
Vessel: A tube-like series of vessel elements with open ends. The walls that join the members have perforations or holes in them to allow water to pass through freely.
Vessel element: Individual cells that make up vessels.

6.6 SELF ASSESSMENT QUESTION

6.6.1 Multiple choice questions:

i) Casparian strips are present in
(a) Cortex (b) Epidermis
(c) Stele (d) Endodermis

ii) The outer most part of the stele consists of one or more layers of parenchymatous cells. The outer layer of this parenchyma is called:
(a) Cortex (b) Epidermis
(c) Stele (d) pericycle

iii) The type of arrangement in which protoxylem lies towards the outside and metaxylem lies towards the inside is called:
(a) Mesarch (b) Endarch
(c) Exarch (d) None

iv) The case in which xylem is present towards the inner side and phloem is present towards the outer side of vascular bundle is:
(a) Collateral (b) Bicollateral
(c) Concentric (d) Bilateral

v) Cambium is absent in:
(a) Monocot (b) Dicot
(c) Gymnosperm (d) None

vi) Exarch and polyarch vascular bundles occur in
(a) Monocot stem (b) Monocot root
(c) Dicot stem (d) Dicot root

vii) The endodermis in dicot stem is also called
(a) Starch sheath (b) Mesophyll
(c) Pili (d) Bundle sheath

viii) Polyarch condition is seen in
(a) Monocot stem (b) Monocot root
(c) Dicot root (d) Dicot stem

ix) Which of the following is seen in a monocot root ?
(a) Large pith (b) Vascular cambium
(c) Endarch xylem (d) Medullary ray

x) Well developed pith is found in
(a) Monocot stem and dicot root (b) Monocot and dicot stems
(c) Dicot stem and dicot root (d) Dicot stem and monocot root

6.6.2 Fill in the blanks:

i) The meristems present at the tips of roots and shoot are called …… meristems.
ii) The meristem situated at the bases of internodes is called …… meristem.
iii) One or More layers of cortex below the epidermis become thick wall to form ……
iv) The inner layer of endodermis is called ……
v) Cells of root caps in many parts form a constant structure called……..
vi) Secondary growth includes the formation of secondary vasculartissues and ……
vii) The walls of epidermal cells of leaves of the xerophytic plantsundergo……

6.6.1 Answer Key:

i. (d), ii. (d), iii. (c), iv. (a), v. (a), vi. (b), vii. (a), viii (b), ix. (b), x (a)

6.6.2 Answer Key:

i) apical, **ii)** Intercalary, **iii)** Endodermis, **iv)** pericycle, **v)** columella, **vi)** Periderm **vii)** lignification

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6.9 TERMINAL QUESTIONS

6.9.1 Short Answer type Question:

1. What are the differences between normal and anomalous secondary growth?
2. Define activity of cork cambium.
3. Define the mechanism of secondary growth.
4. What are spring and autumn wood?

6.9.2 Long Answer type Question:

1. How dicot stem increases in its girth define in detail?
2. Define secondary growth in Dracaena?
3. What is accessary cambium and define its function.
4. How secondary growth occurs in Salvadora?
5. Secondary growth in Boerhaavia occurs due to accessory cambium formation, define this.

UNIT-7 PLANT HORMONES, THEIR FUNCTIONS AND ROLE ON ROOT DEVELOPMENT, SENESCENCE AND POLLEN GERMINATION

7.1 OBJECTIVES

After the study of this unit, students will be able to:

- define the terms growth and development;
- discuss different types of growth hormones and their functions;
- describe the factors affecting plant growth and importance of plant hormones.
- comment on the terms abscission and senescence;
- explain the role of plant hormones in root development, senescence and pollen germination

7.2 INTRODUCTION

You must have noticed that all living organisms grow in size. But have you everthought how do they grow? Growth takes place due to cell division, whichincreases the number of cells in the body. Growth takes place at a faster rate till the plants attain maturity. Then it slows downand at a particular time it stops. Later death occurs. All these changes thatoccur in an organism starting from its beginning till its death may collectively betermed as development.

Plant hormones (also known as **phytohormones**) are chemicals that regulate plant growth. Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and, move to other functional parts of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, the development and ripening of fruit. The term 'Phytohormone' was coined by Thimann in 1948. Synthetic chemicals showing the similar effects as that of plant hormones are called **plant growth regulators** (PGR).

A phytohormone is an organic substance orchemical messengers produced in a small quantity inone part of plant body and capable of moving to other parts to influencethe growth of that part.When hormones reach the target tissue they can:

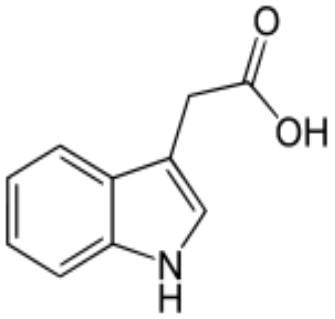
- have a direct effect on the target tissue causing a rapid metabolic response;
- involve the use of a second messenger within target cells; and
- Affecttranscription of nuclear deoxyribonucleic acid (DNA).

Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues. Plants lack glands to produce and store hormones, because, plants use more passive means to move chemicals around their bodies. Hormones are transported within the plant by utilizing different types of movements. For localized movement, cytoplasmic streaming within cells and slow diffusion of ions and molecules between cells are utilized. Vascular tissues are used to move hormones from one part of the plant to another; these include sieve tubes or phloem that move sugars from the leaves to the roots and flowers, and xylem that moves water and mineral solutes from the roots to the foliage.

Classes of plant hormones

In general, it is accepted that there are five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to other. Each class has positive as well as inhibitory functions, and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation. The five major classes are:

1-Auxins



The auxin - indole-3-acetic acid (IAA)

An experiment was performed by Fritz Went on oat seedling to see the effect of auxins. When tip of oat coleoptile (early shoot) was removed, growth stops. Then the removed tip was placed on a block of agar (gelatinous material from sea weeds) for about an hour. This agar block was then placed on the cut end of the seedling. It was observed that the growth of the seedling started again. It shows that there is something that has passed from the cut tip into the agar block, which helped to restart the growth. This was named Auxin, a plant hormone.

Auxin is a growth promoter, generally produced by the growing apex of stem and root of the plants. The most widely studied naturally occurring auxin is indol-3-acetic acid (IAA), which is chemically related to the amino acidtryptophan. IAA can be synthesized from tryptophan in intact cells but other synthetic pathways are available. Auxins have an effect in very low concentrations. Auxins are produced in young shoots and always travel downward in the plant from shoot to root. There are some synthetic auxin like Indole-3-butyric acid (IBA), 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D), and Naphthalene acetic acid (NAA).Synthetic auxins such as NAA is used as rooting hormones. Other synthetic auxins include 2, 4-D and 2, 4, 5-T are used as weed killers.

Functions of Auxin

- Apical Dominance: Removal of apical bud stimulates lateral buds. Auxins inhibit lateral bud formation since they are synthesized in apex. This phenomenon is called apical dominance. e.g.: Potato tubers for apical buds forming.
- Cell Division and Elongation: Shoot and Root elongation is the result of auxin.
- Xylem Differentiation: Auxins helps in the differentiation of different cells of xylem.

- Nucleic Acid Activities of IAA increases total RNA - synthesizes specific enzymes lead to cell enlargement.
- Manifold Activities - Play specific role in seed germination, growth, rooting, flowering (Reproductive phase), abscission, parthenocarpy and tissue culture.

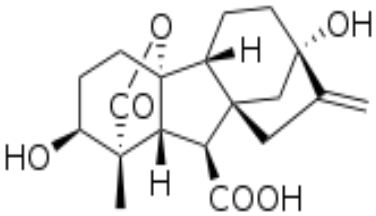
Practical Applications of Auxins

- Germination: IAA, IBA, NAA, 2,4-D are mostly used in soaking seed for germination- at low concentrations promote germination but these effects are subjected to variation depending on form and species of plants.
- Root: NAA, 10% induces 100% rooting in mango, IBA+Sugar application leads to greater number of roots and structure of roots also get changed (Vascular bundles).
- Flowering: Play floragenic role in day neutral plants. IAA promotes formation of female flowers. Increase spikelet number, leaf number and weight and number of grains in wheat. NAA & IAA increase boll-set in cotton (*G.hirsutum*), induce more pine-apples. Fruit weight also increases.
- Parthenocarpy: IBA, NAA produces seed lessfruits, smaller sized fruits, but more in number, hence yield not affected.
- Fruit setting: By using 2,4,5-T fruit setting and yield of berfruit increased. IAA, IBA, and NAA induce high percentage of fruit set.
- Prevention of pre-mature drop of fruits: 2,4-D,IAA,IBA, 2,4,5-T, are used to prevent pre-harvest drop of sweet oranges.
- Tissue and Organ culture: IAA & Kinetin together are used in tissue and organ culture.
- Auxins as inhibitors: High concentrations of auxins inhibit the growth and exert toxic effect on plants. In normal case, self-produced (natural) auxins inhibit the growth and development of lateral buds exerting dominance and as a result apical buds remain dormant.

Auxins use in Agriculture and Horticulture

- Propagation of plants by hormone treatment of cuttings
- Prevention of pre harvest drops of plants.
- Increasing parthenocarpy.
- Increasing fruit set.
- Prevention of sprouting by inhibiting buds.
- Inhibition of prolonged dormancy.
- Control of flowering.
- Defoliation of plants
- Prevention of leaf fall or abscission.
- Thinning of compact fruits.
- Selective weed killer.

2-Gibberellins



Gibberellin A1

Gibberellins, or GAs, include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberellafujikuroi* that produced abnormal growth in rice plants. Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In plants, it is produced in embryos, roots, and young leaves and it enhances growth.

Gibberellins are a very large class of compounds, all with a similar chemical makeup. There have been as many as eighty-four gibberellins identified (named GA1 to GA84), about 51 types are found in higher plants, but GA3 called gibberellic acid is most studied. Gibberellins promote cell elongation, overcome genetic dwarfism, stimulate bolting in biennials, and are involved in seed germination. During the germination of grass seeds the imbibition (intake) of water stimulates the production of gibberellins by the embryo that diffuse throughout the seed.

GAs are involved in many aspects of plant development, including seed germination, trichome development, stem and leaf elongation, flower induction, anther development, and fruit and seed development.

In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue. Absorption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling.

Functions of Gibberellins

- Mechanism of Gibberellins: GA exerts its physiological effect on altering the Auxin status of tissue. It acts at the gene level to cause depressions of specific gene.
- The activated genes by producing new enzymes bring about observed morphologic changes. Alerts the RNA. GA appears to involve in alteration of nucleic acid directed protein synthesis in some long term regulatory action and some other types of activation phenomena in short term regulatory action.

Role of Endogenous Gibberellins

- Apical bud dormancy
- Role in sub apical meristem
- Cell elongation
- Fruit growth
- Flowering
- Metabolisation of food in seed storage cells.

Practical Applications of Gibberellins

- Germination: Increases length of hypocotyl and cotyledonary leaf area.
- Root Growth: Inhibits root growth
- Leaf Expansion: Leaves become broader and enlarged (Cabbage, Sweet corn).
- Hyponasty of leaves: GA treated leaves of chrysanthemum plants holds their leaves more erect.
- Flowering: Induces flowering in long day plants and in plants requiring cold induction. Also promotes formation of male flowers.
- Parthenocarpy: Brinjal, Guava (Allahabad round). Thomson seedless grapes.
- Fruit setting: Increased fruit setting (Phalsa, Sweet lime, Grapes).
- Fruit Drop: Not much effective.
- Stem elongation: In *Chorchoruscapsularis t*here is extension of stem and increased number of internodes. However leaf area, basal diameter of stem and fibre quality is reduced.
- Pollen Germination: Sugar cane 15 out of 34 germinated against normal conditions.
- Breaking Dormancy: In temperate plants, buds become dormant in late summer and do not sprout even when exposed to sufficient moisture, temperature and oxygen. They require low temperatures or long days or red light. GA overcomes this dormancy. Enhanced cell elongation pushes through the endosperm (seed coat). Potato tubers can be made to sprout in winter by GA.
- Other uses: Sprayed on Fruits to prevent rind disorder. Thomson seedless grape bunches if sprayed with GA, causes elongation of bunch, so they are less tightly packed and less susceptible to fungi.
- Initiation of cell division
- Delay of senescence
- Use in tissue culture
- Counteract apical dominance.

Action and application

- Cell division
- Cell enlargement
- Morphogenesis
- Dormancy
- Apical dominance

- Mobility: Immobile obstructs the movement of amino acid, phosphate and various other substances
- Nucleic acid metabolism: Quick increase in the amount of RNA and decreases DNA
- Protein synthesis: Increases DNA
- Protein synthesis: Increased rate
- Florigens: Induction of flowering in short day plants.

3-Abscisic Acid (ABA)

Abscisic acid is one of the most important plant growth regulators. It was discovered and researched in two different names before its chemical properties were fully known; it was called *dormin* and *abscicin II*. Once it was determined that the two compounds are the same, it was named abscissic acid. The name "abscissic acid" was given because it was found in high concentrations in newly abscised or freshly fallen leaves.

In general, it acts as an inhibitory chemical compound that affects bud growth, and seed and bud dormancy. It mediates changes within the apical meristem, causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly abscised leaves, it was thought to play a role in the processes of natural leaf drop, but research has disproven this. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter.

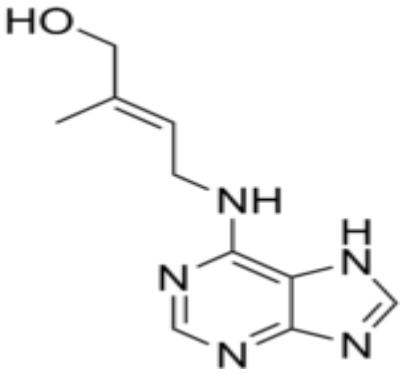
Functions of Abscisic Acid

a) In plants under water stress, ABA plays a role in closing the stomata.
b) ABA exists in all parts of the plant and its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones.
c) Plants start life as a seed with high ABA levels.
d) As plants begin to produce shoots with fully functional leaves, ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant.
e) Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells.

Other roles ABA:

- Induces bud dormancy
- Promotes senescence
- Accelerates leaf abscission in cotton plant
- Induces flowering during long days in certain short day plants - ineffective in short day plants.
- Counteracts GA

4-Cytokinins

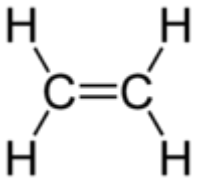


Cytokinins are synthesized in root tips, endosperm of seeds, and young fruits where cell division takes place continuously.Cytokinins work synergistically with auxin in the control of tissue and organ differentiation. Cytokinins are transported in the xylemtoward the shoot. Cytokinins are a group of chemicals that influence cell division and shoot formation. They have a highly synergistic effect with auxins, and the ratio of these two plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins, they in conjunction with ethylene promote abscission of leaves, flower parts, and fruits.Zeatin or isopentenyl adenineis a cytokinin and its name is derived from *Zea*, in which it was first discovered in immature kernels

Functions of Cytokinins

(a) They stimulate cell division, cell enlargement and cell differentiation.
(b) They prevent aging of plant parts.
(c) They inhibit apical dominance and help in growth of lateral buds into branches.
(d)They also help delay senescence of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth.

5-Ethylene



Ethylene

Ethylene is the only gaseous hormone in plants. It is found in ripening fruits, young flowers and young leaves. Ethylene is a gas that forms through the breakdown of methionine, which is present in all cells. Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness.
Ethylene affects cell growth and cell shape, when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object

making its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stem's natural geotropic response, which is to grow upright, allowing it to grow around an object. Ethylene affects fruit-ripening. Normally when the seeds are mature, ethylene production increases within the fruit, resulting in a favourable event just before seed dispersal.

Functions of Ethylene

(a) It induces ripening of fruits.
(b) It promotes senescence and abscission of leaf, and flowers.
(c) In cells it only increases the width not the length.
(d) Ethylene is also considered a growth inhibitor as it may have a role in causing bud dormancy, and it is involved with leaf abscission.
(e) It may determine sex in cucurbits (melon family).
(f) Stimulates formation of aerenchyma (gas transport tissue) in submerged roots and stems.

Experiment No.1. Ethylene, a simple organic gas, is produced in ripe fruits. Accumulation of the gas speed up theaging of plant cells and thus the ripening process of fruits. The old saying that one rottenapple spoils the whole barrel has scientific basis. Bananas, pears, kiwi, tomatoes, and otherfruits can be forced to ripen by exposing them to ethylene gas.

Objective

Students will observe effects of ethylene on ripening fruit.

Materials

i) an apple
ii) two green bananas from the same bunch
iii) two brown paper sandwich bags
iv) a knife

Procedure

1. Tightly seal a green banana in a paper bag.
2. In a second paper bag tightly seal a green banana with a slice of apple.
3. Observe the bananas daily for a week.

Anticipated Results

The cut apple produces and releases small amounts of ethylene gas. Ethylene gas plays a rolein aging of plant tissues and ripening fruit. The presence of ethylene causes fruit to ripen. Thebanana with the apple slice should ripen more quickly than the other.

Role of Phytohormones in RootHair Formation and Root Development

The plant hormone auxin plays important roles in the formation of root hairs. Normally, only one hair arises from a single hair cell because the initiation position of root hair is critically

controlled among the cells. However, the exogenous application of auxin moves the hair position to the rootward end of the cells. Besides an involvement in the process of root hair auxin also plays an important role in the elongation of root hairs (Cho *et al.*, 2007).

The main hormones (intrinsic stimuli) and respective pathways responsible for root architecture development include:

- Auxin – Auxin promotes root initiation, root emergence and primary root elongation.
- Cytokinins – Cytokinins regulate root apical meristem size and promote lateral root elongation.
- Gibberellins – Together with ethylene they promote crown primordia growth and elongation. Together with auxin they promote root elongation. Gibberellins also inhibit lateral root primordia initiation.
- Ethylene – Ethylene promotes crown root formation.

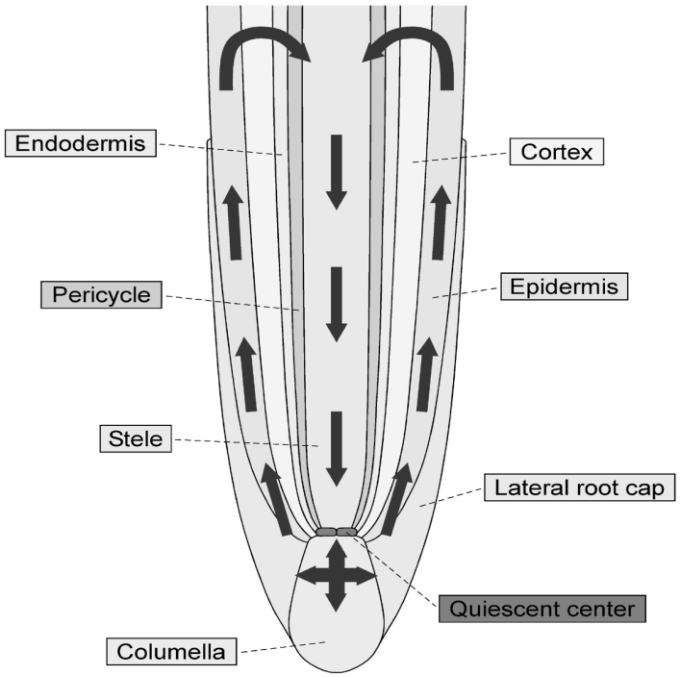


Fig. 7.1. Showing Different Zones of the Root during Development

Experiment for controlling the direction of growth

Auxin is a plant hormone responsible for controlling the **direction of growth** of root tips and stem tips in response to **different stimuli** including light and gravity.You may have noticed that a houseplant grows towards the window and turns its leaves towards the light. It does this because light coming from the window side of the plant destroys the auxin in that side of the stem. So growth on that side slows down.On the shaded side of the plant there is more auxin. So growth on this side is faster. The result in turning the shoots and leaves towards the light for **photosynthesis**.

