INDEX

SEMESTER- III

PRACTICAL: I (Plant Diversity- II)

ALGAE - *SARGASSUM*

Classification

Division: Phaeophyta
Class: Phaeophyceae
Sub class: Cyclosporae
Order: Fucales
Family: Sargassaceae
Genus: ***Sargassum***

THALLUS STRUCTURE:

The plant body is diploid and sporophytic. Sporophyte is thalloid, erect, well branched with a hold fast and main axis. Height of plant varies from species to species. Main axis produces no. of primary and secondary branches. Primary branches produce leaf like secondary laterals which are called leaves. Leaves are simple, flat, broad and with midrib. Their margins may be serrate, dentate or entire with acute apex. Lower portion of main axis produces no. of air bladders instead of leaves. They help in floating of plant and its respiration. From axillary position of some leaves, some long, repeatedly branched, cylindrical or flat structures are produced called receptacles. Receptacles have many flask shaped conceptacles in which sex organs are present.

SEXUAL REPRODUCTION:

Sexual reproduction occurs through production of sex organs inside conceptacles. Male and female sex organs are produced in different conceptacles. Conceptacles open by small openings called ostioles.

Male sex organ:

Male sex organs are antheridia which are club shaped or oval and are produced on paraphyses inside conceptacle

Female sex organ:

Female sex organs are produced on the walls of conceptacle. The oogonium (female sex organ) remains attached to conceptacle wall by means of a mucilaginous stalk. A single egg is developed in an oogonium.

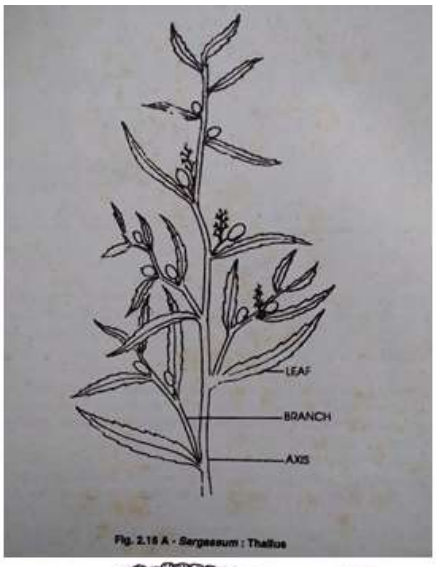


Fig. 2.16 A - *Sargassum* : Thallus

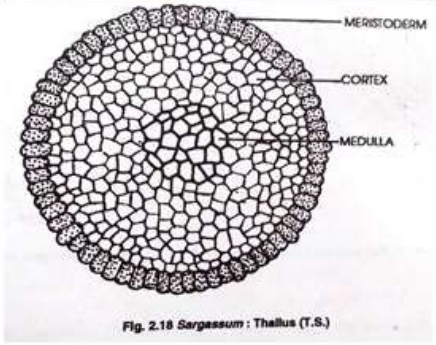


Fig. 2.18 *Sargassum* : Thallus (T.S.)

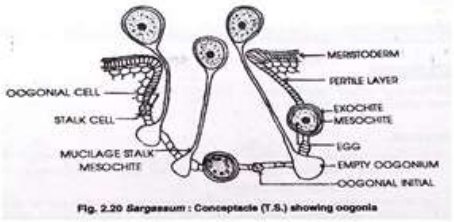


Fig. 2.20 *Sargassum* : Conceptacle (T.S.) showing oogonia

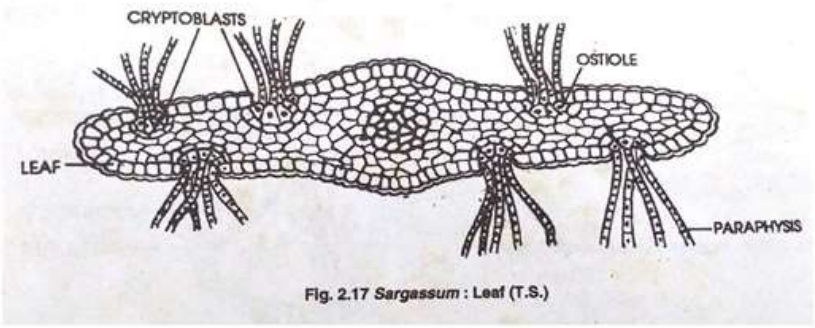


Fig. 2.17 *Sargassum* : Leaf (T.S.)

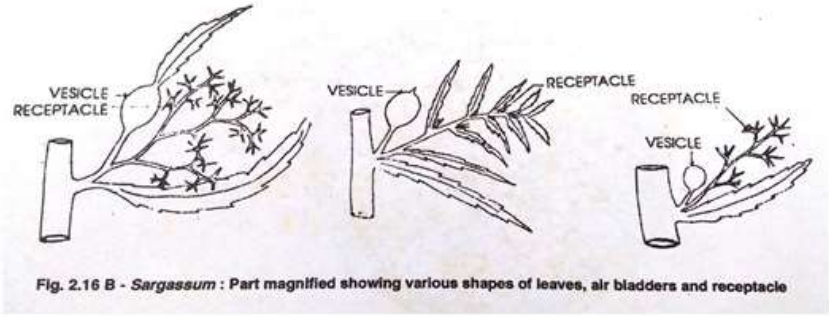


Fig. 2.16 B - *Sargassum* : Part magnified showing various shapes of leaves, air bladders and receptacle

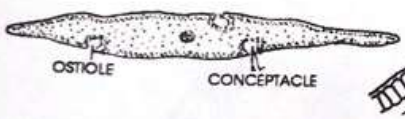


Fig. 2.19 (A) *Sargassum* : Receptacle (T.S.)