- Cutting off the tip and replacing it shows that something produced in the tip is influencing the cells further down the shoot.

- The substance produced in the tip is a chemical that can diffuse through plant tissue or into agar gel. When you cut the tip off, the chemical is still produced and is collected in the agar gel. The chemical then moves out of the gel into the shoot and stimulates growth again. It could be called a 'growth hormone'.
- Normally the tip produces a hormone that spreads down to the cells below. The cells grow evenly all around the shoot and the shoot grows straight up.
- When light coming from one side, growth is faster on the side away from the light. For some reason, the concentration of hormone is less on the light side (or more on the dark side). The part of the shoot on the side away from the light grows more, so the shoot bends towards the light.

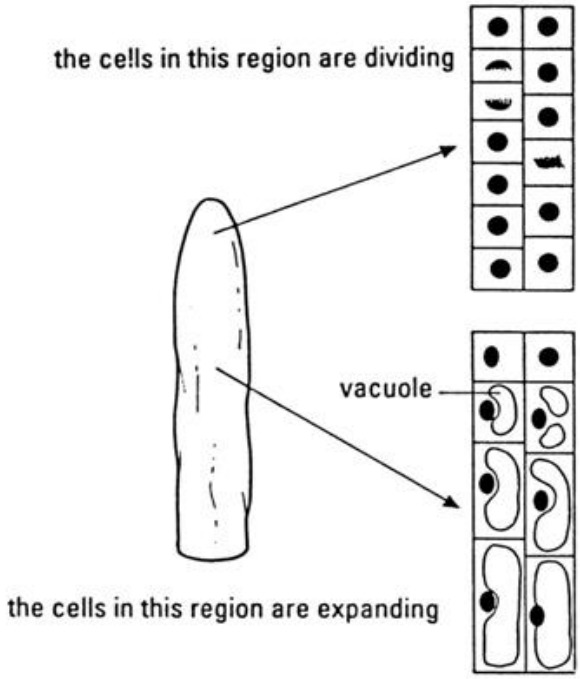


Fig.7.2. Showing How Cell Division Takes Place in Root Cells

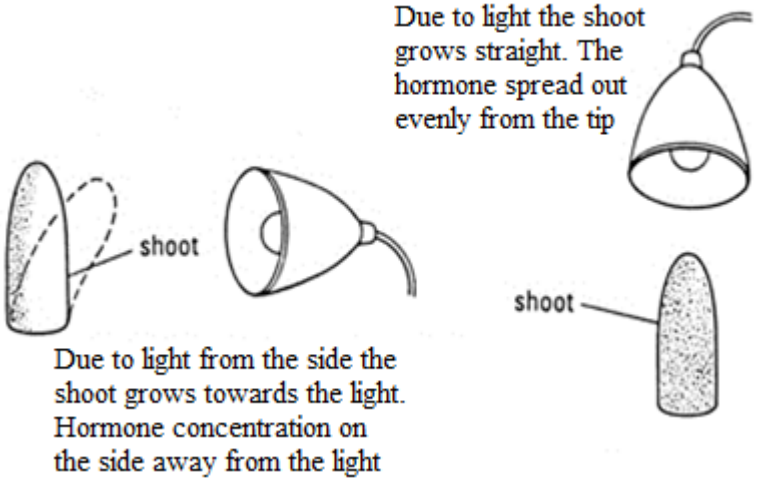


Fig.7.3. Response of Light in Development of Hormone and Growth

7.3 INFLUENCE OF PLANT HORMONES

7.3.1-Root development

Plant hormones are important biotic factors to regulate root growth. Among the seven kinds of plant hormones, auxin and gibberellin (GA) are strong accelerators of shoot growth, but these are not always accelerators for root growth. The endogenous concentration of indole-3-acetic acid (IAA) is inversely proportional to the growth rate. As massive IAA is transported from shoots to roots by polar transport, the influx speed of IAA mainly controls IAA levels in root cells.

Compared to auxins, GA functions in roots are less remarkable. Nevertheless, GA also plays an indispensable role in the normal development of roots. Another interaction of IAA and GA in growth regulation is the enhancement of GA_1 level by IAA.

Experiments on plant hormones

The purpose of this activity is:

- to evaluate an investigation of plant hormones
- to develop an understanding of how plant hormones control their growth

You might have noticed plants growing like those in this picture.

Fig.7.4. Response of Light in Development of Hormone and Growth

These seedlings are growing towards the light – their stems are bent and their leaves are facing the sunlight in a way that exposes as much of the leaf as possible to the light. They are showing a "growing-toward-the-light-tendency" which biologists call "positive phototropism". This is a case of plants responding to a stimulus – the stimulus is light and the response is how they grow.

Experiment-1

Procedure

The investigations described below are examples of work that has been done to try to work out how positive phototropism happens. The seedlings used in these investigations were cereals, such as barley. The shoots do not look the same as cress seedlings. They seem to have no leaves; but in fact the early shoots are closed tubes with long narrow leaves inside. This kind of shoot is called a **coleoptile**.

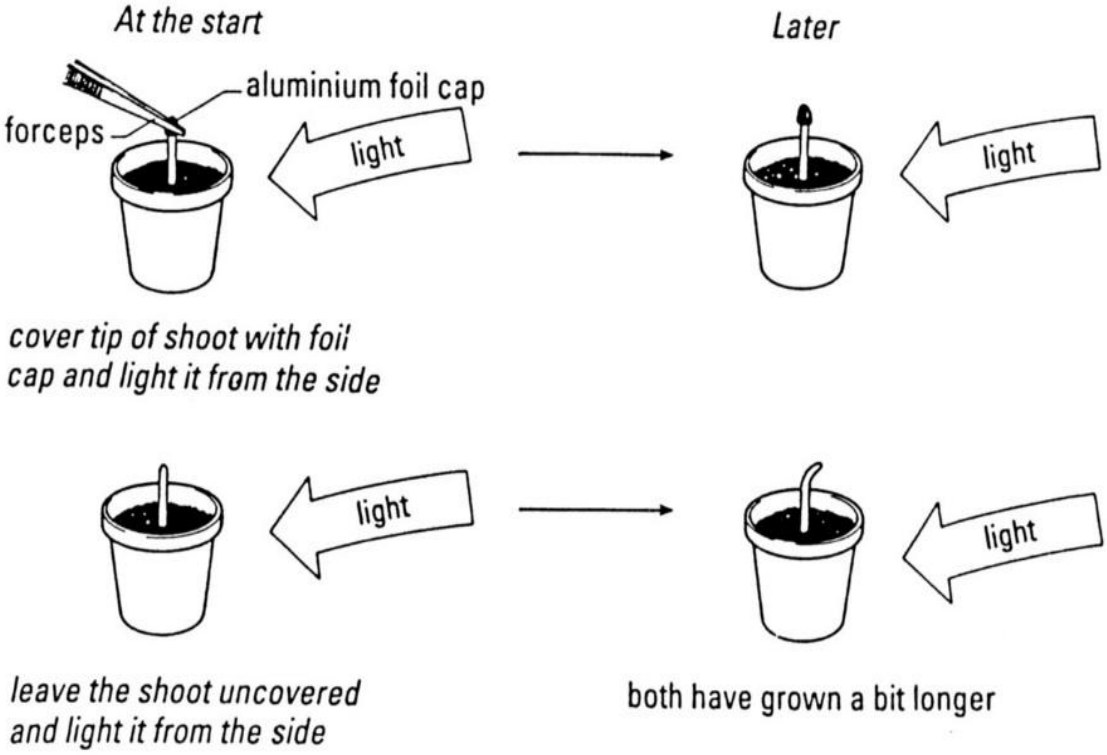


Fig.7.5. Response of Light in Development

a. Two shoots were used. The tip of one was covered with a foil cap. The other was left uncovered.
b. Both shoots were exposed to light from one side.

RESULT: Both shoots grow. One grows straight and the other grows towards the light.

Q.1.What does this investigation tell you about how plants respond to the stimulus of light from one side?

Experiment-2

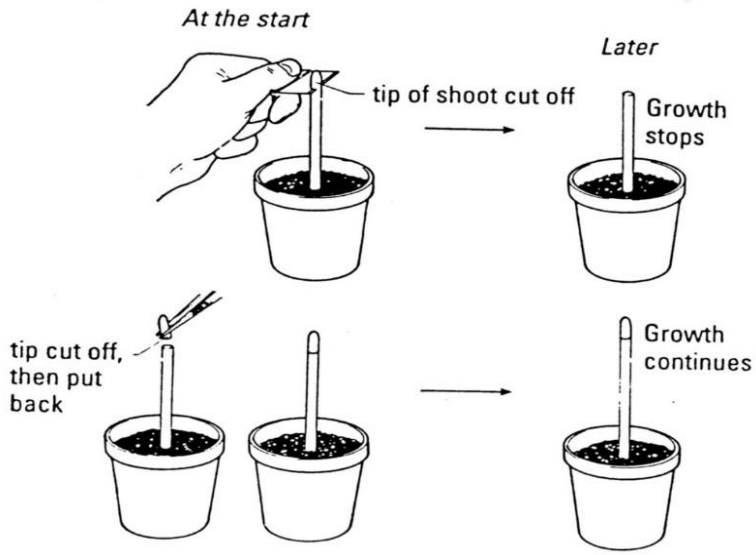


Fig.7.6.ShowingEffect of Hormone on Growth and Development

a. Two shoots were used. From one the tip was cut off and discarded. From the other the tip was cut off and then put back.
b. Both shoots were left to grow with light coming from all sides.

RESULT: The shoot with the discarded tip stopped growing. The shoot whose tip was replaced continued to grow.

Q. 2. What does this tell you about how the tip of a plant shoots control growth?

Experiment-3

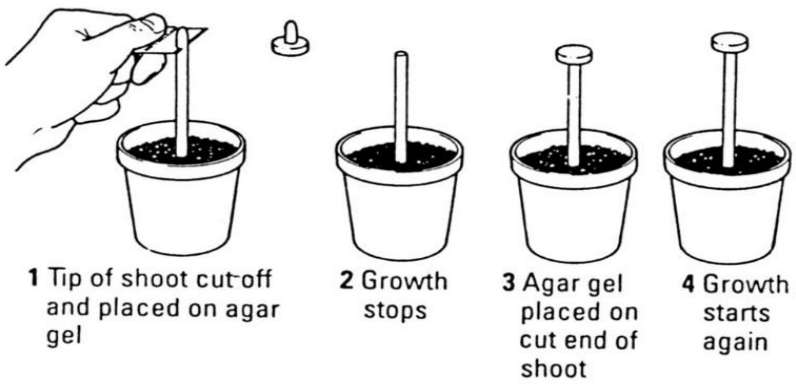


Fig.7.7.Showing Development of Hormone in the Apical Zone and Growth

As in experiment-2, the tip of a shoot was cut off, but this time placed on an agar gel.

RESULT: Growth of the coleoptile stopped.

a. Then the agar gel was placed on the cut end of the shoot.

RESULT: Growth started again.

Q. 3. How would you explain this result?

Experiment- 4

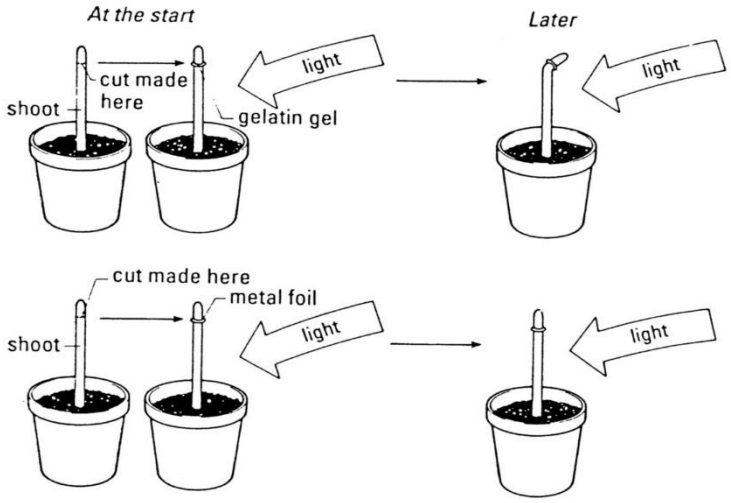


Fig.7.8. Response of Light in Development of Hormone and Growth

i) Two shoots were used. The tips of both were cut.
ii) On one cut coleoptile, a piece of gelatin gel was placed while on the other, a piece of metal foil was kept.
iii) The tips were put again and balanced on top of the gel or foil.
iv) Both shoots were exposed to light from one side.
RESULT: The shoot with the gelatin gel began to grow towards the light.

Q. 4. What does this result tell you about what might be happening within the shoot tip?

Experiment -5

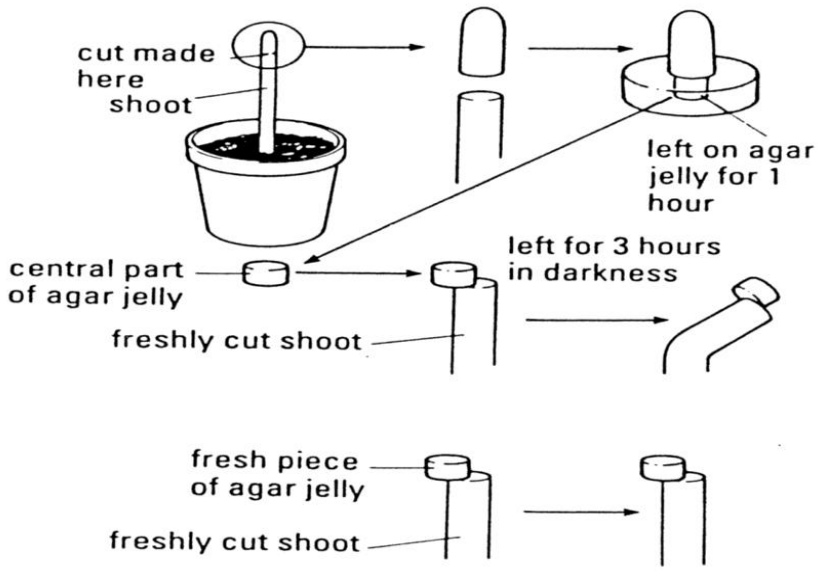


Fig.7.9. Response of Light in Development of Hormone and Growth

i) The tip of a shoot growing in the light was cut off and placed on agar jelly for an hour.
ii) Two more shoots were then cut to remove their tips. The part of the jelly from right under the first tip shoot was then placed on the edge of one cut shoot, and a fresh piece of jelly on the edge of another.
iii) Both were left in the dark for 3 hours.

RESULT: The shoot with fresh jelly did not bend. The shoot with agar from underneath a cut tip bent – with the side underneath the agar elongating compared to the other side.

Q. 5. How would you explain this result?

Answers:
Q. 1. The first experiment suggests that it is the tip of the plant that is sensitive to light. In the absence of light, the shoot keeps growing, but does not grow towards the light.

Q. 2. This tells us that the tip is important in keeping a plant growing. Cutting off the tip stops growth. But replacing it starts growth happening again. If there was an electrical connection (like a nerve impulse) between the tip and the rest of the shoot, cutting it off and replacing it would probably break the connection. So, the message from the tip is likely to be a chemical message (like a hormone) not an electrical message.

Q. 3. This tells us that something from the shoot tip can pass into agar gel and that something (probably a chemical messenger) can re-start growth in a cut shoot.

Q. 4. This tells us that something from the shoot tip (probably a chemical messenger) can pass through gelatin, but not through foil.

Q. 5. This tells us that something from the shoot tip can make a shoot grow unevenly if it is put on one side of the shoot only. So, if a chemical messenger is at a higher concentration on one side of the shoot than the other, the shoot will grow more on one side and bend. It could bend towards a stimulus (such as light). Perhaps, somehow, light changes the concentration of the chemical messenger and this is how the shoot responds to the stimulus.

7.3.2-Senescence

Plant senescence is the process of aging in plants. Plants have both stress-induced and age-related developmental aging. Chlorophyll degradation during leaf senescence is common. Leaf senescence has the important function of recycling nutrients. Senescence occurs due to the deposition of waste material. In some plants the whole plant dies after flowering and producing seeds. This is called whole plant senescence**.** In many other plants, parts above soil die each year and root system stays alive. This is called organ or shoot-senescence.Abscissic acid and ethylene promote senescence of leaves but cytokinin delays senescence and helps leaves remain green for longer period.
Programmed senescence seems to be heavily influenced by plant hormones. The hormones abscissic acid, ethylene, and salicylic acid are accepted by most scientists as promoters of

senescence.Cytokinins help to maintain the plant cell and prevents leaf senescence. Withdrawal of cytokinin, or if the cell cannot perceive the cytokinin, it may then undergo apoptosis or senescence.

7.3.3-Pollen Germination- Hanging Drop Method

Pollen germination and pollen tube growth are key events in the sexual reproduction of plants. After anthesis and pollination, pollen development continues in the stigma with rehydration, germination and pollen tube growth. Both pollen germination and pollen tube growth are dependent on gibberellins synthesized *in situ*. The influence of gibberellins on pollen germination and pollen tube growth has been verified by application to rice flowers (Oryza sativa). Exogenous applications of gibberellinshave also been reported to promote pollen germination and increase pollen tube length *in vitro* in apricot (*Prunus armeniaca*)

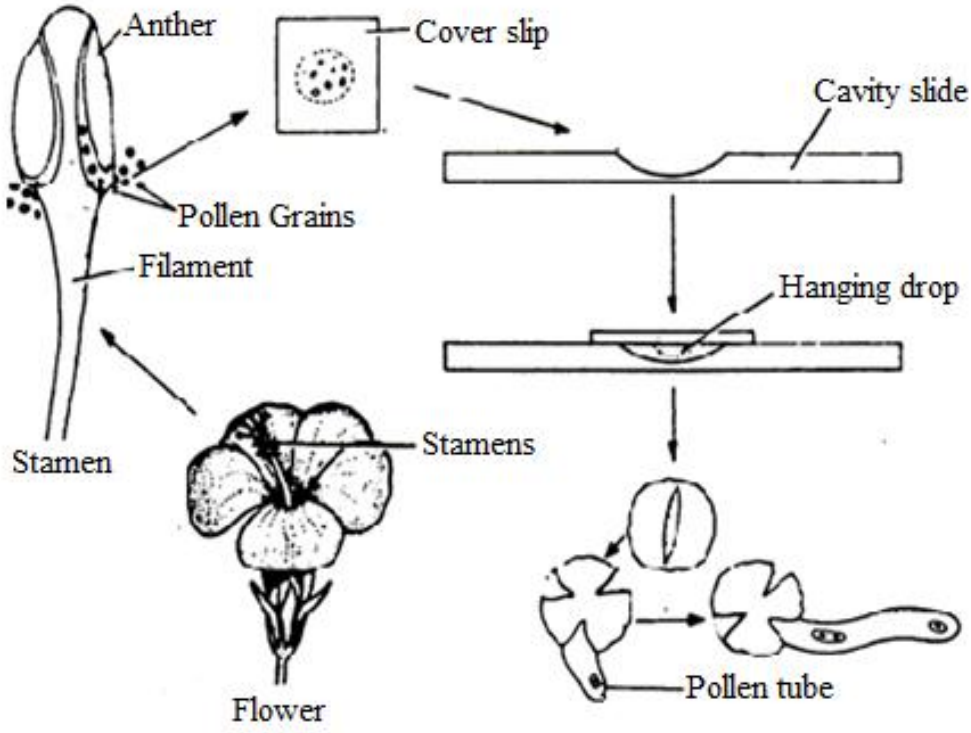


Fig. 7.10.Pollen Tube Germination (Hanging Drop Method)

Materials Required

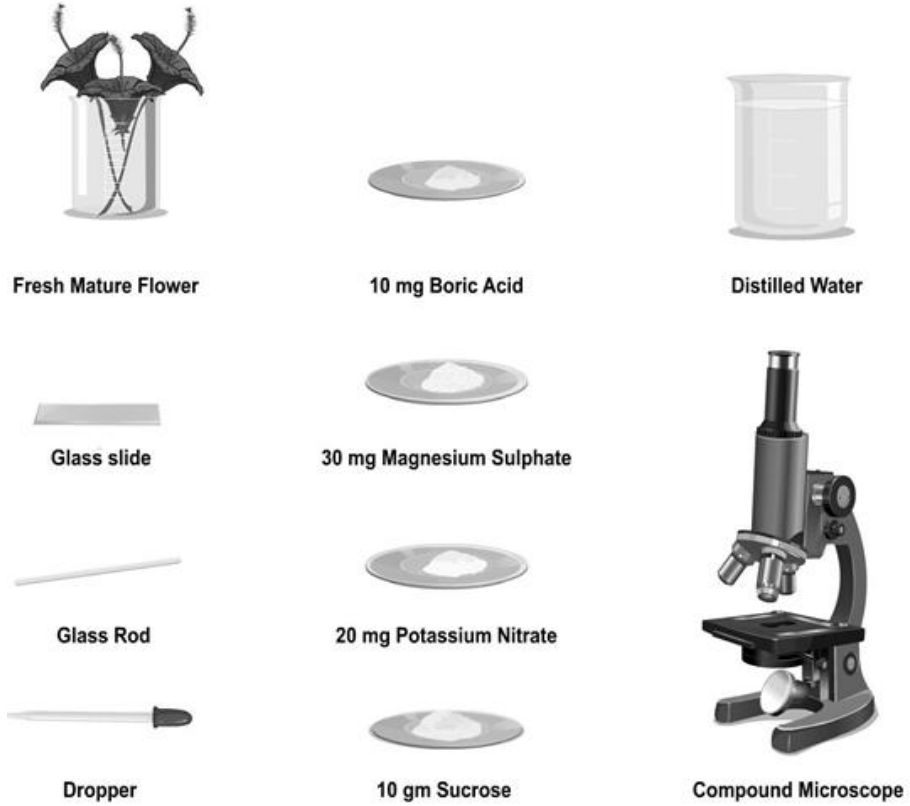


Fig.7.11 Requirements for Pollen Tube Germination

Lab Procedure

- Prepare the pollen germination medium by dissolving 10 grams sucrose, 10 milligrams boric acid, 30 milligrams magnesium sulphate and 20 milligrams potassium nitrate in 100ml of distilled water.
- Using a glass rod, stir the solution to mix it well.
- Using a dropper, take some nutrient solution and put two drops on a clean cavity slide.
- Take a mature flower and dust a few pollen grains from its stamen on to the drop on the cavity slide.
- Place the cover slip over the cavity.
- Allow the slide to remain as such for few hours.
- Remove the cover slip slowly and place on the clean glass slide.The lower side of the cover slip with germinated pollen grains can be seenunder the compound microscope.
- Observe the slide through the microscope regularly for about half an hour.

Observations

The pollen grain is uninucleate in the beginning. At the time of liberation, it becomes 2 celled, with a small generative cell and a vegetative cell.In the nutrient medium, the pollen grain germinates. The tube cell enlarges and comes out of the pollen grain through one of the germ pores to form a pollen tube. The tube nucleus descends to the tip of the pollen tube. The generative cell divides into two male gametes which are also seen in the pollen tube.

7.4 SUMMARY

1. Growth in living organisms results from increase in the number and size of a cell, organ or whole organism.
2. Development is the whole series of qualitative and quantitative changes (growth, differentiation, maturation), which an organism undergoes throughout its life cycle.
3. Plants show three phases of growth - Lag Phase, Log Phase, and Stationary Phase
4. Auxanometer is specially designed equipment used to measure the rate of growth of shoot length of plants.
5. The internal factors responsible for plant growth are auxin, gibberellins, cytokinins, ethylene, and abscissic acid. These are substances produced in a small quantity in one part of plant body and capable of moving to other parts to influence the growth of that part.
6. Florigen is a plant hormone, which is responsible for initiation of flowering in plants.
7. Senescence is a gradual process during which any plant part or the whole plant completely loses its function and ultimately dies.
8. The process of detachment of any leaves, fruits, flower or any part of the plant from the main body after getting older is called abscission.
9. Any change in the environmental conditions that may adversely affect the growth or development in plants is called biological stress. This stress occurs mainly due to temperature, water, salt, shade, light, and various pollutants.
10. During phototropism and geotropism, the plant hormone (auxin) controls cell elongation.
11. The plant hormone (cytokinin) promotes cell division, controlling many developmental processes in plants.
12. Gibberellins control many aspects including shoot elongation, seed germination, fruit and flower maturation, seed dormancy, gender expression, seedless fruit development, and the delay of senescence in leaves andfruits.

7.5 GLOSSARY

Auxin: Growth hormones that is responsible for elongation in phototropism and gravitropism and for other growth processes in the plant life cycle

Chemotropism: Plant growth response to a chemical.

Cytokinin: Plant hormones involved in cell growth and divisionanddelaythesenescence of leaves

Gibberellin: Plant growth hormones that stimulate shoots elongation, seed germination, and fruit and flower maturation.

Geotropism: Plant growth response to gravity.

Nyctinastic: Plant movements in response to the daily cycle of light and dark.

Photoperiodism: it is a plant's response to changes in the length of days and nights.

Phytochrome: Plants monitor changes in day length with a bluish, light-sensitive protein pigment called phytochrome.

Thigmonastic: Nastic movements that occur in response to touching or shaking a plant.

Vernalization: It is the low-temperature stimulation of flowering.

7.6 SELF ASSESSMENT QUESTION

7.6.1 Match the term with the correct definition:

a. abscissic acid	f. gravitropism
b. auxins	g. phototropism
c. cytokinins	h. plant growth regulators
d. ethylene	i. thigmotropism
e. gibberellins	j. growth retardant

1. A colorless gas that speeds the aging of plant parts, particularly fruit
2. A group of hormones that have a primary role in promoting cell elongation
3. A plant's response to the source of light
4. Inhibit cell elongation and keep plants compact
5. A response to mechanical stimuli
6. A plant response to gravity
7. Natural occurring or synthetic chemicals that regulate plant growth and development
8. A growth-inhibiting hormone largely responsible for seed dormancy
9. Plays a key role in the development of flowers and in the production of enzymes during seed germination
10. Hormones responsible for cell division and differentiation

7.6.2 Multiple choice question answers:

1. What term is used to describe stretching of a plant due to low light?

(a) apical dominance (b) elasticity
(c) etiolation (d) phototropism

2. What is a plant response to external stimuli?

(a) abscission (b) reaction
(c) symbiosis (d) tropism

3. What is the meaning of apical dominance?

(a) The apical meristem has dominance over the lateral buds.
(b) The lateral meristem controls growth above.
(c) The meristem tissue takes over surrounding tissues.
(d) The vegetative growth is dominant over root growth.

4. What is a common synthetic root-promoting materials used in the propagation of plants?

(a) Banvel (b) Indoleacetic acid (IAA)
(c) Indolebutyric acid (IBA) (d)None of the above

5. Which plant hormone encourages the growth of lateral shoots, inhibits the branching of the roots, and is an ingredient in tissue culture medium?

(a) auxins (b) cytokinins
(c) ethylene (d) gibberellins

7.6.3 Short Answer Questions

1. List five plant hormones.
2. Define the three plant tropisms.
3. Give five examples of how plant growth regulators are used in the agricultural industry.

7.6.1 Answers Key:

Matching

1. d
2. b
3. g
4. j
5. i
6. f
7. h
8. a
9. e
10. c

7.6.2 Answer Key (Multiple Choice)

1. (c), 2. (d), 3. (a), 4. (c), 5. (b)

7.6.3 Answer Key (Short Answer Questions)

1. Auxins, gibberellins, cytokinins, abscisic acid, ethylene

2. Phototropism is a plant's response to the source of light. Auxins move down the shaded side of the plant stem. The presence of auxins causes the cells on the shady side of the stem to elongate more than cells than the bright side of the stem. The result is a stem that bends towards the light. Under low light or dark conditions cells elongate on all sides of the stem. The result is an appearance of the stem stretching.

Stretching due to low light is known as etiolation. Gravitropism, also referred to as geotropism, is a plant response to gravity. The stems of plants laid on their side curve upward. In this scenario auxins settle to the bottom side of the stem and cause cells to elongate. Thigmotropism is a response to mechanical stimuli. A good example is the tendrils of a cucumber plant. When the tendrils touch an object, the response is to curl around that object.

3. Auxins in the forms of indoleacetic acid (IAA) and indolebutyric acid (IBA) are widely used to speed the rooting of cuttings in the horticulture industry. Naphthaleneacetic acid (NAA) is sprayed on apples to prevent pre-harvest drop of the fruit. Gibberellins are used to induce flowering. Some plants that respond by flowering are carrots, endive, cabbage, turnips, and chrysanthemums. Gibberellins serve as growth stimulants making plants, including sugar cane, grow larger. Gibberellins are used with some plant species, such as

grapes, to produce larger fruit. Cytokinins have been shown to extend the shelf life of lettuce. Cytokinins are also an important ingredient of tissue culture medium, as they promote cell division. Ethylene is used in the ripening of fruits before being placed on grocery shelves. Growth retardants are widely used in the horticulture industry to keep plants compact. Growth regulator herbicides disrupt hormone balance and protein synthesis.

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7.9 TERMINAL QUESTIONS

1. What is auxin? What is its role in the growth of plants?
2. State any two functions of Gibberellin?
3. Explain the role of Cytokinins and Ethylene in growth and development of plants.
4. What is senescence?
5. State any two practical utilities of growth hormones.
6. What is apical dominance? Name the hormone responsible for it.
7. What is meant by plant movement? Describe any two types of movement of plants with example.

UNIT-8 STRUCTURE, ORGANIZATION OF THE SHOOT, AND ANATOMY

8.1 OBJECTIVES

After reading this unit students will be able:

- to explain the stem anatomy of *Hydrilla*, an aquatic plant sps.
- To know morphological and anatomical features of *Ranunculus*sps.
- to describe the stem anatomy of *Euphorbia*sps.
- to understand ecologicaland anatomical differences among *Hydrilla, Ranunculus* and *Euphorbia.*

8.2 INTRODUCTION

The plants which characteristically grow in certain environment often show the structure which is believed to be adapted to that particular environment. In the course of evolution, many species have become adapted in their structural and physiological features to habitats either with an excessive water or deficiency in water.

Plants that live wholly or partly submerged in water or in very wet places are known as hydrophytes while the larger numbers of plants grow under average conditions of moisture and temperature. Plants of habitat that usually show neither an excess nor a deficiency of water are known as mesophytes.Plants that grow in places where the evaporation stress is high and the water supply is low are known as xerophytes.Mesophytes are therefore, intermediate between hydrophytes and xerophytes.

In this section we have chosen one hydrophyte (*Hydrilla*), onemesophyte (*Ranunculus*) and one xerophyte (*Euphorbia*) to study the anatomical detailswith variation in stem anatomy.

Hydrophytes are characterized by large number of air chambers within the tissue of stem and leaves which help them in buoyancy as well as storing oxygen.Root system, vascular tissue, stomata etc are poorly developed. Cuticle layer in leaves is mostly absent.Thick walled tissue is altogether absent.

In mesophytes the root system is well developed with the tap-root system and branched in dicotyledons, while a cluster of fibrous roots in monocotyledons; root hairs are abundantly produced for the absorption of water from the soil.

In mesophytes the stem is solid, erect and normally branched. All the different kinds of tissues, particularly the mechanical and conducting tissues have reached their full development. The aerial parts of plants such as the leaves and the branches are provided with cuticle. In dorsiventral leaves the lower epidermis is provided with numerous stomata, there are few stomata or none at all on the upper surface.

In erect leaves of most monocotyledons, stomata are more or less equally distributed on both surfaces. The stomata are relatively uniform in structure and the guard cells show a maximum

capacity for movement. The anatomy of mesophytic plants is quite normal and no special adaptations are found in them.

Xerophytes growing in very dry places can withstand a prolonged period of drought uninjured, and for this purpose they have certain peculiar adaptations. The xerophytic plants have to guard against excessive evaporation of water, by reducing evaporating surfaces. Plants produce a long tap root which goes deep into the sub-soil in search of moisture. To retain the water absorbed by the roots, the leaves and stems of some plants become very thick and fleshy (e.g., *Aloe, Agave*). Multiple epidermis sometimes develops in the leaf (e.g., *Nerium*). In xerophytes certain structural features are also common. Leaves are thick and leathery, with a well-developed cuticle and abundant hairs. Well differentiated mesophyll and more than one layer of palisade tissue (e.g., *Nerium*). The walls of epidermal and sub-epidermal cells are frequently lignified, and a distinct hypodermis, may be present. They have a well-developed vascular system and often an abundance of sclerenchyma, either in the form of sclereids or fibres. The leaf is sometimes cylindrical or rolled. This organization is to protect the stomata, which may occur in furrows.

8.3 STRUCTURE, ORGANIZATION OF THE SHOOT AND ANATOMY OF-

8.3.1-*Hydrilla verticellata*

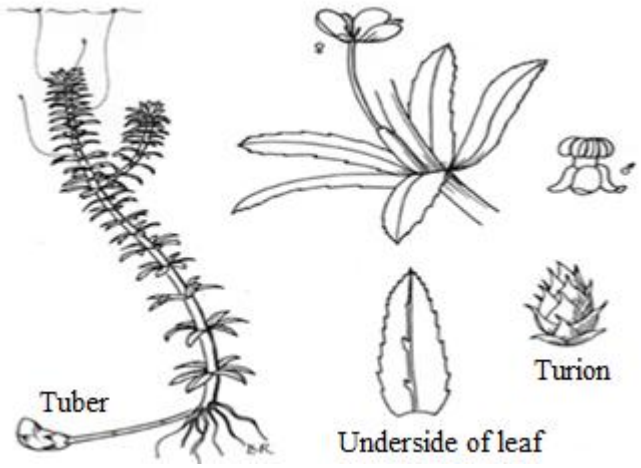


Fig.8.1 *Hydrilla* Plant Morphology

Hydrilla (Waterweed) is a genus of aquatic plant, usually treated with one species, *Hydrilla verticillata*. The stems grow up to 1–2 m long. The leaves are arranged in whorls of two to eight around the stem, each leaf 5–20 mm long and 0.7–2 mm broad, with serrations or small spines along the leaf margins; the leaf midrib is often reddish when fresh. It is monoecious (sometimes dioecious), with male and female flowers produced separately on a single plant; the flowers are small, with three sepals and three petals, the petals 3–5 mm long, transparent

with red streaks. It reproduces primarily vegetatively by fragmentation and by rhizomes, and flowers are rarely seen. They have air spaces to keep them upright.

The inflorescences are unisexual, arising from spathes situated in the leaf axils; each flower has three sepals and three petals. All six perianth parts are clear or translucent green (the sepals usually slightly reddish).The male spathe is about 1.5 mm long, solitary in the leaf axils, somewhat spiny. The female spathe is about 5 mm long, solitary in the leaf axils. There are three petals, three stamens and three styles. The ovary is cylindrical to narrowly conical and is enclosed in the base of a hypanthium; the style is as long as the hypanthium and there are three stigmas.

Chambers and passages filled with gases are commonly found in the leaves and stems of hydrophytes. The air chambers are large, usually regular, intercellular spaces extending through the leaf and often for long distances through the stem (e.g., *Hydrilla, Potamogeton, Pontederia*).

The spaces are usually separated by partitions of photosynthetic tissue only one or two cells thick. The chambers prepare an internal atmosphere for the plant. These air- chambers on the one hand give buoyancy to the plant for floating and on the other they serve to store up air (oxygen and carbon dioxide).

Here, very thin partitions enclose air spaces and the whole structure consists of very feeble tissue. Aerenchyma is phellem formed by a typical phellogen of epidermal or cortical origin. At regular intervals individual cells of each layer of phellem elongate greatly in the radial direction while the other cells of this layer remain small.

However, the term aerenchyma is applied to any tissue with many large intercellular spaces, but such aerenchyma is quite distinct from the typical aerenchyma mentioned above which is of secondary origin.

Stem Apex of *Hydrilla*

Fig.8.2. Stem Apex of Hydrilla Showing Bud Development

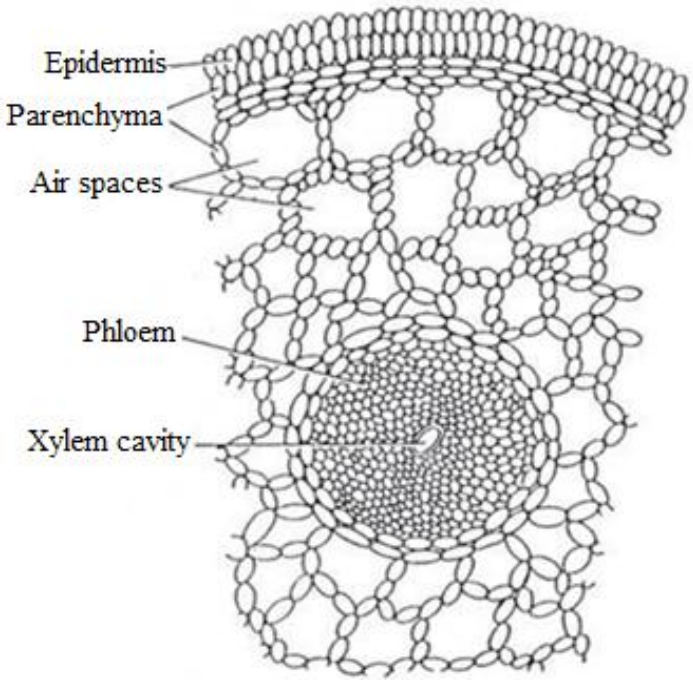


Fig. 8.3.T.S. of *Hydrilla* Root Showing Air Spaces in Cortex and Single Xylem Cavity

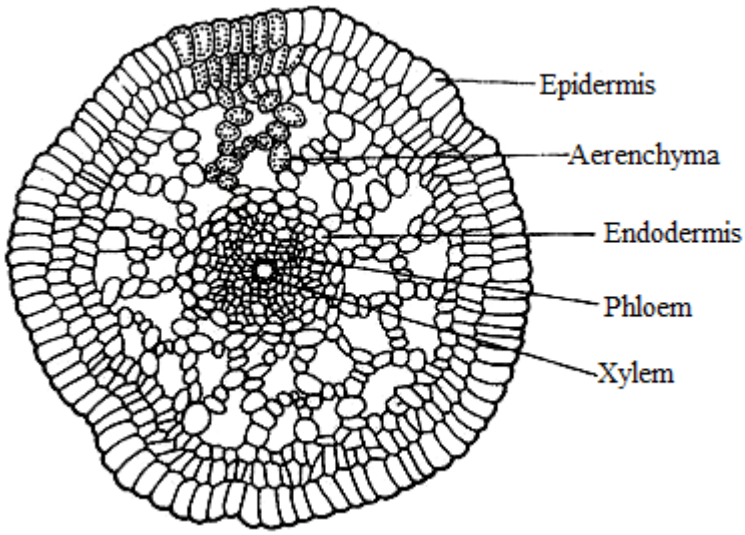


Fig. 8.4.T.S. of *Hydrilla* Stem Showing Aerenchyma

- Stem is usually weak and flexible. Sometimes it is covered by a gelatinous sheath which serves as protection against periodic desiccation.
- The cuticle is either altogether absent or very poorly developed. The epidermis is always single-layered and thin-walled; this character facilitates direct absorption of gases and mineral salts dissolved in water.
- The cortex is very broad and occupies bulk of the stem. The outer layers of the cortex are parenchymatous and usually without inter-cellular air spaces, whereas the inner cortex is aerenchymatous and possesses symmetrically arranged large air spaces. The air filled in

these cavities adds to the buoyancy of the plant and secondly facilitate the exchange of gases during respiration and photosynthesis.