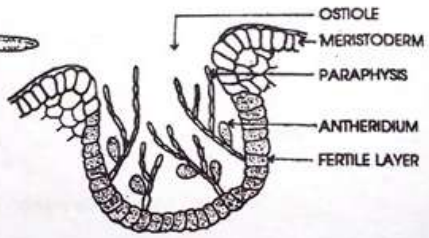


Fig. 2.19 (B) *Sargassum* : Receptacle (T.S.) and conceptacle (section) showing antheridia

RANGE OF THALLUS IN PHAEOPHYTA

The plant body in brown algae is highly complexed and well organized. Unicellular, colonial, coccoid and unbranched filamentous thalli are absent. Except a few microscopic members of Ectocarpales, Tilopteridales and Sphacelariales, most of the brown algae are macroscopic and very large in size. Because of their bulky appearance they have been called 'Giant Kelps'. Brown algae show basically three types of thallus organization – **Heterotrichous, Pseudoparenchymatous** and **Parenchymatous**.

Heterotrichous: Members of Ectocarpales, Tilopteridales and Sphacelariales show the characteristic heterotrichous habit. This is said to be the simplest and most primitive habit. The plant body is differentiated into a creeping prostrate system and an erect projecting system. In *Giffordia loonifera* both the systems are equally developed, but in *Ectocarpus sp*. the erect system is more developed. In *Ectocarpus parasiticus* erect system is extremely reduced.
Many of the brown algae, which are devoid of heterotrichous constructions, show pseudoparenchymatous (haplostichous) or parenchymatous (polystichous) constructions.

Pseudoparenchymatous: Haplostichous ectocarpales (*Hecatonema sargassicola*) form cushion like pseudoparenchymatous growth. The pseudoparenchymatous thallus organization of *Myriatula*, *Myrionema*, *Hecatonema* and *Leathesia* is multiaxial. Band- shaped or fan shaped thalli are found in Cutleriales, whereas cylindrical or flattened thalli are found in Sporochnales. Multiaxial pseudoparenchymatous organization is replaced by uniaxial organization in higher Ectocarpales, Cutleriales, Sporochnales and Desmarestiales. This habit is exemplified by *Nemacystus decipiens*.

Parenchymatous: This thallus habit is shown by many members. Undifferentiated parenchymatous construction is exhibited by *Phloeospora*, but in many members of Dictyo-siphonaceae, Laminariales and Fucales it is well developed and differentiated. Besides the dichotomous ribbon shaped habit of *Dictyota* and *Dictyopteris*, Laminariales attain a highly elaborate external and internal organization in *Dictyota*, *Macrocystis* etc. Plant body is divided into holdfast, stipe and frond or lamina.

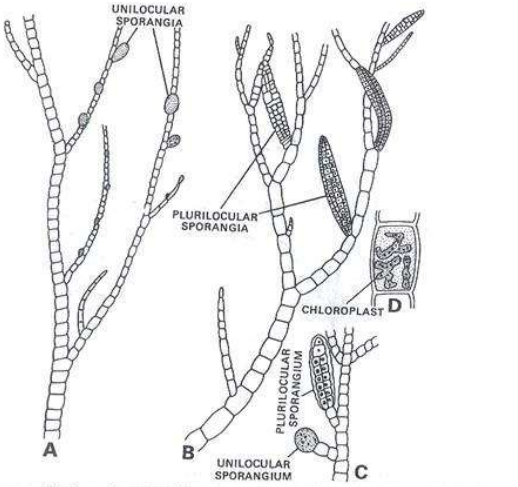
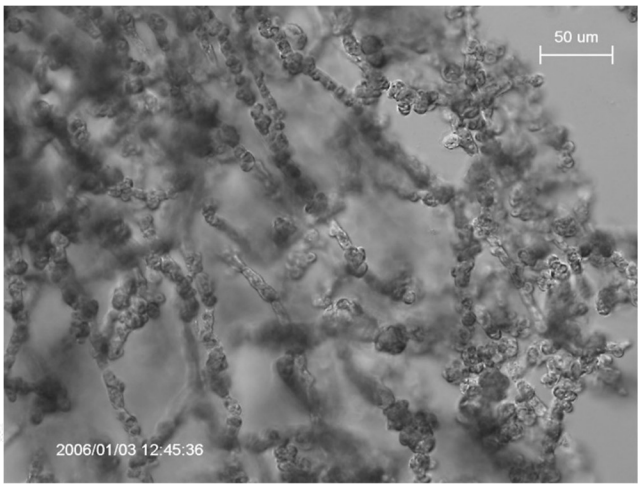
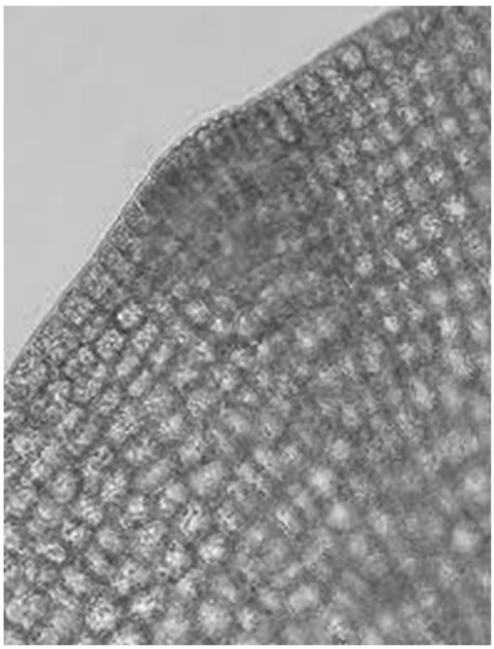


Fig. 6.3. *Ectocarpus siliculosus*. A, a part of the plant with unilocular sporangia; B, a part of the plant with plurilocular sporangia; C, a part of the plant of *E. cylindricus* with both uni-and plurilocular sporangia; D, a cell.