- The cells of the cortex contain chloroplasts and assist in carbon assimilation.
- Usually there is no marked distinction of endodermis and pericycle. Sometimes the inner-most layer of the cortex is regarded as endodermis.
- Vascular tissue is poorly developed and does not show marked differentiation of phloem and xylem. An air cavity is mostly present at the center of the vascular strand that adds to the buoyancy of the plant. Sometimes, xylem is represented by a single strand present in the center of the stele (e.g., *Hydrilla, Potamogeton, Elodea* etc.)
- There is no mechanical tissue present in the stem of the submerged plant. Water column itself provides mechanical support to the plant.

8.3.2-*Ranunculus scleretus*

Ranunculus sceleratus is an annual herb growing up to half a meter tall. The leaves are more or less glabrous (hairless) and have small blades each deeply lobed or divided into three leaflets. They are borne on long petioles. The flowers are 5-10mm across with five or fewer yellow petals a few millimetres long and reflexed sepals. The fruit is an etario ofachenes borne in cluster.

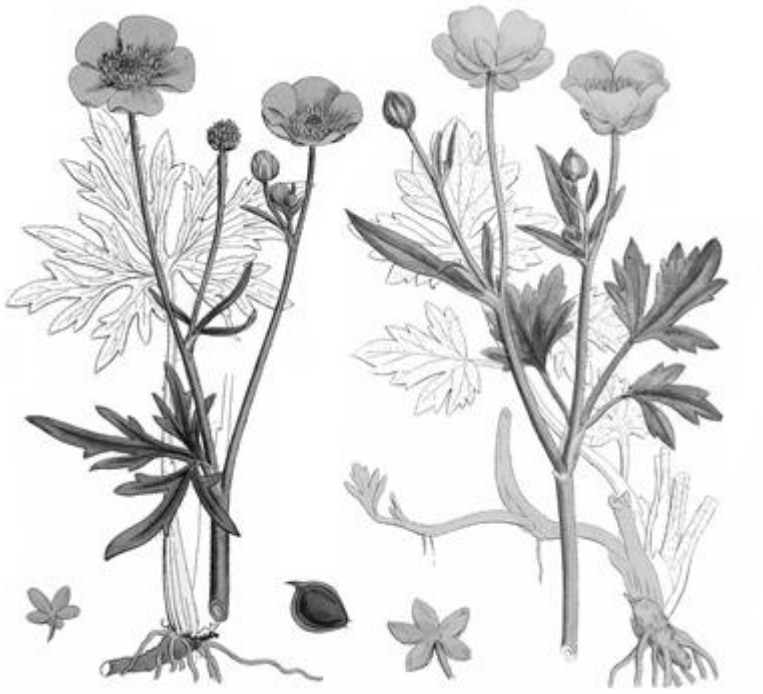


Fig. 8.5 Morphology of *Ranunculusscleretus*

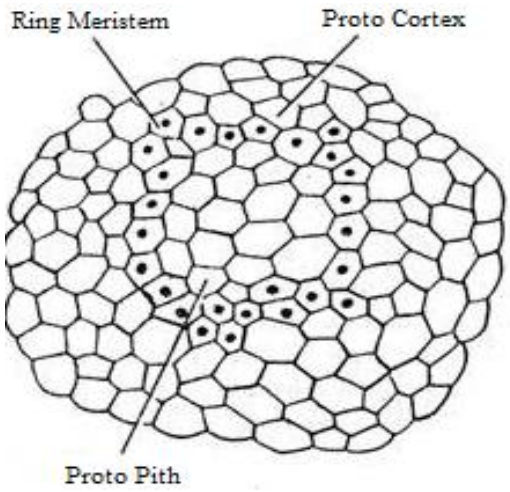


Fig.8.6 T.S.Through Shoot Apex of *Ranunculus* sp

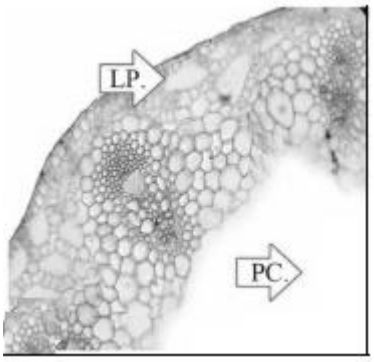
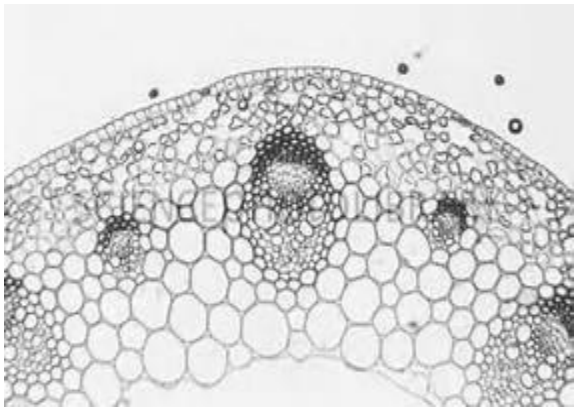
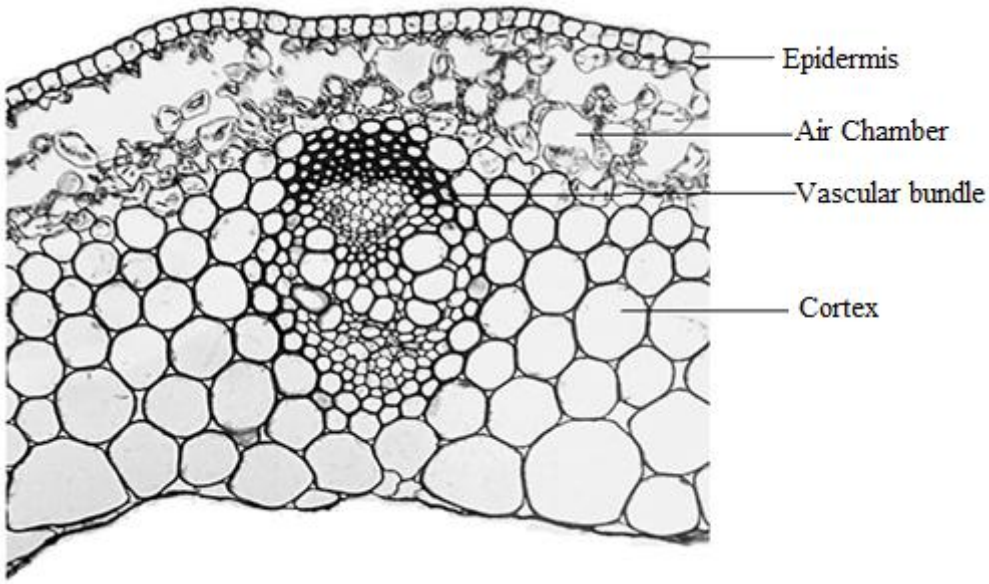


Fig.8.7 T.S. Through Stem of *Ranunculus*sps. Showing Lacunate Parenchyma (LP) & Pith cavity (PC)



(A)



(B)

Fig. 8.8 Stem T.S. of *Ranunculus scleretus* (A&B)

In this low magnification image of a *Ranunculus* stem, attributes of a dicot stem can be viewed. This dicot stem contains vascular bundles arranged in a concentric ring. This is somewhat similar to the arrangement of vascular tissues in a monocot root.

- The epidermis appears thicker due to a cuticle or waterproofing layer. Trichomes and stomata may be present.
- The cortex is made up of the multiple layers of cells between epidermis and pericycle. There are three sub-zones in the cortex. The outer sub-zone is called hypodermis. The hypodermis is composed of a few layers of collenchyma. The middle layer is composed of thin-walled parenchyma with distinct air spaces called aerenchyma. The innermost layer is called endodermis.
- Endodermal cells are rich in starch grains and hence this layer is also called the starch sheath. Pericycle is present on the inner side of endodermis and above the phloem. The pericycle is in the form of semi-lunar patches of sclerenchyma.
- A large number of vascular bundles are arranged in a ring. It is important to remember that the ring-like arrangement of vascular bundles is the characteristic of dicot stem. Each vascular bundle is conjoint, collateral and open. Protoxylem is endarch. Each vascular bundle is capped by sclerenchymatousfibers.
- Usually pith is composed of rounded parenchymatous cells; with large intercellular spaces but in case of *Ranunculus* it is hollow. The deepest parenchyma cells of this stem are fragmented during growth to produce the hollow pith area.

8.3.3-*Euphorbia hirta*

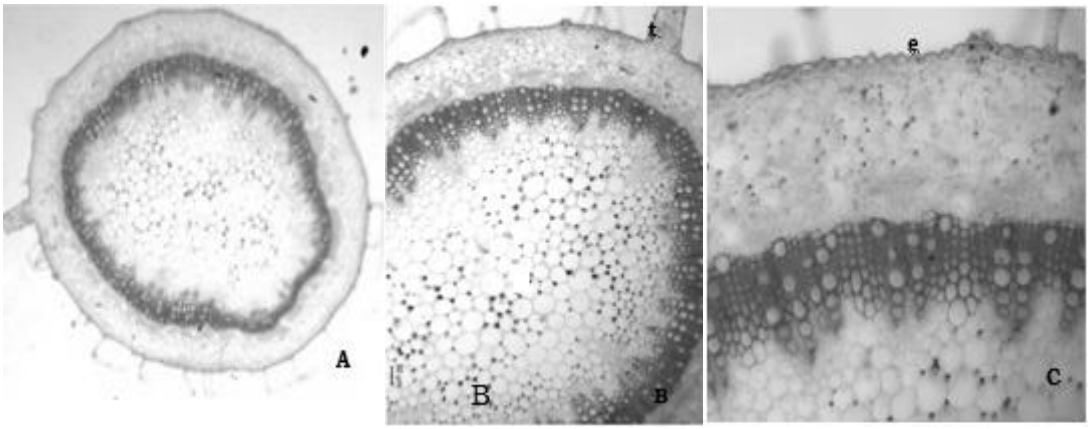
Euphorbia hirta L. belongs to the family Euphorbiaceae, widespread at low altitudes throughout the tropics and subtropics. It prefers sunny to lightly shaded dry conditions. It is an early colonizer of bare ground. *E. hirta* is one kind of weed in cultivated fields of

perennial crops, grasslands, roadsides, gardens, lawns, fallow lands, ditch banks and waste places. It is a slender- stemmed when mature, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in colour.*Euphobiahirta* is used in the treatment of gastrointestinal disorders, bronchial and respiratory diseases, and in conjunctivitis. Hypotensive and tonic properties are also reported in *E. hirta*.

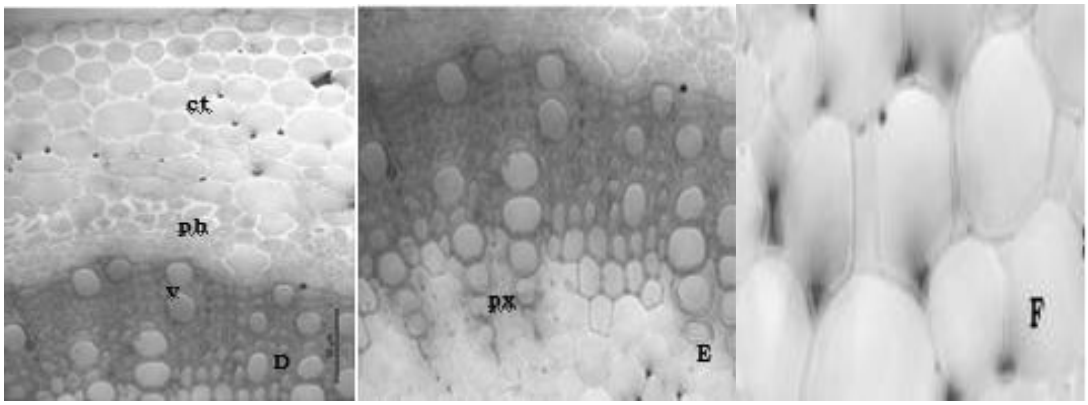
Fig.8.9 Morphology of *Euphorbia hirta* Plant

Macroscopic characters of *E. hirta* leavesshows composition of leaf is simple with dark green color having no odour, about 2-6cm. long in size, shape is ovate, texture is hairy, apex is acute and midrib is distinct on both the sides. T.S. of leaf revealed the presence of stomata on upper and lower epidermis. Powder characteristics revealed the presence of starch granules.

Stem Anatomy:



A. T.S. of Stem B. One Portion Enlarged C. Cortical Region Enlarged



D. Cortical Region E. Vascular Region Showing Protoxylem F. Enlarged View of Pith

Fig. 8.10: Anatomy of *Euphorbia hirta Stem at Different Places*

- Cross section of the stem wasgenerally circular shape.
- Covered with thickcuticle layer, epidermis was uniseriate with epidermal cells elongated, compactly arranged, bearingunicellular and multicellular trichomes (Fig.8.10 B).
- The thickness of the cuticle is greater than normal, like that of plants of semi- xerophytic habitats.
- Cortex was distinctly formed inabout 10-12 rows. Both the parenchymatous and chlorenchymatous cells were present in cortex. The cellswere rich in chloroplasts, therefore it was chlorenchyma.
- Laticifers were also present in the cortex zone.
- The genus *Euphorbia* has got wavy central cylinder usuallybecause of the bundle cap which prolongated and projectedalong the phloem positions. This waving may be initiated bytwo ways:

1) Variations in the vascular bundle positions, there are internal vascular bundles and external ones which alternate with one another.

2) Variations among bundle capdiameters, bundle cap of one isprojected out and the other in.

- In *E. hirta*, the centralcylinder was slightly wavy, wide and with peripheral positionbecause of their similar size and regular positions of the bundlecaps.
- Tracheary elements of wood resembled with vessels and tracheids, which were in radial rows.
- Xylem parenchyma was distinct within thewood. Wood arms (xylary arms) projected clearly toward thepith that will be single, double or triple.
- The phloemwas in external position and usually surrounded by thickfibrous tissue which resemble the bundle caps.
- The phloemwas differentiated by 4-6 narrow parenchymatous layers.
- The sieve elements and other cells of phloem were distributedregularly between the bundle caps and xylem, and there werefew fibers, or these cell elements present as an islands betweenthe bundle cap fibers.
- The pith has adistinct gap or central cavity at maturity.
- At theimmature stage, the pith cells were big, with thin walls anddistinct intercellular spaces. Pith cells were similar in shapeusually spherical or polyhedral.

8.4 SUMMARY

Let's take a look at the anatomy of dicotyledonous and monocotyledonous plants. In this section we have discussed *Hydrilla* a monocot, *Ranunculus* and *Euphorbia* spp. the dicots

Hydrilla stem is usually hollow with no secondary growth. The anatomy of monocot and dicot stem are similar, however some notable differences are as follows:

- The hypodermis of the cortex in monocots is made of sclerenchymatous cells.
- Vascular bundles are numerous but scattered, conjoint and closed, surrounded by the ground tissue.
- Phloem parenchyma is absent.
- Plant shows hydrophytic characters as the vascular tissue is poorly developed and does not show marked differentiation of phloem and xylem. An air cavity is mostly present at the center of the vascular strand in addition to air chambers in the cortical region that adds to the buoyancy of the plant.

Dicotyledonous stem of *Ranunculus* and *Euphorbia* is usually solid. The transverse section of their stems consists of the following parts:

- Epidermisis the outermost protective layer which is covered with a thin layer of cuticle.
- Epidermis possesses trichomes and a few stomata.
- Cortex is multi-layered sandwiched between epidermis and pericycle.
- The outer hypodermis, the middlecortical layers and the inner endodermis together make the three subzones of cortex.
- Next to endodermis is the pericycle which is constituted of semi-lunar patches of sclerenchyma.
- Ring arrangement of vascular bundles is present (only in dicot stem).
- Vascular bundle is conjoint, collateral and open with endarch protoxylem.
- Pith is evident and is made of parenchymatous cells but hollow in *Ranunculus*.

8.5 GLOSSARY

Adaxial Surface-The upper surface of a leaf, harvests light. This side is closer to the meristem in the leaf primordia.
Aerenchyma- Parenchyma with large intercellular air spaces.
Angular -Ridged along its length, these ridges appearing as angles in the cross-section.
Antrorse -Projecting forwards; used for an arrangement of hairs, the anther or less commonly the column wings.
Apex -The tip or end.
Bilocular -With two cavities or locules.
Bisexual -Both male and female sexes present.
Bristly - With stiff hairs or bristles.
Dimorphic -Existing in two different forms.

Dimorphism -The non-flowering plants are strikingly different to the flowering plants.
Dissected -Deeply divided into segments.
Distal - Away from the base towards the apex.
Distichous -In two ranks; usually applied to the arrangement of leaves or flowers.
Dorsal -The upper or outer surface or edge.
Epidermis -The outermost layer of cells covering the leaves.
Mesarch - A type of xylem maturation in which the protoxylem is embedded in the metaxylem and development proceeds both centripetally (from the outside in) and centrifugally (from the inside out); compare to endarch and exarch
Mesophyll -Parenchyma tissue between the upper and lower epidermis of a **leaf**
Metaxylem -Type of primary xylem that differentiates and matures later than the protoxylem; generally metaxylem tracheids are longer than protoxylem
Parenchyma -The most common type of plant cell; thin-walled cells varying in size, shape, and function
Periderm -A tissue primarily consisting of cork cells; outer bark
Phloem -Photosynthate conducting tissue of vascular plants
Pith -The central parenchymatous tissue in a vascular plant axis
Prostrate - Lying flat.
Proximal - Situated near the point of attachment.
Sessile - Without a stalk, pedicel or petiole.
Sheath - The base of a leaf or bract which embraces a bud or axis.
Shoot -A horticultural term used by growers for a new growth.
Terminal - The apex or end.
Vascular -Said of plants which have water-conducting tissue.

8.6 SELF ASSESSMENT QUESTIONS

8.6.1Objective type Questions:

1. A bicollateral vascular bundle is characterized by
(a) phloem being sandwiched between xylem (b) transverse splitting of vascular bundle
(c) longitudinal splitting of vascular bundle (d) xylem sandwiched between phloem.

2. A narrow layer of thin walled cells found between phloem/bark and wood of a dicot is
(a) cork cambium (b) vascular cambium
(c) endodermis (d) pericycle

3. Casparian strip occurs in a
(a) endodermis (b) exodermis
(c) pericycle (d) epidermis

4. Vascular bundles in a dicot stem are
(a) Open, collateral, exarch (b) Closed, collateral, endarch

(c) Closed, collateral, exarch (d) Open, collateral, endarch

5. Annual rings are distinct in plants growing in
(a) Temperate regions ` (b) Tropical regions
(c) Grasslands (d) Arctric region

6. The lateral roots generally originate in
(a) Cork cambium (b) cortex
(c) pericycle cells lying against protoxylem (d) Endodermal cells lying against protoxylem

8.6.2 Fill in the blanks:

1. The inner most layer of the cortex is distinct and well developed in primary roots. It is called_____.
2. A band of suberin develops all around the cell in the middle of the transverse and radial walls. This suberin band is called _____ strip.
3. The outer most part of the stele consists of one or more layers of parenchymatous cells. The outer layer of this parenchyma is called _____
4. In case _____ xylem is present towards the inner side and phloem is present towards the outer side of vascular bundle.
5. In case_____ , phloem is present on both side of xylem.
6. In case of_____ bundles, on type of vascular tissue (xylem or phloem) completely surround the other type of tissue.
7. Vascular bundles having cambium between xylem and phloem are called _____ type.
8. A narrow strip of meristematic cells is present between the xylem and phloem in the vascular bundles of dicots and gymnosperms. This strip of meristematic cells is called vascular……..
9. The phellogen produces a group of loosely placed cells at certain points. These loosely placed cells are called ____
10. The tissues in which the cells are undifferentiated and capable of division are called _____

8.6.1 Answers Key: 1. (d), 2.(c), 3. (a), 4. (d), 5. (a), 6.(c)

8.6.2 Answers Key: **1.** Endodermis, **2.** Casparian, **3.** Pericycle,
4. Collateral, **5.** Bicollateral, **6.** Concentric**, 7.** Open, **8.** Cambium, **9.** Lenticels,
10. Meristem

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8.9 TERMINAL QUESTIONS

8.9.1. Short Answer type Question:

1. Describe the cortex of *Hydrilla*.
2. Explain the vascular bundles in *Hydrilla.*

3. Describe waving in central cylinder of *Euphorbia* stem.
4. Is there any similarity in *Ranunculus* stem and monocot root anatomy?
5. Define the anatomical difference in vascular bundles of *Hydrilla* and *Euphorbia.*

8.9.2 Long Answer type Question:

1. Draw a well labeled diagram of *Hydrilla* stem T.S. and describe in detail.
2. Draw and comment on the anatomical features of *Euphorbia* stem.
3. How *Ranunculus* shows some specific features in its stem anatomy? Describe and draw its anatomical features.

BLOCK: PLANT ECOLOGY AND BIOSTATISTICS

UNIT-9 DETERMINATION OF THE MINIMUM SIZE AND NUMBER OF QUADRATS BY SPECIES AREA CURVE METHOD FOR THE COMMUNITY ANALYSIS OF THE GIVEN AREA

9.1 OBJECTIVES

After reading this unit students will be able -

- To determine the minimum size of quadrat by species curve method.
- To determine the minimum number of quadrats to be laid down for vegetational analysis of the given area.

9.2 INTRODUCTION

Vegetation, as you know, includes all plants of an area. It is made-up of small groups of populations which forms a community. Study of entire plant community or segment thereof cannot be measured, even if it is small. Therefore, we have to depend on samples, based on sampling methods drawn from a community to approximate the structure of entire community. So we have to select the samples cautiously in such a way that the data so generated could be utilized to estimate the nearly true value as accurately as possible. The minimum size and minimum number of samples are determined to get the accuracy of data and to avoid the waste of energy of the worker involved in analysis. Mainly three types of sampling methods are used for vegetation study. These are: Quadrat, Transect and Point methods. Of these, quadrat method is commonly used for vegetation sampling.

We must know what is a quadrat? It is a sampling unit of varying shapes and sizes, the selection of which depends on convenience and usefulness. It can be rectangular, circular or square. Compared to rectangular and circular quadrats, square quadrat is preferable. Though square quadrats are often used, rectangular quadrats are also better because most plant distributions are clumped, and a rectangle can best encompass patches of different species. But a rectangular quadrat should not be more than two to four times as long as it is wide; as the ratio of length to width increases, the amount of border relatives to area increases, causing increased error from the edge effects (generally too many individuals near border are counted) (Singh *et al.* 2006). A quadrat may be of either types: (i) *List* (for listing of all species present), (ii) *Count* (for determining the individuals of species), (iii) *Cover* (for determining basal area or canopy of species), (iv) *Chart* (for mapping the plants within the quadrat), (v) *Clip* or *Harvest* (for determining biomass or the weight of plants) and, (vi) *Denuded quadrat* (for determining the sequence or development of vegetation overtime, following a treatment (Singh *et al.* 2006)).

The size of quadrat varies in accordance with the size of plants to be sampled. Commonly used quadrats for density measurements are: 10 x 10 m for tree layer, 5 x 5 m for woody under growth upto 3.0 m height, and 1 x 1 m or less for herb layer (Oosting 1956). The number of quadrats should be such as to sample about 20 per cent of the vegetation of an area.

The size of quadrat in which maximum number of diversity of species can be recorded, is called as "*minimum size of quadrat*" for a particular area. Similarly, the number of quadrats

in which maximum number of diversity of species can be recorded, is called as "*minimum number of quadrat*" for a particular area.

9.3 INSTRUMENTS USED

Thread, scale or meter tape, needle or nails for determining minimum size of quadrat, and quadrat size so determined for finding the minimum number of quadrats to be laid down.

9.4 SPECIES AREA CURVE METHOD FOR VEGETATIONAL ANALYSIS

(a) For determining minimum size of quadrat

In case of grasslands or herbaceous vegetation using thread and needle, small quadrat of 10 x 10 cm is laid randomly in an area, the number of species present therein are recorded. The size of quadrat is then increased gradually from 20 x 20, 30 x 30 …….. 100 x 100 cm, and the number of species in each quadrat are noted as shown in Fig. 9.1.a and b, and Table 9.1. A graph is drawn between the size of quadrat (X axis) and number of species (Y axis). The point at which the curve starts flattening up is the minimum size of quadrat required for sampling of the vegetation in that site (Fig. 9.2). 40X40cm size seems to be the minimum size of quadrat in the present example.

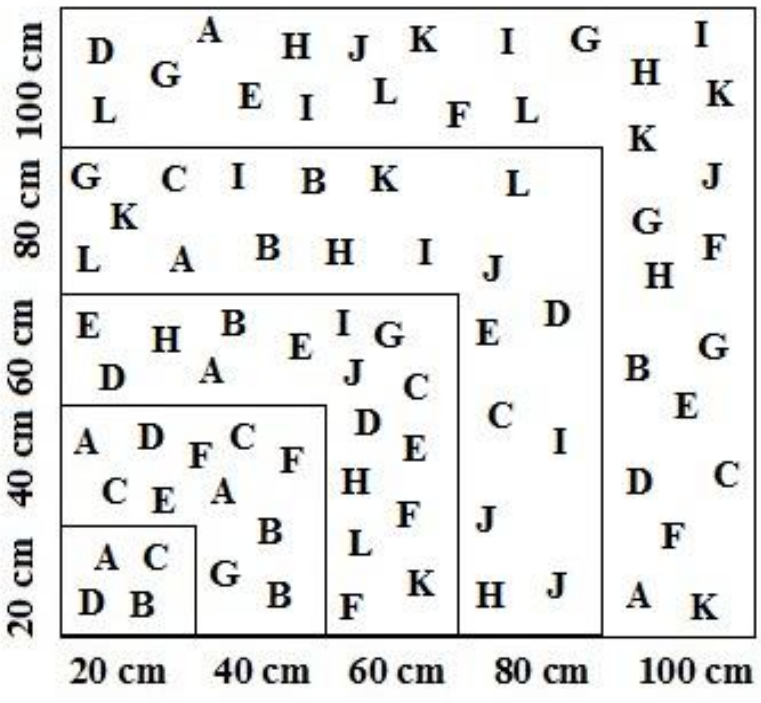

Fig. 9.1.a. Increasing size of quadrats and the number of species in a stand

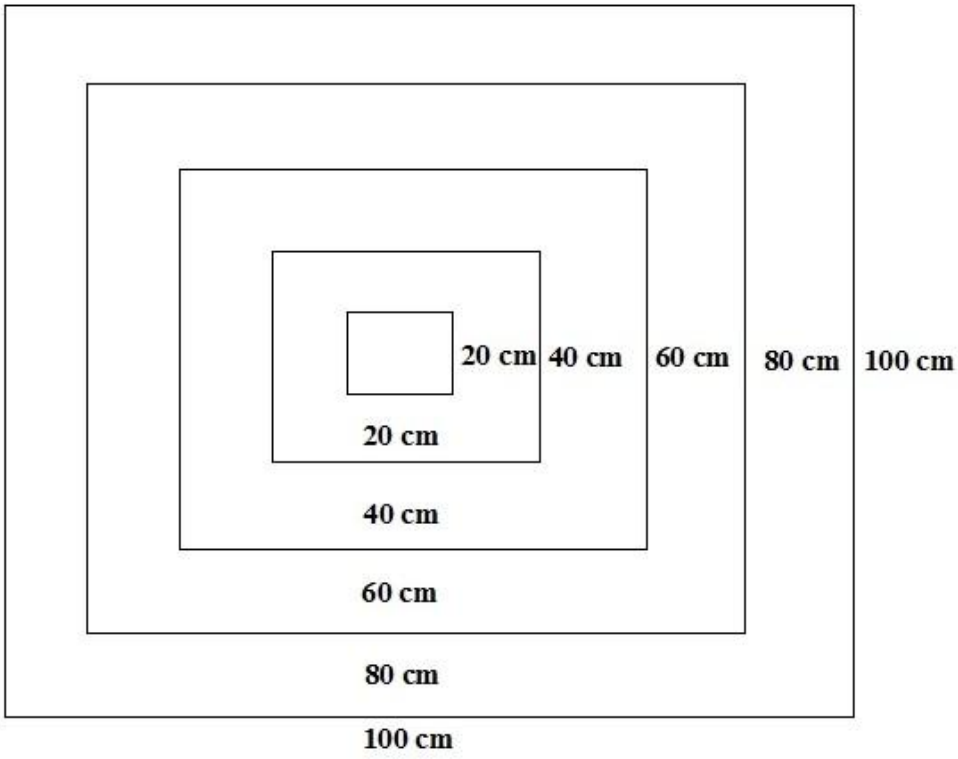

Fig. 9.1.b. Increasing size of quadrats in a stand

Table 9.1 Relationship between size of quadrat and number of species

Quadrat size (cm x cm)	Species (No.)
10 x 10	4
20 x 20	16
30 x 30	26
40 x 40	30
50 x 50	30

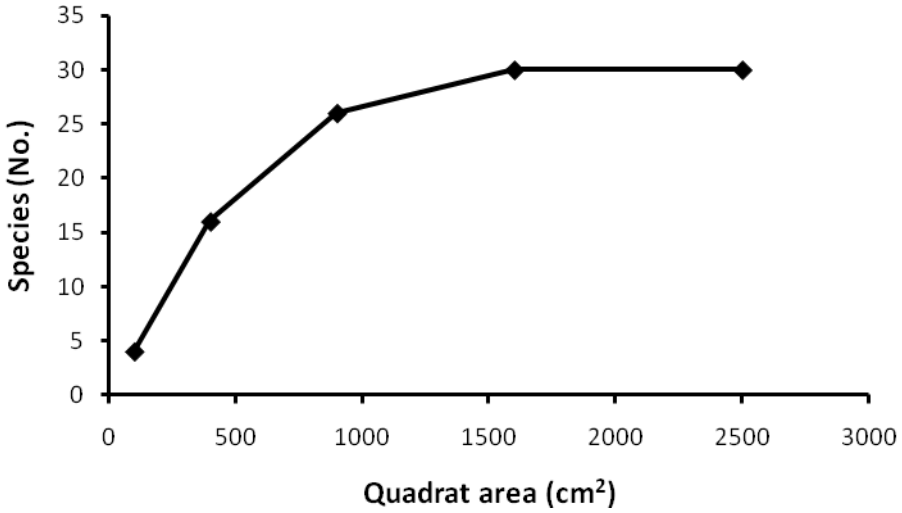

Fig. 9.2. Species area curve

(b) For determining minimum number of quadrats- Quadrat of minimum size, say 40 x 40 cm, is laid randomly in the field and the number of species occurring in each quadrat is recorded. By increasing the number of quadrats, the effect on number of species is noticed. Thus, about 25 quadrats are studied (Table 9.2). A graph between number of quadrats on X axis and number of species on Y axis is drawn (Fig. 9.3). The point, where the graph starts flattening indicates the minimum number of quadrats required for study of vegetation at particular site.

Table 9.2. Relationship between size of quadrats and number of species

Quadrat (No)	Species (No.)
1	7
2	7
3	8
4	8
5	10
6	11
7	12
8	12
9	13
10	13

11	15
12	15
13	16
14	17
15	17
16	19
17	19
18	20
19	20
20	22
21	22
22	22
23	22
24	22
25	22

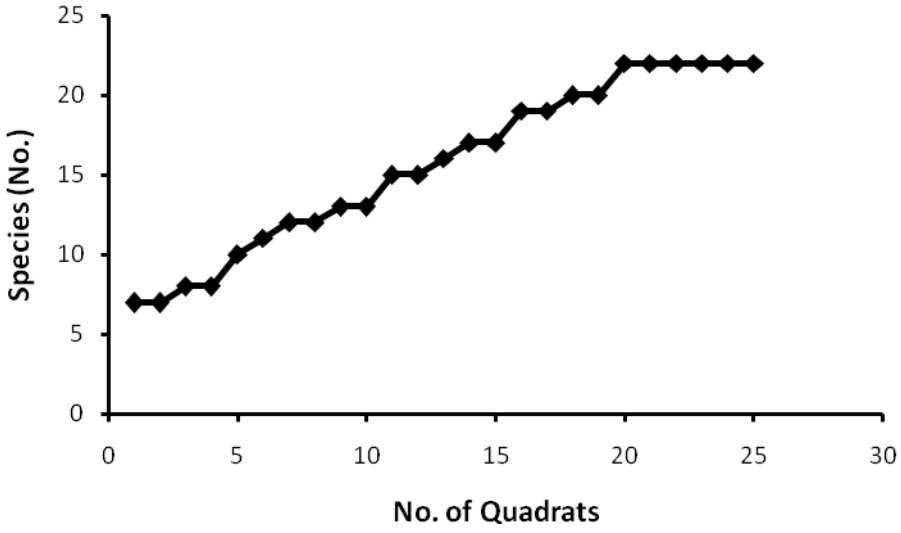

Fig. 9.3. Species area curve

Twenty (20) quadrats minimum are required for studying vegetation at this site.

9.5 SUMMARY

From the above description, you now understand how to identify the minimum size and number of quadrats for studying the vegetation of a given area. It needs careful observation of species count and proper placing of the quadrats of varying sizes. The human bias in locating quadrat in the field must be as negligible as zero to obtain accuracy in findings. The minimum size and number of quadrats so determined could be used for studying vegetation for different analytical characteristics.

9.6 GLOSSARY

Cover: Ground area occupied by a species
Canopy: Crown of a species
Quadrat: A sampling unit of definite shape and size
Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.
Stand: The vegetation of a plot of suitable size is a stand.
Association: It is a product of artificial synthesis of stands and an abstract unit of vegetation.

9.7 SELF ASSESSMENT QUESTIONS

Q.1. What do you understand by quadrat?
Q.2. Name the shape of the quadrat commonly used for vegetation study.
Q.3. What do you mean by species area curve?
Q.4. Discuss briefly the limitation of random sampling.

9.8 REFERENCES

- Oosting, H.J. 1956. *The Study of Plant Communities*: *An Introduction to Plant Ecology*, Second Edition, W.H. Freeman, San Francisco.
- Singh, J.S., S.P. Singh and S.R. Gupta. 2006. *Ecology, Environment and Resource Conservation,* (Reprinted 2008), Anamaya Publishers, New Delhi.

9.9 SUGGESTED READINGS

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- Curtis, J.T. 1959. *Plant Ecology Work Book, Laboratory, Field and Reference Manual,* Burgers Pub. Co., Minnesota, USA.
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9.10 TERMINAL QUESTIONS

Q.1. Why do we decide a minimum size and number of samples for vegetation analysis?
Q.2. What is a quadrat? Discuss various types of quadrats.
Q.3. Discuss briefly the point in species area/number curve that indicate the minimum size/number of sample or quadrat required for vegetation analysis.
Q.4. Discuss the effect of sample size on the quantitative characteristics of a community.

UNIT-10 DETERMINATION OF FREQUENCY, DENSITY AND ABUNDANCE OF SPECIES IN A COMMUNITY BY QUADRAT METHOD

10.1 OBJECTIVES

After reading this unit students will be able -

- To determine frequency, density and abundance of plant species in a community by quadrat method
- Comparison of observed frequency with Raunkiaer's normal frequency diagram

10.2 INTRODUCTION

A plant community or stand is generally studied using qualitative and quantitative characteristics. The qualitative characteristics are descriptive. These prominently include presence/absence of the given species, genera and family among sites. These describe community structure, composition and other features using visual observations without actual measurements. The quantitative analysis deals with the structure and composition of vegetation across vegetation types or communities, and compares them in terms of attributes, such as, frequency, density, abundance, etc.

10.3 QUADRAT METHOD

10.3.1 Frequency: It is the percentage of quadrats in which a species is present or it is the number of sampling units (as %) in which a particular species occurs. Frequency by quadrat method is non-absolute measure as it varies with the size of quadrat. Frequency denotes the homogeneity of distribution of different species in a stand. If a species, e.g., *Solanum nigrum* occurs more abundantly all over the area, it will have the opportunity of occurring in all the samples/quadrats. Thus, frequency of this species will be cent-per-cent. Species occurring poorly (even though many individuals of the same species occur at one spot) will have a chance of occurrence only in few samples/quadrats. In that case, frequency of the species will be low. High value of frequency denotes a greater uniformity of its occurrence or dispersion. It can be stated that "frequency gives the idea of degree of dispersion of individual in an area and is expressed in terms of percentage occurrence".

$$\text{Frequency (\%)} = \frac{\text{No. of quadrats of in which of a species occurs}}{\text{Total number of quadrats sampled}} \times 100$$

Frequency class: Among the early plant ecologists, C. Raunkiaer (1934) concentrated on plant frequencies. Raunkiaer's law of frequency includes five frequency classes based on frequency value (20% interval) as follows:

Class A 1-20 % frequency value

Class B 21-40 % frequency value

Class C 41-60 % frequency value

Class D 61-80 % frequency value

Class E 81-100 % frequency value

Accordingly, the frequency law suggested:

>

A>B>C=D<E

<

In general, higher values of classes B, C and D indicate heterogeneity of the stand, and greater value of class E shows homogeneity of the stand. If the value of the ratio (E+D)/(B+C) is < 1.0, the stand is heterogeneous, whereas the stand is homogenous if the value is > 1.0.

On the basis of the study of large samples (some 8087 frequency percentages), Raunkiaer prepared a Normal Frequency Diagram in which frequency classes A included 53% of the species, B 14%, C9%, D8% and E16% as shown in Fig. 10.1.

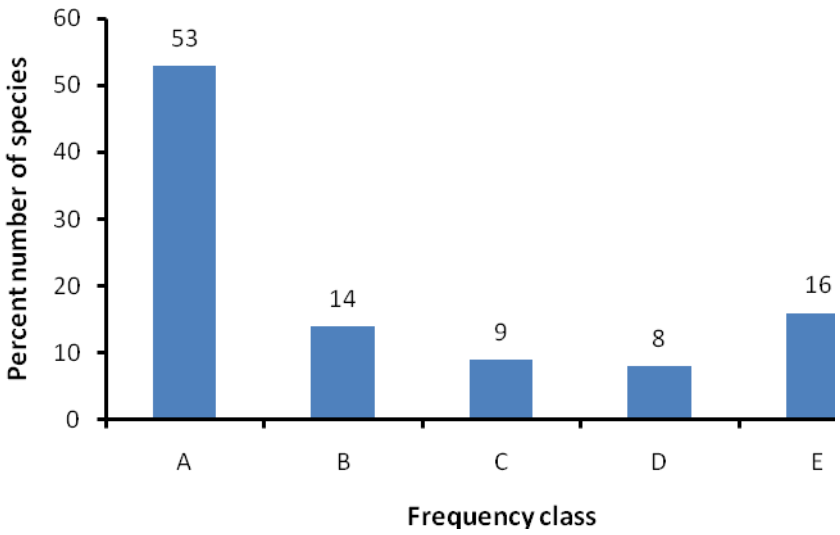

Fig. 10.1 Raunkiaer's normal frequency diagram

After studying the frequency of different species in a locally available stand, students may assign frequency class to each species calculating the percentage of each class mentioned by Raunkiaer and a comparison with Raunkiaer's normal frequency diagram can be made and drawn in the graph sheet.