Heterotrichous thallus



Psuedoparenchymatous thallus



Parenchymatous thallus

ECONOMIC IMPORTANCE OF PHAEOPHYTA

The brown algae provide food and shelter for fish and other marine animals. In different parts of world, these algae are used as food or in food preparation and as fodder. They are also good source of carbohydrates, vitamins and minerals. Few examples are as follows –

i) *Laminaria*:

It is a marine alga commonly known as **'Kelp'**. Lamina or blade is terminal expanded leaf- like borne terminally on stipe. The thallus is rich in proteins, fats and vitamins. It acts as good laxative and helps in digestion. Laminarin lowers the level of lipids in blood. Algin is obtained from *Laminaria* is used in a number of industrial processes. It has the property of thickening and gelling mixtures. Alginic acid helps to treat lead poisoning. Young stipe is edible.

ii) *Sargassum*:

It is a marine alga commonly known as **'Gulf Weed'**. It looks like aquatic angiosperm.

The thallus is rich in proteins, fats and vitamins. It contains dietary fibre and minerals such as Iron, Calcium, Magnesium etc. Some are consumed fresh, others are cooked in coconut milk or a little vinegar or lemon juice. It is smoke-dried to preserve it. It contains antibacterial fatty acids, antioxidants and is mild diuretic. In Chinese medicine, it is dried, powdered and used to make tea to control phlegm. It is used as raw material for biodiesel production. Oil can be easily converted to biodiesel fuel by transesterification of triglycerides.

iii) *Fucus*:

It is a marine alga commonly known as **rockweed'**. It grows up to 30 cm. Thallus is dark brown, leathery and slimy to touch. The frond (lamina) is a repeatedly branched, ribbon like with a distinct midrib. Air bladders are present on the surface of the frond. Primary chemical constituents of this orga nism include mucilage, algin, mannitol, beta carotene, zeaxanthin, iodine, bro mine, potassium, volatile oils, and many other minerals. In Scotland and Norw ay, up until the mid 19th century, several seaweed species from *Fucus* and other genera were harvested, dried, burned to ash, and further processed to become "kelp", which was a type of soda ash. It has an alkali content of about 2.5%–5% that was mainly sodium carbonate (Na_2CO_3). Alkali is essential to soap-making, glassmaking, and other industries. The seaweed was also used as fertilizer for crop land in the same areas in which it was harvested.

In 2005, it was announced that bacteria grown on *Fucus* have the ability to attack and kill the MRSA superbacterium. Some people may suffer an allergic reaction to the iodine in bladder wrack. *Fucus* to have the highest antioxidant activity from a range of edible seaweeds, possibly due to the presence of Fucoxanthin. *Fucus* species have often been reported to have a direct effect on the metabolism of the human body which shows potential for controlling weight and cellulite deposits.

iv) Macrocystis: Also known as 'Giant Kelp' or 'Giant Bladder Kelp'. This genus contains the largest of all the brown algae. It is widely distributed in subtropical and temperate oceans. It grows upto 60 mts. (200 ft.) and has the fastest linear growth. Stipes are unbranched and each blade has a gas bladder at its base. ***Macrocystis pyrifera*** is a commercially important seaweed. Humans harvest this kelp for its rich iodine, potassium, polysaccharides, and other minerals. The primary product obtained from giant kelp is algin for alginates. Algin is an emulsifying and binding agent used in the production of many foods and cosmetics, such as salad dressings, ice

cream, and toothpaste. Because *Macrocystis pyrifera* lacks tannins, it is more palatable than some other species of brown algae, and is sometimes eaten raw.

The demand for *Macrocystis pyrifera* is increasing due to the new found uses of these plants in commercially available products like these below:

- In anti-aging cream.
- cleansing milk, cleansing gel, and as a skin cleanser.
- Body polish, facial mask, and makeup remover.
- Shampoo and conditioner.
- Nutritional and dietary supplements.
- Seasonings and spices.
- Animal feed
- Fertilizers and soil treatments