10.3.2 Density: It represents numerical strength of a species in a stand and is expressed as number of rooted individuals per unit area. It is calculated as:

$$\text{Density (Indv./Quad.)} = \frac{\text{Total number of individuals of the species recorded in all quadrats studied}}{\text{Total number of quadrats studied}}$$

10.3.3 Abundance: For this attribute, only quadrats where a species present are taken into consideration. Thus:

$$\text{Abundance} = \frac{\text{Total number of individuals of the species recorded in all quadrats studied}}{\text{Total number of quadrats in which the species occurred}}$$

Method

After understanding the concept, you would like to apply it. To study frequency, density and abundance, first determine the minimum size of quadrat (sample plot) and quadrat numbers (sample numbers) using species area curve and species number curve, respectively as described in Unit 09 earlier. Generally, a quadrat of minimum size 50 x 50 cm for herbaceous vegetation, 5 x 5 m for shrub species and 10 x 10 m for tree species is used. The number of samples to be studied is generally a minimum of 10. The quadrat is laid randomly in the stand. For the study of frequency, the presence (+) and absence (-) of the species is recorded in each quadrat. The data so collected are put in a tabular form (Table 10.1) to draw the frequency diagram using different frequency classes.

Table 10.1 Frequency (%) of plant species studied in a given stand

Name of the species	Quadrat studied										No. of quadrats of occurrence	Total No. of quadrats studied	Frequency (%)	Frequency class
	1	2	3	4	5	6	7	8	9	10				

For measurement of density and abundance the number of rooted individuals of the species are recorded and tabulated as presented in Table 10.2.

Table 10.2 Density and abundance of plant species in a given stand

Name of the species	No. of rooted individuals in each quadrat										Total No. of rooted individuals	No. of quadrats of occurrence	No. of quadrats studied	Density	Abundance
	1	2	3	4	5	6	7	8	9	10					

After arranging data in tabular form, analyze the data and present them as "Results". It is to be followed by discussion which must justify the findings with valid site-specific reasons.

10.4 SUMMARY

Community is characterized by species diversity, different growth forms and successional stages. For the study of any community or stand a number of parameters are taken into consideration. These are then used to express the characteristics of a community. Any community can be studied by analyzing various attributes and these analytical characters may be qualitative or quantitative. Quantitative characters include frequency, density, abundance cover and basal area of which first three attributes have been discussed in this chapter.

10.5 GLOSSARY

Quadrat: It is a sample plot of known area.

Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.

Stand: The vegetation of a plot of suitable size is a stand.

Association: It is a product of artificial synthesis of stands and an abstract unit of vegetation.

10.6 SELF ASSESSMENT QUESTIONS

Q1. While locating site for the study, ask to self, are you choosing a representative of the entire area.
Q2. Assess the randomness of the random sampling. Examine bias while placing the quadrat (s).
Q3. Judge the correctness of the identification of species with past workers or through herbarium.

10.7 REFERENCES

- Raunkiaer, C. 1934. *The Life Forms of Plants and Statistical Plant Geography*, Clerendon Press, Oxford.

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10.9 TERMINAL QUESTIONS

Q1. Discuss the significance of such investigations for society.
Q2. Obtain data of similar stand from other sites in your area/distinct/state and compare them with your own observations.
Q3. How far can you correlate the abiotic factors with the plant community in your area?
Q4. Why should you take samples at random?
Q5. Using the method outlined above, study the vegetation in a lawn, a grassland, a forest and a cultivated field. Compare your results and explain similarities and differences.

UNIT-11DETERMINATION OF THE MEAN BASAL AREA (COVER) AND TOTAL BASAL AREA (COVER)

11.1 OBJECTIVES

After reading this unit students will be able-

- to determine the mean basal area (cover) and total basal area (cover) of grassland.
- to determine the mean basal area (cover) and total basal area (cover) of woody (tree) community.

11.2 INTRODUCTION

Cover or coverage or area of a plant species is an expression of the ground area occupied or covered by that species. It is expressed in two ways:

I. *Canopy cover or crown cover*: It refers to the ground area covered by the crown or canopy or aboveground parts of a species when canopy is projected vertically to the ground.
II. *Basal area or cover*: It is the ground area actually penetrated by the stem or shoot of a species.

Compared to the density values, cover is given a greater ecological significance as it provides a better estimate of plant biomass. It is also the most suitable measure for recording the change in a stand. The basal cover or basal area is regarded as an index of dominance of a species. Thus, a higher basal area is an expression of dominance of a species. The grassland species are entirely different than woody species. Therefore, methods for determining basal area (cover) for these species are described separately as follows:

11.3 INSTRUMENTS USED

Vernier caliper, Screw guage, thread, meter tape, note book etc.

11.4 DETERMINE THE MEAN BASAL AREA (COVER) AND TOTAL BASAL AREA (COVER) OF GRASSLAND

Method

Basal area is measured either 2.5cm above ground or actually on the ground for herbaceous vegetation. In case of grassland 8-10 stems of a species are clipped at the ground level or 2.5cm above the ground and diameter of each individual at its base is measured with the help of Vernier caliper or Screw guage and average diameter of one stem can be obtained. This value is divided by two (2) to get the value of radius. This procedure is repeated for each species present in the grassland where the study is being conducted. The basal area of each species present in the grassland can be obtained as follows:
Basal area or cover = πr^2 , where we know the value of π and r (radius).

Total Basal area or cover = Mean basal area X Density

11.5 DETERMINE THE MEAN BASAL AREA (COVER) AND TOTAL BASAL AREA (COVER) OF WOODY (TREE) COMMUNITY

Method

The basal area of woody species is estimated by measuring either the diameter or circumference of the average tree at breast height, i.e., 1.37 m, using the following formula:

Circumference = $2\pi r$

$r = \text{Circumference}/2\pi$

Basal area or cover = πr^2

The basal area (cover) os each tree species is either calculated or recorded directly through conversion table as given in Tables 11.1 and 11.2. The basal area of an average tree when multiplied by density gives the value of total basal area.

Table 11.1. Circumference to basal cover table

Circumference (inch)	Basal Cover (inch^2)	Circumference (inch)	Basal Cover (inch^2)
12.0	11.46	16.5	23.00
12.5	12.43	17.0	24.37
13.0	13.45	17.5	25.58
13.5	14.50	18.0	27.23
14.0	15.60	18.5	28.73
14.5	17.90	19.0	30.26
15.0	19.12	19.5	31.83
15.5	20.37	20.0	33.46
16.0	21.66	20.5	35.27

Table 11.2. Diameter to basal cover conversion table

Diameter (inch)	Basal Cover (inch^2)	Diameter (inch)	Basal Cover (inch^2)
1.00	0.78	3.25	7.30
1.25	1.23	3.50	9.62

1.50	1.47	3.75	11.04
1.75	2.41	4.00	12.57
2.00	3.14	4.25	15.90
2.25	3.98	4.50	19.63
2.50	4.91	4.75	23.75
2.75	5.94	5.00	28.27
3.00	7.07		

Source: Kapur and Govil (2000)

11.6 SUMMARY

You must have now understood mean and total basal area (cover) of a species and the canopy cover. The area occupied by the stem or shoot of an individual species on the ground of a stand is the basal area (cover) whereas area covered by crown of the species is canopy cover. The value of basal cover gives you information about dominance of the species or change in the area occupied by a species over a period of time.

11.7 GLOSSARY

Breast height: Height at breast level of a normal human. It is generally considered as 1.37 m above the flat ground.

Canopy: Crown of a species

Cover: Ground area occupied by a species

Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.

Stand: The vegetation of a plot of suitable size is a stand.

Quadrat: A sampling unit of definite shape and size

11.8 SELF ASSESSMENT QUESTIONS

Q.1. What do you understand by cover.

Q.2. What is canopy cover?

Q.3. What is basal area?

Q.4. In what way, the value of density is important for determining total cover?

11.9 REFERENCE

- Kapur, P. and S.R. Govil. 2000. *Experimental Plant Ecology,* CBS Publishers and Distributers, New Delhi.

11.10 SUGGESTED READINGS

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11.11 TERMINAL QUESTIONS

Q.1. Why do we study cover? Explain its importance in regard to vegetational change at a site along time series.

Q.2. Why estimate of cover is given more importance in ecological studies, compared to density?

Q.3. What is the difference between mean basal area (cover) and total basal area (cover) of a species.

Q.4. Explain basal cover and canopy cover in case of a wood species.

UNIT-12 STATISTICAL PROBLEMS ON CENTRAL TENDENCIES-MEAN, MEDIAN, MODE AND STANDARD DEVIATION AND CHI-SQUARE TEST

12.1 OBJECTIVES

After reading this unit student will be able:

- to know about mean
- to know about Median
- to know Mode
- to understand standard deviation
- to know about the chi-square test

12.2 INTRODUCTION

Statistics refers to the subject of scientific activity which deals with the theories and methods of collection, analysis and interpretation of such data. Term biostatistics is used when tools of statistics are applied to the data that is derived from biological field.

Characteristics of Biostatistics

1. Biostatisticsis the aggregate of facts.
2. Biostatistics is numerically expressed.
3. Biostatistics is affected by multiplicity of causes and not by single cause.
4. Biostatistics must be related to the same field of inquiry.
5. Biostatistics should be capable of being related to each other, so that some causes and effects on relationship can be stabilised.
6. The reasonable standard of accuracy should be maintained in statistics.

Importance and Usefulness of Biostatistics

1. Statistics help in presenting large quantity of data in a simple and classified form for research as well as teaching.
2. It gives the method of comparison of data.
3. It helps in finding the condition of relationship between the variables.
4. It serves a guide in planning and shaping in field designing for agricultural trial.

Limitations of Statistics

1. Statistics can be used to analyse only collective matters not individual events
2. it is applicable only to quantitative data
3. Statistical results are ascertained by samples. If the selection of samples is biased errors will accumulate and results will not be reliable
4. The greatest limitations of statistics is that only one who has an expert knowledge of statistical methods can efficiently handle statistical data

Application and Uses of Biostatistics

1. To define what is normal or healthy in a population and to find limits of normality in variables

2. To find the differences between the means and proportions of normal at two places or in different periods
3. To find out correlation between two variables X and Y such as height and weight.

Central Tendency or Average

The word 'average' denotes a representative of a whole set of observations. It is a single which describes the entire series of observations with their varying sizes. It is a typical value occupying a central position where some observations are larger and some others are smaller than it. Average is a general term which describes the center of a series. The values of variable tend to concentrate around the central value. It is the central part of the distribution and therefore also called the measures of central tendency.

Characteristics of Central Tendency

1. An average should be properly defined so that it has one and only one interpretation. The average should not depend on the personal prejudice and bias of the investigator.
2. The average should depend on each and every item of the series. So that if any of the item is dropped, the average itself be altered.
3. The desirable property of an average is that it can be readily understood and then only it can be made popular
4. The average should depend on each and every figure, so we must be aware that no extreme observations could unduly influence the central value
5. Due to an extreme observation, the central value changes or distorts and it cannot be typical for the group values.
6. It should be least affected by the fluctuations of the sampling: If we select different groups of samples we should expect some central value approximately in each sample.
7. It should be easy to interpret: The average can become popular only because of its access for easy computation.

Importance of Central Tendency

Averages have important place in statistics. According to Dr. Bowley, "Statistics is the science of averages". The word average is very commonly used even in everyday life. Such as average income of a family, average production of sugarcane in a farm, average production of seed for particular crop. Average height of trees in an agricultural land, average production of rice or wheat in a state/country, or even average rainfall in an area during a particular month or season.

Objectives of Central Tendency

There are four main objectives of study of averages:

1. The main objective of calculating or measuring averages is to determine a single value for the whole series.
2. Average describes the characteristic of the entire group.
3. It gives a bird's eye-view of the entire data.
4. Averages help in the comparison of different data within one or between groups of data.

Measures of Central Tendency

The most common measures of central tendency are:
(i) Mean or Arithmetic mean, (ii) Median, (iii) Mode

(i) Mean: This measure implies arithmetic average or arithmetic mean which is obtained by summing up all the observations and dividing the total by the number of observations.

(ii) Median: When all the observations of a variable are arranged in either ascending or descending order, the middle observation is known as median. It implies the mid value of series. Median is an indicator of central value when one or more of the lowest or the highest observations are wide apart or not so evenly distributed.

(iii) Mode: This is the most frequently occurring observation in a series, i.e. the most common or most fashionable**.** Out of the three measures of central tendency mean is better and utilized more often because it uses all the observations in the data and is further used in the tests of significance.

12.3 STUDY OF MEAN

Introduction- It is obtained by summing up all the observations and dividing the total by the number of observations. Arithmetic mean for ungrouped data or individual observations:
In$x_1, x_2, x_3,\ldots\ldots x_n$ be 'n' observations for a variable x, the arithmetic mean $\bar{x}$ is given by

$$\bar{x} = \frac{x x_1 + x_2 + x_3 + \cdots x_n}{n}$$

To further simplify the writing of a sum, the Greek letter∑ (sigma) is used as a short hand. The sum $x_1 + x_2 + x_3 + \cdots x_n$ is denoted,

$$\bar{x} = \sum_{i=1}^{n} xi/n$$

and read as "the sum of all x_i with i ranging from 1 to n". We can now formally define the mean as follows
Definition: The sample mean of the variable is the sum of observed values $x_1, x_2, x_3,\ldots,x_n$ in a data divided by the number of observations n. The sample mean is denoted by $\bar{x}$ and expressed operationally

$\frac{\sum_{i=1}^{n} x_i}{n}$ or $\sum x_i/n$

Steps of Calculation:

1. Add together all the values of x and get $\sum x$.

2. Divide this total by the number of observations

In case of frequency distribution:

$\bar{X} = f_1x_1+f_2x_2+----------+f_nx_n/f_1+f_2+------f_n$

$\bar{X} = \sum fx/\sum f$

$\bar{X} = \sum fx/N$

N=$\sum f$, total frequency

In case of continuous or grouped frequency list, the value of x is taken as the mid value of the corresponding class.

Object 1: The intelligent quotient (IQ) of 10 boys in a class are given as 70,120,110,101,88,83,95,98,107,100. Calculate mean.

Work procedure

Mean ($\bar{X}$) = $X_1+X_2+X_3+X_4+X_5+------X_n/ N]$

So $\bar{X}$= 70+120+110+101+88+83+95+98+107+100/10 = 972/10=97.2

Object 2: The following is the frequency list of the number of telephone calls received at 245 successive one minute intervals in an exchange.

No of class	0	1	2	3	4	5	6	7
Frequency	14	21	25	43	51	40	39	12

Calculate mean no. of calls per minute.

Work Procedure

Number of class: 0, 1, 2, 3, 4, 5, 6, 7

Frequency (f) = 14+21+25+43+51+40+39+12

N=245

fx =0+21+50+129+204+200+234+84

$\sum fx$= 922

$\bar{X}$=$\sum fx/N$

So $\bar{X}$= 922/245

$\bar{X}$= 3.763

Step Deviation Method for Computing Arithmetic Mean

Let d=X-A

$fd = f(X-A) = fX - A.f$

$\bar{X}=A+\frac{\sum fd}{N}$

In case of grouped or continuous frequency distribution with class intervals of equal magnitude, the calculations are further simplified by taking

$d = \frac{X-A}{h}$

$\bar{x}=A + h\frac{\sum fd}{N}$

Steps:-

1. $d = \frac{X-A}{h}$, A=arbitrary number and h = common magnitude of the classes
2. Determine fd
3. Find $\sum fd$
4. Determine $\frac{\sum fd}{N}$
5. $\frac{\sum fd}{N}$X h
6. Add $A + h\frac{\sum fd}{N}$

   The resulting value gives the arithmetic mean of the given distribution

Object 3: Calculate the mean for the following frequency distribution

Marks: -	0-10	10-20	20-30	30-40	40-50	50-60	60-70
No. Of student-	6	5	8	15	7	6	3

I. By the direct formula
II. By the step deviation method

Work Procedure:-

Computation of Arithmetic mean

Marks	Mid value (x)	Number of students (f)	fx	d= (x-35)/10	fd
0-10	5	6	30	-3	-18
10-20	15	5	75	-2	-10
20-30	25	8	200	-1	-8
30-40	35	15	525	0	0
40-50	45	7	315	1	7

50-60	55	6	330	2	12
60-70	65	3	195	3	9
		N=∑*f*=50	∑*fx*=1670		∑*fd*=-8

a) Direct Formula
Mean $\bar{x}$ = ∑*fx*/∑*f*=1670/50=33.4 Marks

ii) Step Deviation method

A=35, h=10

$\bar{x}$=A + h $\frac{\sum fd}{N}$ = 35+$\frac{10(-8)}{50}$= 33.4 Marks

Mathematical Properties of Arithemeic Mean

1) ∑($\bar{x}$-$\bar{x}$)=0

2) $\bar{x}$= $n_1\bar{x}_{1+}$ $n_2\bar{x}_2$/ (n_{1+} n_2)

3) S= ∑*f* (x-A)^2
The sum of squares of the deviations of the given set of observations is minimum when taken from arithmetic mean

4) $\bar{x}$=∑*fx*/N

Result is useful in following processes:-

a) If we are given mean wages ($\bar{x}$) of a no of workers (N) in a factory, then by the above formula we can determine the total wage bill of the factory.
b) In general if r observations are misread as x_1',x_2'….x_n' while the correct observations are x_1,x_2…….x_n then the corrected observations are x_1,x_2….$x_n$then the corrected sum of observation is given by N($\bar{x}$) – (x_1'+$_{x2}$'+…xr') +(x_1+x_2+….xr) Dividing this sum by N, we get the corrected mean.

Object 4: The number 3.2, 5.9, 7.9 and 4.5 have frequency x,x+2, x-3 and x+6 respectively . If arithmetic mean is 4.876, find the value of x.

Work Procedure: Mean=∑*f*x/∑*f*

Number(x)	Frequency (*f*)	*fx*
3.2	X	3.2x

5.8	X+2	5.8(x+2)
7.9	x-3	7.9(x-3)
4.5	X+6	4.5(x+6)

$\sum f$=4x+5

$\sum f$= 3.2x+5.8 (x+2) +7.9 (x-3)+4.5 (x+6)

$\sum fx$=21.4x+ 14.9

Mean=$\sum fx/\sum f$= 4.876

21.4x+ 14.9=4.876(4x+5)
1.896x=9.480
X=9.480/1.896=5

Object 5: The mean salary paid to 1000 employees was found to be Rs. 180.40.After distribution of salary, it was discovered that the salary of two employees was wrongly entered as 297 and 165. The correct salary was Rs 197 and Rs 185. Find the correct arithmetic mean.

Work Procedure:

$\bar{x}$= salary of an employee

$\bar{x}=\frac{\sum x}{1000} X\ 180.40$

$\sum x$ = 180400

Salary of Employees in the establishment is Rs 180400.

After incorporating the corrections we have, corrected = 180400- sum of wrong + sum of correct salaries

180400- (297+165)+(197+185) =180320

Corrected mean salary= 180320/1000

=180.32Rs

Weighted Arithmetic Mean

The formulae discussed so far for computing the arithmetic mean are based on the assumption that all the items in the distribution are of equal importance. However, in practice we might come across the situation where relative importance of all the items of the distribution is not same. If some items in a distribution are more importantthan others, then this point must be borne in mind, in other words average computing is representative of the distribution.