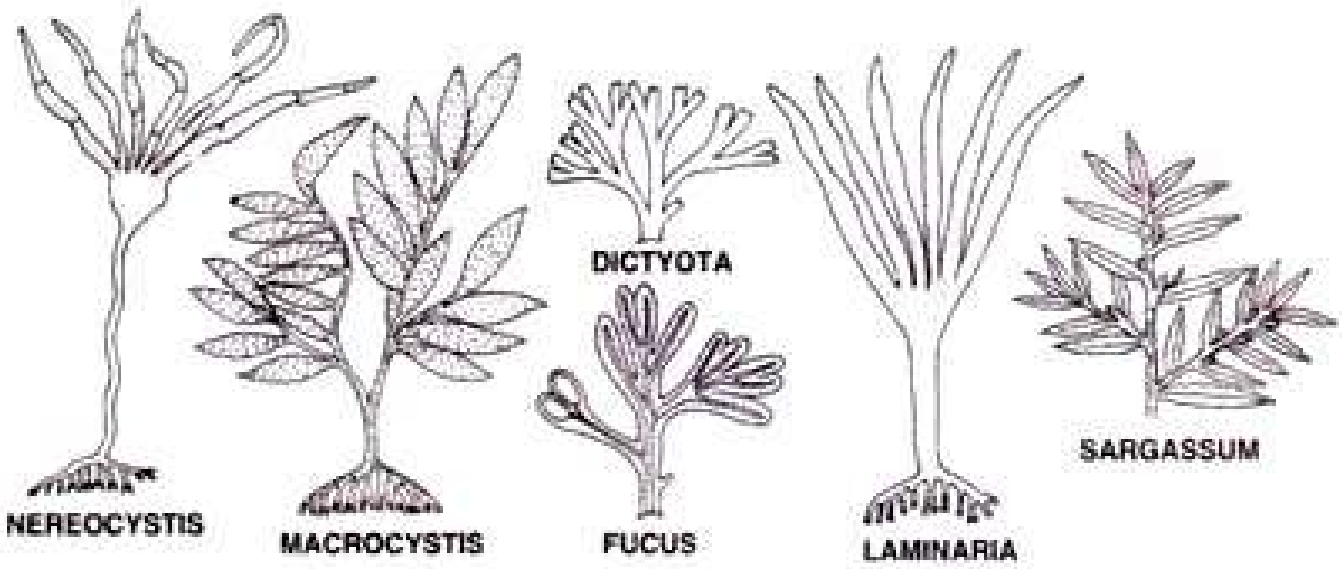


Fig. 3.2. Some Brown Algae.

BRYOPHYTA - *ANTHOCEROS*

Classification:

Division: Bryophyta

Class: Anthocerotae or Anthocerotopsida

Order: Anthocerotales

Family: Anthocerotaceae

Genus: ***Anthoceros***

STRUCTURE STUDY:

The thallus is dorsiventrally flattened, irregularly lobed and has folded margins. It is yellowish to dark green in colour and prostrate. The upper surface of the plant is smooth. There is no distinct mid rib. The scales are absent. Areolae are absent. On the ventral side develop simple, rhizoids. The tuberculated rhizoids are absent. Also on the lower surface are small rounded dark coloured areas. These are Nostoc colonies. Present on the upper surface are horn-like sphorophytes.

V. S. OF THALLUS:

Internally, the thallus shows no differentiation of tissues into photosynthetic and storage regions. The outermost layer is formed of compactly arranged cells forming upper epidermis. Next to the upper epidermis is storage region formed of parenchymatous cells. Each cell possesses a single chloroplast with a single pyrenoid in the centre of the chloroplast. Ventral portion shows mucilage-filled schizogenuous cavities having Nostoc colonies.

In some species there are stomata-like pores or slits called the slime pores which are guarded by two bean-shaped guard cells. Slime pores lead into the large mucilage cavities. The guard cells do not control the opening of the pores. They are always open. From the lower epidermis a number of unicellular, simple rhizoids develop.

SEXUAL REPRODUCTION: Sex organs are embedded in gametophytic tissue.

L.S. of thallus showing antheridia:

The antheridia are present in the antheridial cavity or chamber. The roof of the chamber is of two layers. Each antheridial cavity contains 1 to 4 antheridia. The secondary antheridia may develop from the buds formed on the stalks of primary antheridia.

Antheridium:

The male sex organ (antheridium) consists of a stalk and a body. The body has a single jacket layer on its outer side which encloses numerous androcytes. The androcytes metamorphose into biflagellate antherozoids. The antheridia are enclosed in antheridial cavities or chambers having a two-celled thick roof.

L.S. of thallus showing archegonia:

The archegonia are embedded in the thallus, only the cover cells project beyond the surface of the thallus. The archegonia are in direct contact with vegetative cells.

Archegonium:

The female sex organ (archegonium) consists of four to six neck canal cells, a ventral canal cell and an egg. There is no sterile jacket layer, but four cover cells form the lid cells. The lid cells project above the surface of the thallus.

L.S. of sporophyte:

The sporophyte is formed due to division and redivision of the zygote. It can be differentiated into foot and capsule. Present between the foot and the capsule is the intermediate zone formed of meristematic tissues, ensuing continuous formation of spores. The foot is parenchymatous and is embedded in gametophytic tissue. A capsule can be differentiated into central sterile columella formed of sixteen rows of cells. It gives mechanical support and helps

in conduction. Sporogenous tissue is cylindrical and surrounds the columella. It possesses spores at various stages of development; the terminal region has mature spores and elaters, central region has sporocytes and elaters and the basal zone has sporogenous tissue. Elaters are simple or branched and multicellular. Wall of the capsule is formed of four to six layers of cells. The outermost layer of this wall is epidermis which possesses stomata. Inner layers form the photosynthetic region with cells possessing chloroplasts. The sporophyte of *Anthoceros* is thus a partial parasite.

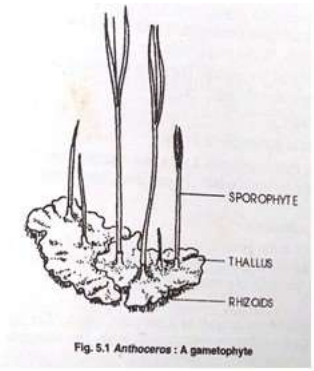


Fig. 5.1 *Anthoceros* : A gametophyte

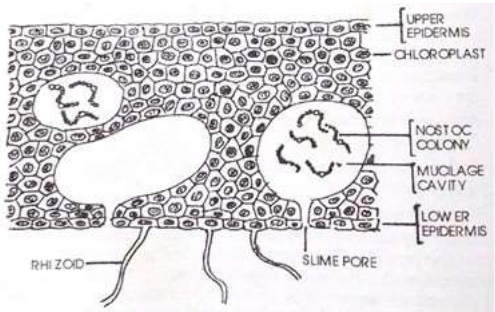


Fig. 5.2 *Anthoceros* : Thallus V.S.

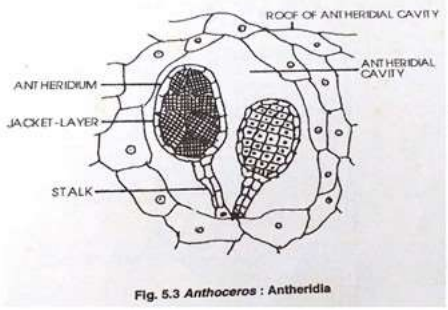


Fig. 5.3 *Anthoceros* : Antheridia

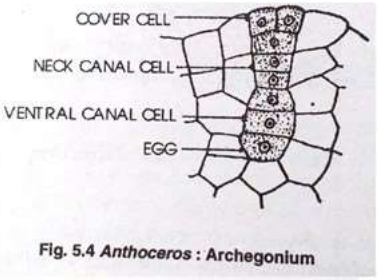


Fig. 5.4 *Anthoceros* : Archegonium

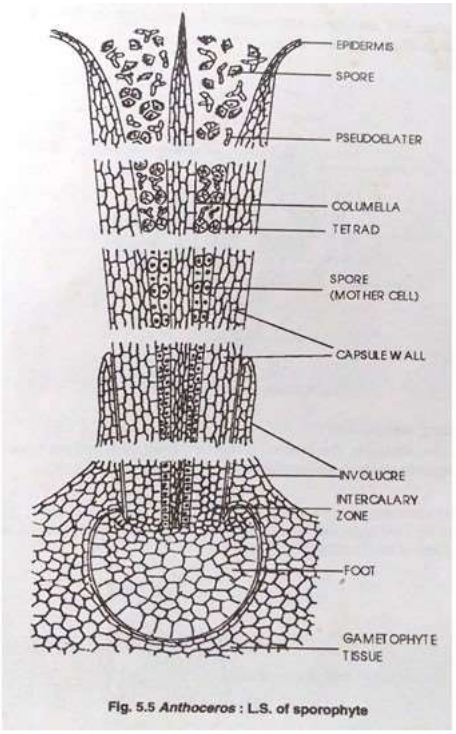


Fig. 5.5 *Anthoceros* : L.S. of sporophyte

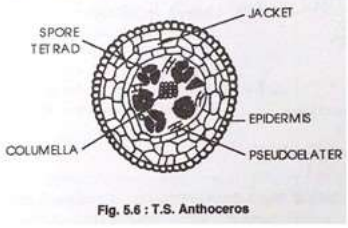


Fig. 5.6 : T.S. Anthoceros

BRYOPHYTA - *FUNARIA*

Classification:

Division: Bryophyta

Class: Musci (Bryopsida)

Sub Class: Eubrya (Bryidae)

Order: Funariales

Family: Funariaceae

Genus: ***Funaria***

EXTERNAL MORPHOLOGY:

Thallus of *Funaria* consists of two stages, the juvenile stage called protonema and the adult stage called gametophore. The gametophore can be differentiated into three regions; (1) rhizoids (2) cylindrical stem and (3) sessile leaves

Rhizoids: They arise from the base of the stem. They are slender, multicellular and branched. The multicellular rhizoids are one-cell thick and possess oblique septa. Functions of rhizoid are fixation and absorption.

Stem (cauloid): The stem of moss is cylindrical and branched. The branches are extra axillary and develop below the leaves.

Leaves (phylloides): They are spirally arranged on stem and branches, and are sessile. Leaves are crowded at the apex forming bud-like structure called the head. The leaf is thin, flat and one cell in thickness except the mid rib.

SEX ORGANS OF MOSS:

L.S. of Antheridial head

Club-shaped antheridia (male sex organs) are intermingled with sterile, colourless paraphyses. The terminal cell of paraphysis is larger than the rest of the cells. The green foliage leaves surrounding the antheridia and paraphysis are called perigonial leaves. Each antheridium is club-shaped consisting of stalk and body. The outermost sterile layer of body is called jacket-layer which encloses androcytes. The antheridia are intermingled with paraphysis.

L.S. of Archegonial head

The flask-shaped archegonia, the female reproductive organs, are intermingled with sterile paraphysis. The archegonia with paraphysis are surrounded by green foliage leaves called perichaetial leaves. The flasked shaped archegonium has basal round venter and a terminal neck. The stalk of the archegonium is short and massive. The venter has ventral canal cell and an egg while neck possesses neck canal cells

EXTERNAL MORPHOLOGY OF SPOROPHYTE:

The sporophyte is seen at the apex of the female branch. It consists of foot, seta and capsule.

Foot: It is multicellular, embedded part of the sporophyte. It is dagger-like and helps sporophyte in attaching to the gametophyte.

Seta: It is a long, slender, stalk-like structure which elevates the capsule above the gametophore.

Capsule: It is pear-shaped body present at the tip of the seta. Capsule at its distal end bears the remenant of archegonial neck called calyptra.

L.S. of Capsule

The capsule shows three distinct regions apophysis, theca and operculum.