Let $W_1, W_2, W_3 \ldots W_n$ be the weights attached to variable values, $x_1, x_2, \ldots x_n$ respectively. Then the weighted arithmetic mean usually denote by

$$(\bar{x}w) = \frac{w_1x_1 + w_2x_2 + \cdots\cdots w_nx_n}{w_1 + w_2 \pm \cdots - w_n} = \frac{\sum wx}{\sum w}$$

In case of frequency distribution, if f_1, $f_2, \ldots f_n$ are the frequencies of variable values $x_1, x_2, \ldots x_n$ respectively then the weighted arithmetic mean is given by

$$\bar{x}_w = \frac{w1(f1x1) + w2(f2x2) + \cdots\cdots wn(fnxn)}{w1 + w2 + \cdots + wn}$$

$$= \frac{\sum w(f_x)}{\sum w}$$

Where w_1, w_2------ W_n are the respective weight of x_1, x_2---x_n

Object 6: Comment on the performance of the students in the universities given below, using simple and weighted average

University	**Bombay**		**Calcutta**		**Madras**	
Course of study	% of pass	Number of student	% of pass	Number of student	% of pass	Number of student
M.A	71	3	82	2	81	2
M.Com.	83	4	76	3	76	3.5
B.A..	73	5	73	6	74	4.5
B.Com.	74	2	76	7	58	2
B.Sc.	65	3	65	3	70	7
M.Sc.	66	3	60	7	73	2

Work Procedure

Computation of simple and weighted Average

University	Bombay			Calcutta			Madras		
Course of study	% of pass (x_1)	No. of stud (w_1)	w_1x_1	% of pass (x_2)	No. of stud. (w_2)	w_2x_2	% of pass (x_3)	No. of stud. (w_3)	w_3x_3
M.A.	71	3	213	82	2	164	81	2	162
M.Com	83	4	332	76	3	228	76	3.5	266
B.A.	73	5	365	73	6	438	74	4.5	333
B.Com.	74	2	148	76	7	532	58	2	116
B.Sc.	65	3	195	65	3	195	70	7	490
M.Sc.	66	3	198	60	7	420	73	2	146
Total	432	20	1451	432	28	1977	432	21	1513

Univ.	Simple Average	Weighted average
Bombay	$\sum x_1/6=432/6 =72$	$\sum w_1x_1/\sum w_1= 1451/20 =72.5$
Calcutta	$\sum x_2/6=432/6 =72$	$\sum w_2x_2/\sum w_2= 1977/28 =70.6$
Madras	$\sum x_3/6=432/6 =72$	$\sum w_3x_3/\sum w_3= 1513/21 =72.0$

On the basis of simple arithmetic mean which comes out to be same for all the three universities 72, we cannot distinguish between the pass % of students in the 3 universities. However, the weighted averages show that the results are best in Bombay (which has highest weighted average of 72.55), followed by Madras university (72.05) and while Calcutta university shows the lowest performance

12.4 STUDY OF MEDIAN

Introduction : In the words of L.R Connor "The Median is the value of that item in a series which decides the series into two equal parts, one part consisting of all values less and the other all values greater than it".

Thus median of a distribution may be defined as the value of the variable which exceeds and is exceeded by the same number of observation i.e. it is the value such that the number of observations above it is equal to the number of observation below it. It is the middle most point or the central value of the variable in a set of observations when observations are arranged either in ascending or in descending order of their magnitudes.

Calculation of Median

Ungrouped data (Simple series)

Procedure:

1) Arrange the data in either ascending or descending order of magnitude.

2) If the number of observations be odd, the value of the middle-most items is the median. However, if the number be even, the arithmetic mean of the two middle most items is taken as median.

When 'n' is odd. In this case $\frac{n+1}{2}$th value is the median. M = $\frac{n+1}{2}$th term.

When 'n' is even. In this case there are two middle terms $\frac{n}{2}$th and $\left(\frac{n}{2}+1\right)$th .

The median is the average of these two terms.

$$M=\frac{\frac{n}{2}+\left(\frac{n}{2}+1\right)}{2}$$

Grouped data (Discrete series)

Procedure:

I. Arrange the data in either ascending or descending order of magnitude.

II. A table is prepared showing the corresponding frequencies and cumulative frequencies.
III. Now median is calculated by the following formula

$$M = \left(\frac{n+1}{2}\right) th \text{ N} = \sum f$$

Continuous series

Procedure

1) Here data is given in the form of a frequency table with class interval.
2) Cumulative frequencies are found out for each value.
3) Median class is then calculated where cumulative frequency $\frac{N}{2}$ lies is called median class.
4) Now median is calculatedby applying the following formula.

$$M = L + \frac{\frac{N}{2} - c.f}{fm} Xi$$

L = lower limit of class in which median lies.

N = Total no of frequencies

*f*m = frequency of the class in which median lies.

C = cumulative frequency of the class preceding the median class.

i = width of the class interval in which the median lies.

Advantages (merits) of median

a. It is easily understood although it is not so popular as mean.
b. It is not influenced or affected by the variation in the magnitude of the extreme items.
c. The value of median can be graphically ascertained to ogives.
d. It is the best measure for qualitative data such as beauty, intelligence etc.
e. The median indicates the value of middle items in the distribution.

Disadvantages (demerits) of median

a. For the calculation of median, data must be arranged.
b. Median being a positional average can't be dependent on each and every observation.
c. It is not subject to algebraic treatment.
d. Median is more affected by sampling fluctuation than the arithmetic mean.

1. Object- Find the median of the following numbers

(a) 21,12,49,37,88,46,55,74,63
(b) 88,72,33,29,70,86,54,91,61,57.

Work Procedure: Let us arrange the data in order : 12,21,37,46,49,55,63,74,88

In this data the number of item is n = 9 (odd)

Median (M) = $\frac{(n+1)}{2} = \frac{(9+1)}{2}$th term = 5^{th} term

Now the 5^{th} value in the data is 49.

Median is 49.

b. Let us arrange the data in order ; 29,33,54,57,61,70,72,86,88,91

In this data the number of item is n = 10 (even)

Median = average of $\left(\frac{n}{2}\right) th + \left(\frac{n}{2}+1\right) terms.$

Average of $\left(\frac{10}{2}\right)$th and $\left(\frac{10}{2}+1\right)$th terms.

= Average of 5^{th} and 6^{th} terms

M = $\frac{61+70}{2} = \frac{131}{2}$ = 65.2.

Median is 65.2

2. Object- Find the median class of the data given below

Class boundaries	15-25	25-35	35-45	45-55	55-65	65-75
Frequency	4	11	19	14	0	2

Work Procedure

Class boundary	Mid value (m)	Frequency (*f*)	Cumulative frequency (c*f*)
15-35	20	4	4
25-35	30	11	15
35-45	40	19	34
45-55	50	14	48
55-65	60	0	48
65-75	70	2	50

$\frac{N}{2} = \frac{50}{2} = 25$

$L_1 = 35$ *f*m = 19 C = 15 i = 10

$M = 35 + \frac{25-15}{19}$ X 10

$= 35 + \frac{10}{19}$ X 10 = 35 + 5.263 = 40.263

Median class: 35-45

Object 3: **Calculate the median for the following data**

Number of plants	6	16	7	4	2	8
Height	20	25	50	9	80	40

Procedure

Let us arrange the data (height) in ascending order and then form cumulative frequencies

Height	No. of plants (*f*)	Cumulative Frequency
9	4	4
20	6	10
25	16	26
40	8	34
50	7	41
80	2	43

Here $\sum f = n = 43$

Median (M) = $\frac{n+1}{2}$= 22nd value

Object: Calculate the Mean deviation from the median for the following data:

Marks less than	80	70	60	50	40	30	20	10
Number of students	100	90	80	60	32	20	13	5

Work Procedure

Convert *c.f* into ordinary frequency

Marks	*c.f*	*f*	Mid-value	|x-Md|	*f*|*x*-Md|
0-10	5	5	5	41.43	207.15
10-20	13	8	15	31.43	251.44
20-30	20	7	25	21.43	150
30-40	32	12	35	11.43	137.16

40-50	60	28	45	1.43	40.04
50-60	80	20	55	8.57	171.14
60-70	90	10	65	18.57	185.70
70-80	100	10	75	28.57	285.70
	∑F=100				∑*f*|*x*-Md|=1428.6

Md=l +h/f(N/2-c)=40+10/28(50-32)=46.43

M.D about median= 1/N∑*f*|X-Md|= 1428.6/100=**14.29**

12.5 STUDY OF MODE

Mode: mode is considered as the value in the series which occur most frequently and has a maximum frequency.

Calculation of mode

A. Ungroup data

Procedure:

1) In the case of simple series mode can be determined by locating the value which occurs the maximum number of times.
2) It can be determined by the inspection only.
3) It is the value of the variable which correspond to the largest frequency.

B. Group data

1. Discrete series
2. Continuous series

Discrete series: mode can be determined by the inspection method or by grouping method. The inspection method is similar to the one discussed in ungrouped data. This method is used in cases where there is regularity and homogeneity in the series. Inspection method is usually not reliable and grouping method is applied. It involves preparation of grouping method as follows:

Procedure

1) In the grouping table first of all, the values of variables are arranged in ascending order.
2) In column 1, corresponding frequencies are written
3) In column 2, frequencies are grouped in two's beginning with the first value.
4) In column 3, the frequencies are grouped in two's beginning with the second value of the series.
5) In column 4, the frequencies are grouped by three values(i.e,1,2&3)starting with the first value Write them as shown in the table
6) In column 5, frequencies are grouped into three values beginning with the second value.
7) In column 6, frequencies are grouped into three values beginning with the third value.

8) Underline the maximum grouped frequencies in each column.

Mode of a Continues Series

Determination of mode in case of continues frequency distribution is complicated and involves following two steps:

Step 1. First of all, the modern class is ascertained either by inspection method or by grouping method.

Step 2. After determining the model class, the exact value of mode is calculated by using the following formula:

Mode (Z) $= L_1 + \left(\frac{f_m - f_1}{2fm - (f_1 - f_2)}\right) Xi$

Where L_1 = Lower limit of model class

f m = Frequency of model class or the maximum frequency

f_1 = Frequency of class just preceding the model class

f_2 = Frequency of class just succeeding the model class

i = Class interval or class width of the class

Advantages of mode

1) It can be obtained by the inspection.
2) It is not affected by the extreme value.
3) This average can be calculated from open ends classes.
4) It can be easily understood.
5) It can be used to describe qualitative phenomenon.
6) The value of mode can also be found graphically.

Disadvantage of mode

1) Mode has no significance unless a large number of observationsare available.
2) It can't be treated algebracally.
3) It is a peculiar measure of central tendency.
4) For the calculation of Mode, the data must be arranged in the form of frequency distribution.
5) It is not rigidly defined measure.

Object 1: The temperature recorded for the growth of plants in a plant tissue culture room in Celsius. What is the mode of following data?

20,25,35,25,40,36,29, 30, 22

Variable(X)Temperatures in $^{\circ}C$	20	25	35	25	40	36	29	30	22

Work procedure: Let us prepare table showing frequency

Value (Temperature)	**20**	**22**	**25**	**29**	**30**	**35**	**36**	**40**
Number of frequency	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**1**

Because after arranging the above in increasing order 25 Celsius is recorded maximum times Here 25 repeat 2 times and is most frequent hence 25 is mode.

Object 2- Study of mode in the following series of the number of coloured flowers in a hybrid flowering plant.

7 – pink, 13 – yellow, 18 – white, 24 – red, 3- maroon

Work procedure: mode is 24 because 24 is the highest occurring number of red flowers in the plant.

Object 3: The apple trees in an orchard gave different number of apples with following distribution. Find the mode of distributions

Variable (X) fruits	100	200	50	500	150
Frequency (*f*) trees	40	30	10	25	20

Work procedure

Number of trees ($\sum f$) = 40+30+10+25+20=125

Number of fruits on 125 trees varies from 1 to 5. This is called variable.

Trees bearing 100 flowers are maximum i.e., 40 in number. This represents highest frequency.

100 is value with highest frequency of 40.

Therefore, Mode= 40 per tree.

Objective 4: Fifty baby carrots were grown using special soil. They were dug and their lengths were measured (to the nearest mm) with following groups. Calculate mode

Length	150-154	155-159	160-164	165-169	170-174	175-179	180-184	185-189	

(mm)									
Frequency	5	2	6	8	9	11	6	3	

Work procedure: The model group is the one with the highest frequency, which is 175-179:

a. L = 174.5 (the lower class boundary of the 175-179 group)
b. *f*(m-1) = 9
c. *f*m = 11
d. *f*(m+1) = 6
e. i = 5

Mode (Z) = $L_1 + \left(\frac{fm-f_1}{2fm-(f_1-f_2)}\right) \times$ i

Estimated mode = 174.5 + 11 – 9/ (11 – 9) + (11 – 6) X 5
174.5 + 1.42
= 175.9 mm.

12.6 STUDY OF STANDARD DEVIATION

Standard deviation usually denoted by letter σ (sigma) was suggested by Karl Pearson measure of depression in 1893. It is defined as positive square root of the squares of deviations of the various items from the arithmetic mean of the series. It is also called root mean square deviation. It is represented in short form by SD. It represents the extent to which individual values differ from the average or mean.

Merits of Standard Deviation

1) It summarises in one figure the deviation of a large distribution from mean.
2) It is most reliable and dependable measure of dispersion.
3) It helps in finding the suitable size of sample for valid conclusions.
4) It is less affected by fluctuations in sampling.
5) Standard deviation is rigidly defined and its values are always definite.

Demerits of Standard Deviation

1) It gives more weightage to extreme values and less to the values that are closer to mean.
2) The process of squaring deviations and then taking square root involves lengthy calculations. Hence, its calculation is not easy.

Significance of Standard Deviation

1) Standard deviation is based on all the observations.
2) The squaring of the deviation $(X - \overline{X})^2$ removes the drawbacks of ignoring the signs of derivations in computing the mean deviation.

3) A large standard deviation shows that the measurements of frequency distribution are widely spread out from the mean, while small standard deviation shows that observations are closely spread in the neighbourhood of mean.
4) Standard deviation indicates whether the variation of differences of any individual observation from the mean is natural or real due to some specific reasons.
5) It helps in finding the standard error which determines whether the difference between the means of two similar samples is by chance or real.

Computation of Standard Deviation

Standard deviation is computedin the following steps:

Firstly arithmetic mean is calculated using the formula:

Standard deviation (σ') = $\sqrt{1/n\sum (X-\bar{X})^2}$

$\bar{X}=\frac{\sum X}{N}$

Deviation or difference of each observation from the mean is found out using the formula: dx=X-$\bar{X}$ .This difference between observation and mean is squared

$dx^2= (X-\bar{X})^2$

All the squared values are added to calculate the sum of squared deviations i.e, $\sum dx^2$ or $\sum(X-\overline{X})^2$/n

X= value of variable

$\bar{X}$= arithematic mean

N= total no of observation

Merits of standard deviation

- it is based on all the observation.
- It is rigidly defined.
- It is less effected by fluctuation of sample as compared to other measure of dispersion.
- It is extremely used in correlation.

Demerits of standard deviation

- It is difficult to compute unlike other measure of dispersion.
- It is not simple to understand.
- It gives more weightage to extreme values.

Uses of Standard Deviation

- It summarises the deviation of a large distribution from mean.

- It indicates whether the variation of differences of an individual from the mean is by chance. i.e., natural or real due to some special reason.
- It helps in finding the standard errors which determines whether the difference between means of two similar samples is by chance or real.
- It also helps in finding the suitable size of sample for valid conclusion.

Standard deviation may be used:

- When arithmetic mean is used for central tendency.
- When the statistics with greater stability is sought.
- When the correlation coefficient and other statistics which depend upon the standard deviation are to be computed.
- The standard deviation is used in preference to other deviation as it is a neat method of removing the negatives.

Coefficient of Standard Deviation

For comparative study, coefficient of standard deviation is obtained by the following formula:

$$\text{Coefficient Of standard Deviation} = \frac{Standard Deviation}{Arthmetic Mean} = \frac{SD}{\bar{X}}$$

Computation of Standard Deviation From Grouped Data:

There are two methods to compare standard deviation from available data, one islong method and other is short method.

(a) Long Method: Following formula is used to obtain standard deviation.

$$SD\ (\sigma') = \sqrt{\frac{\sum fx^2}{\sum f}} \text{ or } \sqrt{\frac{\sum f(X-\bar{X})^2}{\sum f}}$$

SD = standard deviation

$X = (X-\bar{x})$

Steps in calculating standard deviation from grouped data by method involves following step:

Step1. Find arithmetic mean value of the series using formula $\frac{\sum f.X}{\sum f}$

Step 2. Find each deviation from mean or X.

Step 3. Square each deviation, finding x^2

Step 4. Multiply each squared deviation with corresponding frequency finding fx^2

Step 5. Sum the squared deviation multiplied by frequency, finding $\sum fx^2$

Object 1- Find arithematic mean and standard deviation from data given in the form of variables 2, 4, 7, 11 and 15

Work Procedure-

x= 2, 4, 7, 11 and 15

Mean ($\bar{X}$)= $X_1+X_2+X_3+X_4+X_5+$------X_n/ N]

So $\bar{X}$= 2+4+7+11+15/5 = 7.9

Standard deviation (σ') = $\sqrt{1/n}\sum(X-\bar{X})^2$

$(X-\bar{X})$	$(X-\bar{X})^2$
2-7.9= -5.9	34.81
4-7.9= -3.9	15.21
7-7.9= -.9	0.81
11-7.9= 3.1	9.61
15-7.9= 7.1	50.41
	∑=110.85

$\sigma'=\frac{\sqrt{1}}{5} \in (110.85)^2= \sqrt{22.17} = 4.70$

Object 2: Calculate mean and standard deviation of each data set of plants variety A, B and C

Plant (A)	**9**	**10**	**11**	**17**	**13**
Plant (B)	**10**	**10**	**10**	**10**	**10**
Plant (C)	**1**	**1**	**10**	**19**	**19**

Work Procedure-

Mean ($\bar{X}$) = $X_1+X_2+X_3+X_4+X_5+$------X_n/ N]

So Mean ($\bar{X}$) of Data set A = (9+10+11+7+13)/5 = 10

Mean ($\bar{X}$) of Data set B = (10+10+10+10+10)/5 = 10

Mean ($\bar{X}$) of Data set C = (1+1+10+19+19)/5 = 10

Standard deviation (σ') = $\sqrt{1/n}\in(X-\bar{X})^2$

Standard deviation of Plant A

$(X-\bar{X})$	$(X-\bar{X})^2$
9-10	1

10-10	0
11-10	1
7-10	9
13-10	9
	∑=20

So standard Deviation Data set of Plant A = √[($(9-10)^2+(10-10)^2+(11-10)^2+(7-10)^2+(13-10)^2$)/5]=2

Standard deviation of Plant B

$(X-\overline{X})$	$(X-\overline{X})^2$
10-10	**0**
10-10	**0**
10-10	**0**
10-10	**0**
10-10	**0**
	∑= 0

So Standard Deviation Data set B = √[($(10-10)^2+(10-10)^2+(10-10)^2+(10-10)^2+(10-10)^2$)/5] = 0

Standard Deviation Data set C

$(X-\overline{X})$	$(X-\overline{X})^2$
1-10	81
1-10	81
10-10	0
19-10	81
19-10	81
	∑=324

So Standard Deviation Data set C = √ [($(1-10)^2+(1-10)^2+(10-10)^2+(19-10)^2+(19-10)^2$)/5] = 8.05

(c) Data set C has the largest standard deviation

Object 3- Take 20 plants under observation and observed the growth of plants on the basis of plant height, which is given- as height (X) 10, 13, 17 and 20 cm. Calculate standard deviation of following.