Apophysis: It is a basal region of the capsule. The central cells are elongated, colourless and form conducting region. The conducting region is surrounded by parenchymatous cells containing chloroplasts. Sporophyte thus is a partial parasite. The chlorophyllous tissue is surrounded by single layered epidermis having stomata.

Theca: It is the middle region of the capsule, between apophysis and operculum. The central region of theca consists of sterile cells and is called columella. The upper part of columella projects into the operculum. Surrounding the columella is a spore sac containing many spores. The spore-sac is surrounded by an air chamber. The air chamber is traversed by a number of trabeculae. The trabeculae connect the innermost layer of capsule wall to the outer wall of the spore-sac. Epidermis, the outermost layer of the capsule wall is continuous with that of the apophysis but stomata are absent.

Operculum: It consists of three parts operculum, peristome and annulus. Operculum is the lid of the capsule and present at the apex of the capsule. Peristome lies below the operculum and consists of peristomal teeth. Peristomal teeth are in two rings, each ring consisting of 16 teeth. The teeth of outer ring show bars of lignosuberin. The teeth of inner ring are smaller in size and without lignosuberin bars. The peristomal teeth are hygroscopic and help in spore dispersal.

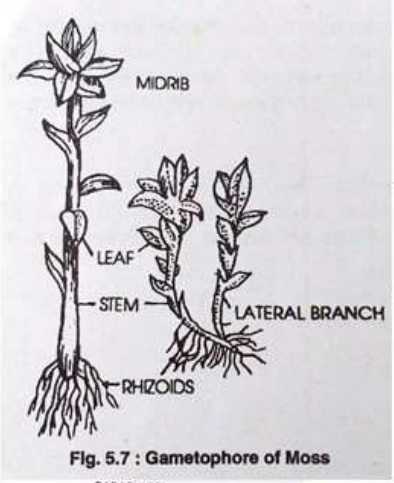


Fig. 5.7 : Gametophore of Moss

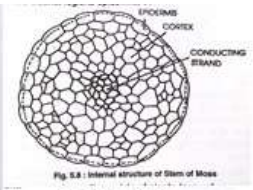


Fig. 5.8 : Internal structure of Stem of Moss

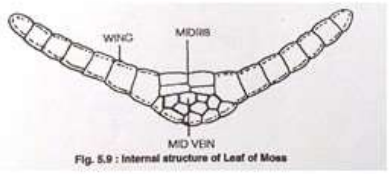


Fig. 5.9 : Internal structure of Leaf of Moss

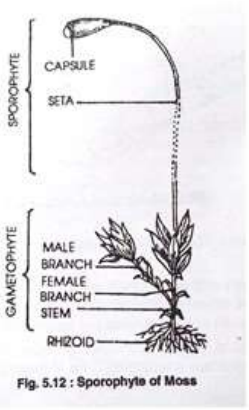


Fig. 5.12 : Sporophyte of Moss

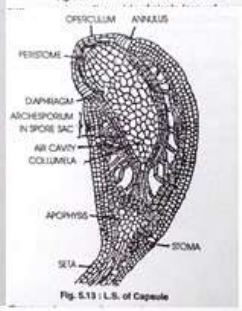


Fig. 5.13 : L.S. of Capsule

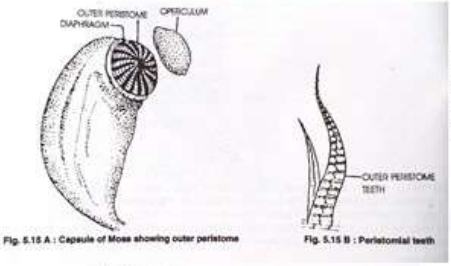


Fig. 5.15 A : Capsule of Moss showing outer peristome
Fig. 5.15 B : Peristomial teeth

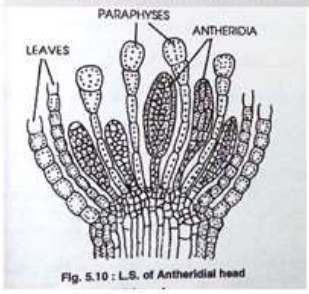


Fig. 5.10 : L.S. of Antheridial head

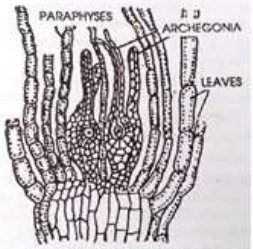


Fig. 5.11 : L.S. of Archegonial head

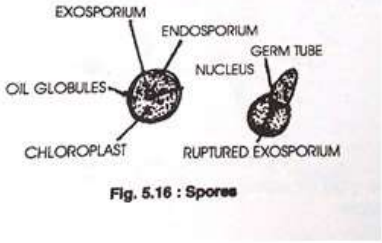


Fig. 5.16 : Spores

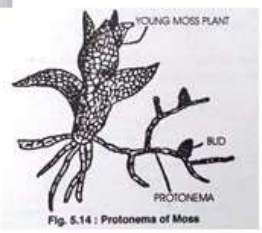


Fig. 5.14 : Protonema of Moss

STUDY OF PLANTS FOR ANATOMY IN RELATION TO TAXONOMY

Aim: To authenticate the leafy vegetable "Takla" (*Senna tora* (L.) Roxb. = *Cassia tora* L.) leaf by anatomical evidence

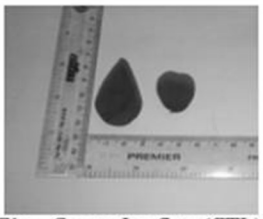
Background: 'Takla', botanically known as *Senna tora* (L.) Roxb. (Syn. *Cassia tora* L.) from family Caesalpiniaceae, is one of the leafy vegetables that is eaten in the beginning of rainy season. In the urban market, this vegetable is available. Many a times, along with *Cassia tora* L. leaves, other species of *Cassia* is mixed.

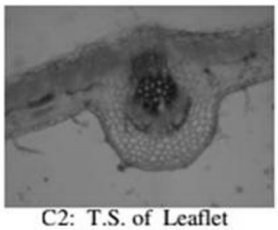
In order to authenticate the *Senna tora* (L.) Roxb. leaf, the microscopic analysis of the market specimen is carried out and compared with the authentic sample collected from the field.

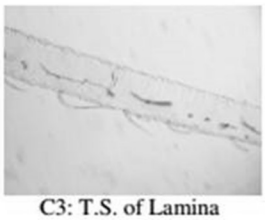
Observation: The authentic sample of *Senna tora* (L.) Roxb. (Syn. *Cassia tora* L.) leaf shows the following characters.

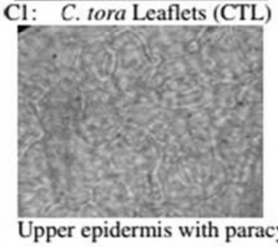
T.S of leaflet through midrib and lamina region is dorsiventral in structure and shows two main regions lamina and midrib.

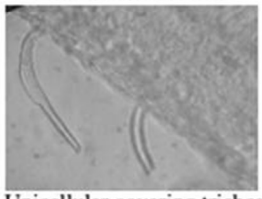
- Lamina region in transverse section shows the upper and lower epidermis covered by cuticle.
- Epidermis exhibits uni- to multicellular uniseriate covering trichomes with constricted uppermost cell on both the surfaces.
- Under the upper epidermis single layer of elongated palisade cells which is followed by 3-4 layers of loosely arranged spongy parenchymatous cells are present.
- Paracytic stomata are present on both the surfaces. But, the abaxial surface shows both anisositic stomata in addition to parasitic stomata
- The midrib is biconvex. The epidermal cells covered with cuticle are present in the midrib region. The palisade parenchyma is continuous in the midrib region below the upper epidermis.
- In the centre of midrib vascular bundles are present which are surrounded by sclerenchymatous pericyclic fibres.
- Above the lower epidermis 3-4 layers of collenchymatous cells are present.

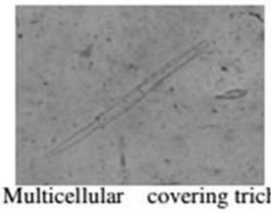

C1: *C. tora* Leaflets (CTL)

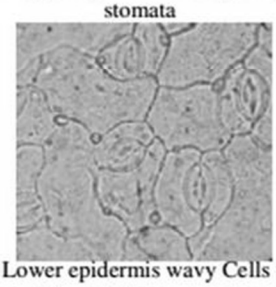

C2: T.S. of Leaflet

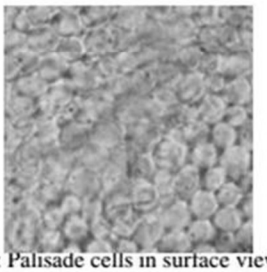

C3: T.S. of Lamina

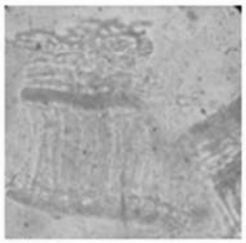

C4: Upper epidermis with paracytic stomata


C5: Unicellular covering trichomes


C6: Multicellular covering trichome


C7: Lower epidermis wavy Cells with paracytic and Anisocytic stomata


C8: Palisade cells in surface view


C9: Palisade cells in transverse view

T. S. of *Senna tora* (L.) Roxb. leaf – Authentic sample

Comparative study of both the samples:

Parameters	Senna tora (L.) Roxb.	Market sample of 'Takla'
Trichomes	Unicellular and Multicellular uniseriate covering trichomes and uppermost cell is constricted	
Palisade parenchyma in midrib region	Present	
Midrib	Biconvex	
Stomata Adaxial surface	Paracytic	
Stomata Abaxial surface	Paracytic and anisocytic	

Conclusion: From the comparative microscopical study, it is concluded that the market sample "Takla" is authenticated and is identified as *Senna tora* (L.) Roxb. OR it does not belong to *Senna tora* (L.) Roxb.

Estimation of Phenols and flavonoids from given plant material

Requirement: *Adhatoda vasica* leaves (dried powder), ethanol, distilled water, Folin –Cicolteau reagent ,Gallic acid, 7.5% sodium carbonate.

Principle: Polyphenols in plant extracts react with Folin-Ciocalteu reagent to form a blue complex that can be quantified by visible-light spectrophotometry The reaction generally provides accurate and specific data for several groups of phenolic compounds,

Preparation of extract: 20 g of dried powder of *Adhatoda* leaves is extracted with 250 ml of ethanol: water (50 : 50) for 8 hrs., reflux at 50^0 C. Filter, evaporate the filtrate to dryness. Prepare the extract in methanol and make volume to 10 ml.

Standard- Gallic acid. Prepare concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml in methanol.