Work Procedure- X= 10, 13, 17, 20

Mean ($\overline{X}$)= $X_1+X_2+X_3+X_4+X_5+------X_n/ N$]

Mean ($\bar{X}$)=10+13+17+20

Mean ($\bar{X}$) =15

Standard deviation (σ') = $\sqrt{1/n} \sum (X-\bar{X})^2$

$(X-\bar{X})$	$(X-\bar{X})^2$
10-15= -5	25
13-15= -2	4
17-15= 2	4
20-15= 5	25
	∑=58

Standard deviation (σ') = $\sqrt{1/n} \sum (X-\bar{X})^2$

Object 4: Calculate the mean and Standard deviation from the following data

Value	90-99	80-89	70-79	60-69	50-59	40-49	30-39
Frequency	2	12	22	20	14	4	1

Work Procedure

Calculation for mean and S.D

Class	*x*	*f*	d=(x-64.5)/10	*Fd*	fd^2
90-99	94.5	2	3	6	13
80-89	84.5	12	2	24	48
70-79	74.5	22	1	22	22
60-69	64.5	20	0	0	0
50-59	54.5	14	-1	-14	14
40-49	44.5	4	-2	-8	16
30-39	34.5	1	-3	-3	9
		N=75		∑ *fd*=29	∑ fd^2=127

$\bar{x}$=A + h $\frac{\sum fd}{N}$

=64.5+ (10X27)/75=68.1

S.D= h $(\sum(fd^2/N)-(\sum fd/N)^2)^{1/2}$

= 10X $[127/75-(27/75)^2]$

=10X1.2505= **12.505**

12.7 STUDY OF CHI-SQUARE TEST

The chi-square test is an important test amongst the several tests of significance developed by statisticians. Chi-square, symbolically written as $\chi 2$ (Pronounced as Ki-square), is a statistical measure used in the context of sampling analysis for comparing a variance to a theoretical variance. As a non-parametric* test, it "can be used to determine if categorical data shows dependency or the two classifications are independent. It can also be used to make comparisons between theoretical populations and actual data when categories are used." Thus, the chi-square test is applicable in large number of problems. The test is, in fact, a technique through the use of which it is possible for all researchers to (i) test the goodness of fit; (ii) test the significance of association between two attributes, and (iii) test the homogeneity or the significance of population variance. The chi square is computed on the basis of frequencies in a sample and thus the value of chi square so obtained is a statistic.

Chi-square (X^2) test

A statistical test to determine if the "observed" numbers deviate from those "expected" or

"Theoretical" number under a particular hypothesis.

$$\mathbf{X^2} = \sum_{i=1}^{n} \left[|(O - E)| - \frac{1}{2} \right]^2 / \text{E}$$

Where O= Observed frequency

E= expected frequency

½=.5= Yates correction

Chi-square (x^2) test was first used in testing statistical hypothesis by *KARL PAERSON* in the

year 1900.

Calculation:

a. Calculate all the expected frequencies i.e, E for all values of i = 1,2,3…….n.
b. Take the difference between each observed frequency 'O' and the corresponding expected frequency 'E' for each value of i.e ,(O-E).
c. Square the difference for each value i.e., $(O-E)^2$ for all values of i=1,2,3,..n.
d. Divide each square difference by the corresponding expected frequency i.e, calculate $(O-E)^2\backslash E$ for all values of i=1,2,3...n.
e. Add all these quotients obtained in steps '4' i.e,

$\mathbf{X^2} = \sum_{i=1}^{n} \left[|(O - E)| - \frac{1}{2} \right]^2 / \text{E}$ $\frac{1}{2}$ =Yates correction It is the required value of chi-square.

Important characteristics of chi-square

1) The value of chi-square is always positive as each pair is squared up.
2) X^2 (Chi-square) will be zero if each pair is zero and it may be assume any value extending to infinity, when the difference between the observed frequency and expected frequency in each pair are unequal .Thus chi-square lies between 0&∞.
3) Chi-square is a statistic not a parameter.

A. Test for Goodness of Fit (Pearsonian-X^2)

As a test of goodness of fit, $\chi 2$ test enables us to see how well does the assumed theoretical distribution (such as Binomial distribution, Poisson distribution or Normal distribution) fit to the observed data. When some theoretical distribution is fitted to the given data, we are always interested in knowing as to how well this distribution fits with the observed data. The chi-square test can give answer to this. **If the calculated value of $\chi 2$ is less than the table value at a certain level of significance, the fit is considered to be a good one** which means that the divergence between the observed and expected frequencies is attributable to fluctuations of sampling. But **if the calculated value of $\chi 2$ is greater than its table value, the fit is not considered to be a good one.**

B. Test For Independence of Attributes or Contingency Chi –square:

1) It is applied to test the association between the attributes when the sample data is presented in the form of contingency table with any number of rows or columns.
2) It is occasionally desirable to compare one set of observation taken under particular condition to those of a similar nature taken under different condition.
3) In this case there are no definite expected values; the question is, whether the results are dependent (contingent upon) or independent of condition under which they are observed. The test is therefore is called a test for independence or contingency test.

Calculations

1) Set up the null hypothesis (Ho): No association exit between the attributes.

2) Calculate the expected frequency 'E' corresponding to each cell by the formula

$Eo = R_i \times C_j / n$

Where Ri = Sum total of the row in which E_{ij} is lying

C_j = Sum total of the column in which E_{ij} is lying

n = total sample size

I. Calculate formula for determining chi-square (x^2)

$$x^2 = \sum \frac{(O-E).(O-E)}{E}$$

E = Expected value
O = Observed value

Here degrees of freedom
df = (R-1)(C-1)

R = Number of rows
C = Number of columns

II. Find from the table the value of chi-suare for the given value of the level of significance (α) and for degree of freedom (df).

III. If no value for α is mentioned, then the table α = 0.05.V. (i).Compare the computed value of chi square with with the tables values of x^2

(ii).If the calculated value of X^2<Tabulated value then accept the hypothesis (Ho)

If X^2> Tabulated value then reject the null hypothesis & accept the alternative hypothesis

N.B= It is used in (2×3) contingency tests.

OR

Other calculations

I. Set up (a) null hypothesis (H_o): There is no association exist between the attributes.

(b) Alternative Hypothesis (H_1): An association exists between the attributes.

II. Here contigency table has only 2 rows and 2 columns with four cell frequency viz a,b,c,d as shown below:

a	b	R_1
c	d	R_2
C_1	C_2	N

I. The frequency ‘a’ is placed in the upper left cell and d’ is placed in the lower right cell

The frequency ‘b’ & “c’ are placed in other diagonal position.

For simplicity R_1 and R_2 denote row totals and C_1 and C_2 denote the number of columns totals.

Therefore

$$x^2=\frac{N\left\{(ad-bc)-\frac{N}{2}\right\}^2}{R_1 \cdot R_2 \cdot C_1 \cdot C_2}$$

C. Homogeneity Chi-Square

A test of "homogeneity" must be performed to decide whether the separate samples are sufficiently uniform to be added together.

Calculations

1) The chi-square of each individual sample should be calculated based on expected ratio. since these chi- square are to be added, they Yates correction factor should not be used, even though only 1 degree of freedom may be involved in each calculation.

2) The individual chi-square should be summed to give a total chi-square. In this process total chi-square accumulated a number of degrees of freedom equal to the sum of the degrees of freedom in the individual chi- square.

The total chi-square value has two components.

(a) The chi-square contributed by the departure of the pooled data from the expected ratio.

(b) The chi-square contributed by the difference between individual samples.

To calculate 'b',& 'a' and subtract the value from total chi-square.

3) Calculate chi-square for the summed data of all samples.

Subtract the chi-square of for the summed data from the summed chi-square to obtain the homogeneity chi-square.

Accompany this, by subtracting the number of degrees of freedom between these respective values to obtain degrees of freedom for homogeneity chi-square.

Object 1: A genetics engineer was attempting to cross a tiger and a cheetah. She predicted a phenotypic outcome of the traits she was observing to be in the following ratio 4 stripes only: 3 spots only: 9 both stripes and spots. When the cross was performed and she counted the individuals she found 50 with stripes only, 41 with spots only and 85 with both. According to the Chi-square test, did she get the predicted outcome?

Work procedure

Chi-square = $\Sigma (O-E)^2 /E$

D.F.	Value
1	3.841
2	5.991
3	7.815

Expected ratio	Observed #	Expected #	O-E	$(O-E)^2$	$(O-E)^2/E$
4 stripes	50	44	6	36	0.82
3 spots	41	33	8	64	1.94
9 stripes/spots	85	99	-14	196	1.98
16 total	176 total	176 total	0 total		Sum = 4.74

4/16 * 176 = expected # of stripes = 44

3/16 * 176 = expected # of spots = 33

9/16 * 176 = expected # stripes/spots = 99

Degrees of Freedom = N-1 = 3 - 1 = 2 (3 different characteristics - stripes, spots, or both)

Since 4.74 is less than 5.991, the null hypothesis put forward by the engineer.

1. **Object 2:** In the garden pea, yellow cotyledon color is dominant to green, and inflated pod shape is dominant to the constricted form. Considering both of these traits jointly in self-fertilized dihybrids, the progeny appeared in the following numbers 193 green, inflated, 184 yellow constricted, 556 yellow, inflated 61 green Do these genes assort independently? Support your answer using Chi-square analysis.

Work procedure:

Genes assort independently (are NOT on the same chromosome and NOT linked) if they follow the 9:3:3:1 rule (on the 16 square Punnett square) resulting from a dihybrid cross. In this dihybrid cross

Observed	556	184	193	61
Expected	559	186	186	62

The total observed is 994, the expected values are:

9/16= x/994 x= 559
3/16= x/994 x= 186

1/16= x/994 x= 62

Chi square= $[(556-559)^2 /559] + [(184-186)^2/186] + [(193-186)^2/ 186] + [(61-62)^2/62]$

= (0.016) + (0.02) + (0.26) + (0.016)

= 0.312

df= 3

p value from table at 0.05 is 7.815

Since the calculated value is much lower than the p value from the table, so we cannot reject the null hypothesis. The genes assort independently according to a 9:3:3:1 ratio and are not on the same chromosome.

Object 3: A poker-dealing machine is supposed to deal cards at random, as if from an infinite deck. In a test, you counted 1600 cards, and observed the following:

Spades	404
Hearts	420
Diamonds	400
Clubs	376

Could it be that the suits are equally likely? Or are these discrepancies too much to be random?

Work procedure

	Expected	expected	
observed	(percent)	(counts)	z
404	0.25	400	0.200
420	0.25	400	1.000
400	0.25	400	0.000
376	0.25	400	-1.200

chi-square-> 2.480

critical value-> 7.815

Compute each z from its own row as (observed-expected)/sqrt (expected). Using the counts in this formula, not the percentages. The chi-square statistic is the sum of the squares of the z-values.

The number of degrees of freedom is 3 (number of categories minus 1). The critical value is from a table you'll have on the exam (using $\alpha = 0.05$).

12.8 SUMMARY

Statistics is a very broad subject, with applications in a vast number of different fields. In generally one can say that statistics is the methodology for collecting, analyzing, interpreting and drawing conclusions from information. Putting it in other words, statistics is the methodology which scientists and mathematicians have developed for interpreting and drawing conclusions from collected data. Everything that deals even remotely with the collection, processing, interpretation and presentation of data belongs to the domain of statistics, and so does the detailed planning of that precedes all these activities.

Statistical analyses need condensation and manipulation of huge amount of data to a meaningful representative form at the very first sight. Summarisation, condensation and classification simplify the huge data, improve its main characteristics and to a comparison of data obtained from different sources. In some cases, data is condensed to a single value, especially in cases where a direct comparison is to be made between data obtained from different sources. Such a single value expression or presentation of data is called central value. It is used to represent a whole series. It neither includes the lowest value of the series nor the highest value, but a value somewhere between these two limits and possibly in the centre, where most of the values of the series cluster. This central value of the series is also called the central tendency or average. The measures devised to calculate the central tendency are known as measures of central tendency. It may be easily subjected to further mathematical calculations by using mean, median mode standard deviation and chi square

test'. An average value could be preferred to others if it is capable to be used for further statistical computation.

12.9 GLOSSARY

Analysis bias (for data)-Gearing data analysis to support a particular hypothesis and ignoring aspects that contradict the hypothesis, e.g., using an inflated P-value for some statistical tests, using proportions when mean is appropriate, etc.

Analysis of variance- Breaking variance into its components such as within groups and between groups. This method is used in regression analysis and in various other situations but more commonly for comparing three or more means.

Analysis -The process of going into the deep of a phenomenon, data-set, thought, etc., and looking at its various components.

Arithmetic mean -Same as mean.

Binary variable: A variable whose only two possible values, usually zero and one.

Biostatistics -The science dealing with medical uncertainties in one or more groups of subjects-their identification, measurement, and control—leading to decision with least error.

Chi-square test-A versatile statistical procedure that is used to test different types of hypothesis on proportions, such as equality, trend and relationship.

Data- A set of observations, generally in numerical format but can be in text format also (plural of datum).

Degree of freedom: The number of values in the final calculation of a statistic that are free to vary

Frequency distribution: It is a table that displays the frequency of various outcomes in a sample. Each entry in the table contains the frequency or count of the occurrences of values within a particular group or interval, and in this way, the table summarizes the distribution of values in the sample.

Frequency- Used in two senses: 1. Frequency of occurrence per unit of time (per month, per year, etc.). 2. Number of subjects with a particular characteristic or with values in a particular interval, such as how many have fruits in apple trees.

Goodness of fit - How well the actual observations fit into a specified pattern. The goodness of fit is statistically tested mostly by chi-square method.

Mean: Arithmetic average, i.e., the sum of all the values divided by the number of observations. The mean of a binary variable is equal to the proportion of ones because the sum of all the zero and one values equals the number of ones. The mean can be heavily influenced by outliers.

Median-The most middle value obtained after arranging values in increasing or decreasing order. Median seeks to divide the group in two equal halves, each with n/2 individuals. Sometimes in practice exactly equal halves are not possible, and they are divided into nearly equal halves.

Mode- The most commonly occurring value, i.e., a value seen in highest number of subjects

Null hypothesis: Customarily but not necessarily a hypothesis of no effect, e.g., no reduction in means blood pressure or no correlation between age and blood pressure. The null

hypothesis, labeled H0, is often used in the frequents branch of statistical inference as a "straw person"; classical statistics often assumes what one hopes doesn't happen (no effect of a treatment) and attempts to gather evidence against that assumption (i.e., tries to reject H0). H0 usually specifies a single point such as 0mmHg reduction in blood pressure, but it can specify an interval, e.g., H0: blood pressure reduction is between -1 and +1 mmHg. "Null hypotheses" can also be e.g. H0: correlation between X and Y is 0.5.

Observational study: Study in which no experimental condition (e.g., treatment) is manipulated by the investigator, i.e., randomization is not used.

Quantitative data-Collection of observations on characteristics that could be numerically expressed for an individual such as number of fruites, plant species, and weight of seeds. Most common summary measures for quantitative data are mean and standard deviation (SD).

Sample -A part of the target population, which is actually studied. Sample size — The number of subjects or units in a sample.

Sampling-Choosing a part from the whole, such as choosing 300 child births out of 5000 in a hospital in one year for studying the intrauterine growth retardation. See random sampling, purposive sampling.

SD -Short for standard deviation

Standard deviation (SD)-Most common and generally most appropriate measure of dispersion obtained as positive square root of variance.

Standard error: The standard deviation of a statistical estimator. For example, the standard deviation of a mean is called the standard error of the mean, and it equals the standard deviation of individual measurements divided by the square root of the sample size. Standard errors describe the precision of a statistical summary, not the variability across subjects.

Statistic - A summary measure for any characteristic in the sample or the group actually studied, such as mean, median or standard deviation of a sample, or proportion of subjects found affected in a sample.

Statistical analysis-Subjecting data to the rigours of statistical methods so that the uncertainty levels are either quantified or minimized, or both.

Variable - A characteristic that varies from person to person, or from situation to situation. Platelet count in different persons is variable but number of eyes or number of fingers is not a variable. See quantitative variable, qualitative variable, discrete variable, continuous variable, dependent variable, and independent variable.

Variance -A measure of dispersion or scatteredness of quantitative data obtained as average of the squared deviations from mean

12.9 SELF-ASSESSMENT QUESTIONS

12.9.1 Very Short Answer Type Questions:

1. Give formula for calculating mode in a continuous series.
2. How can relationship between mean, median and mode be depicted by an equation?
3. Give one difference between median and mode.
4. How many types of central tendencies are used in statistics?

5. What is the measure of averages of position called?
6. What is combined arithmetic mean?
7. When is mode calculated?
8. Why is median value required?

12.9.2 Short Answer Type Questions:

1. Describe requisites for an ideal measure of central tendency.
2. Describe merits and demerits of mode.
3. Write a note on arithmetic mean.
4. Difference between mean and median
5. Explain role of statistics in botany
6. Write various step involved in formation of frequency distribution table
7. What is standard deviation? How you would calculate equation for standard deviation.
8. Define ogives.
9. Describe merits and demerits of the four important measures of central tendency
10. What is statistical average? What are criteria for good average? Examine them for each.
11. Why average are called measures of central tendency

12.9.3 Fill in the Blanks:

1. Average obtained arithmetically is called …….. arithmetic.
2. An average is a measure of …….. .
3. The sum of deviations of individual items from arithmetic mean is always …….. .
4. The most popularly used measure of central tendency is …….. mean.
5. An average reduces the large number of observations to ……
6. Harmonic mean is …….. of arithmetic mean of a given observation.
7. …… is the quickest way to measure the central tendency.
8. The reciprocal number in arithmetic progression is called …….. progression.
9. …… is greatly affected by fluctuations.
10. …… is the positional average.

12.9.3 Answer Key:

1. mean, 2. central tendency, 3. zero, 4. arithmetic, 5. a single figure, 6. reciprocal, 7.mode, 8. harmonic, 9. arithmetic mean, 10. median

12.10 REFERENCES

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12.12 TERMINAL QUESTIONS

Example 1 .Calculate mean of following data

Trees	0-10	10-20	20-30	30-40	40-50
Frequency	22	38	46	34	20

Example 2: Calculate standard deviation for following data

Flowers	60-62	63-65	66-68	69-71	72-74
Number of plant	5	18	42	27	8

Example 3: From following find mean and median:

Age	200	230	405	321	175	600	520
No. of Workers	12	30	35	65	45	25	18

Example 4: Calculate mean and standard deviation of following data
20, 22, 27, 30, 48, 45, 32, 31, 35

Example 5: Calculate the Mean for the following frequency distribution:

Marks	0-10	10-20	20-30	30-40	40-50	50-60	60-70
No. of Students	6	5	8	15	7	6	3

Example 6: Find the mean of the following set (. 8, 11, 6, 22, 3) of integers

Example 7: The mean score of a group of 20 students is 65. Two other students whose scores are 89 and 85 were added to the group. What is the new mean of the group of students?

Example 8: Find the median in the set of numbers given below
15,16,15,7,21,18,19,20,11

Example 9: Find the Mode of the following data set.
3,12,15,3,15,8,20,19,3,15,12,19,9

Example 10: Find the median of the following set of points in a game:
15, 14, 10, 8, 12, 8, 16

Example 11: Find the mean, median and mode of the following set of data:
23, 29, 20, 32, 23, 21, 33, 25

Example 12: What is the mode of this group of numbers?
2, 2, 4, 6, 3, 4, 2, 5, 8

Example 13: Find the mode of the following set of scores: 14 11 15 9 11 15 11 7 13 12

Example 14: Find the standard deviation for the following data series: 12,6,7,3 15,10,18,5

Example 15: Find the standard deviation for the following series of numbers:
2, 3, 6, 8, 11
12, 6, 7, 3, 15, 10, 18, 5

Example 16: Find the standard deviation for the series:
3, 5, 2, 7, 6, 4, 9
3, 5, 2, 7, 6, 4, 9, 1

Example 17: Given the statistical distribution of the table Calculate the standard deviation.

x_i	**61**	**64**	**67**	**70**	**73**
f_i	**5**	**18**	**42**	**27**	**8**

Example 18: A statistical distribution is given by the following table. Calculate the standard deviation

x_i	**[10, 15)**	**[15, 20)**	**[20, 25)**	**[25, 30)**	**[30, 35)**
f_i	**3**	**5**	**7**	**4**	**2**

Example 19: Find the mode for each of the following frequency tables: The frequency table below shows the weights of different bags of rice.

Weight (kg)	45	50	55	60	65	70	75	80
Bags of rice (Frequency)	8	11	7	10	9	10	12	8