Sr. No.	Concentration (mg/ml)	Folin-Cicolteau reagent (ml)	Sodium carbonate (ml)
1	0.01	2.5	2
2	0.02	2.5	2
3	0.03	2.5	2
4	0.04	2.5	2
5	0.05	2.5	2
6	(0.5 ml) Plant extract	2.5	2

Prepare the tube as given in the table, Cover the tube with aluminium foil and allow to stand for 30 min at room temp. Read OD at 760 nm.Plot the Standard graph and calculate the concentration of phenols in plant material

Procedure II

1 ml pf plant extract and 9 ml of distilled water was taken in a 25 ml volumetric flask .Add 1 ml of Folin- Ciocalteu phenol reagent to the mixture

and shake well. After 5 min, 10 ml of 7% sodium carbonate solution was added to the mixture. The volume was made up to 25 ml.

Prepare set of standard solutions of gallic acid (20, 40, 60, 80 and 100µg/ml), proceed in the same manner as described earlier.

Incubated for 90 min at room temperature and read the absorbance for test and standard solutions at 550nm.Plot the standard graph and calculate the phenols in plant material

Re:sult- Total phenols in the given plant material is_____ mg of gallic acid/gm of plant material.

Test for phenols

i) **Test for Water-Soluble Phenols: 2-3 ml Hydro-alcoholic plant extract +** 1 to 2 drops of 1% aqueous iron (III) chloride solution - A red, blue, green, or purple color indicate presence of water soluble phenols.

ii) **Test for Water-Insoluble Phenols:**. **2-3 ml Hydro-alcoholic plant extract +**0.5 mL of di chloro methane and 3-5 drops of a 1% solution ferric chloride in methylene chloride (di chloro methane). Add a drop of pyridine and stir, will produce a color if phenols s are present.

$$3\ ArOH + FeCl_3 \rightarrow Fe(OAr)_3$$

The ferric chloride forms a complex with the phenol, provided they are both very dilute, and the complex, acts as a colour test for phenols

Flavonoid (Aluminium Chloride method)

Principle: The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. . Quercetin is reported to be suitable for building the calibration curve.

Requirement- 10% aluminium chloride (10gm in 100 ml DW), 1M Potassium acetate (98.15gm/100ml),suitable plant material(neem bark)

Preparation of plant extract- 10gm of dried powder of suitable plant material in 100 ml of methanol, keep it overnight. Filter, evaporate filtrate to dryness. Make volume to 10 ml with methanol.

Standard- 10 mg of quercetin dissolved in 100 ml of methanol (0.1mg/ml)

Sr. No.	Concentration (mg/ml)	Methanol (ml)	$AlCl_3$ (ml)	K acetate (ml)	DW
1	0.1	0.9	0.1	0.1	
2	0.2	0.8	0.1	0.1	
3	0.4	0.6	0.1	0.1	
4	0.5	0.5	0.1	0.1	
5	0.6	0.4	0.1	0.1	
6	0.8	0.2	0.1	0.1	
7	1.0	0.0	0.1	0.1	
8	(0.5 ml) Plant extract	0.5	0.1	0.1	

Prepare the tubes as described in the table, wait for 30 minutes Filter the extract. Read OD at 415 nm. against blank. Plot the standard graph and calculate the flavonoid in plant material

Test for Flavonoids:

Ferric chloride test – Test solution + few drops of Ferric chloride solution → blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution + sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test – Test solution + few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

ANGIOSPERMS

Leguminosae

Family Leguminosae is further divided into three sub-families namely; Papilionaceae, Caesalpinae and Mimosae.

CLASSIFICATION:

Division: Spermatophyta: Seed bearing plants.

Sub-Division: Angiospermae: Ovules enclosed in an ovary.

Class: Dicotyledonae: Seeds contain two cotyledons, venation reticulate, flowers tetra or pentamerous.

Sub-class: Polypetalae: Petals are free, stamens not epipetalous.

Series: Calyciflorae: Sepals united to form a prominent calyx.

Order: Rosales: Leaves stipulate and alternate.

Family: Leguminosae: Fruit a legume, placentation marginal.

Sub-family: **A) Papilionaceae**
(corolla papilionaceous; stamens10, diadelphous i.e. 9+1

Genus: ***Clitoria***

Species: ***ternatea*** **(Eng-butterfly pea)**

Habit: A twiner

Leaves: Alternate, stipulate, impair-pinnately compound; leaflets 5-7, elliptic-oblong,leaflets show presence of stipels. Venation reticulate unicostate.

Inflorescence: Solitary axillary.

Flower: Zygomorphic, bisexual, hypogynous, pedicilate, bracteate.

Calyx: Sepals 5, gamosepalous (united),tubular.

Corolla: Petals 5, polypetalous, papilionaceous – posterior petal largest, outermost called standard or vexillum;two lateral petals called wings or alae and the anterior two petals forming a boat like structure called keel or carina. Aestivation vexillary.

Androecium: Stamens 10 diadelphous (9 united & one free) staminal tube encloses the gynoecium. Anther bilobed, basifixed.

Gynoecium: Carpel 1 (monocarpellary) superior, unilocular elongated with marginal placentation; style terminal, curved, with papillate (hairy) stigma.

Fruit: A legume or pod.

Economic importance- A twiner with beautiful blue or white flowers grown in gardens as hedge.

Other examples of economic importance:-

1. ***Pisum sativum***: Commonly called green peas. Used mainly as vegetable.

2. ***Butea monosperma***: Commonly called flame of the forest or palas. The flowers yield yellow colour dye. Leaves joined together to serve as plates and are used in religious functions.

CLASSIFICATION:

Division: Spermatophyta: Seed bearing plants.

Sub-Division: Angiospermae: Ovules enclosed in an ovary.

Class: Dicotyledonae: Seeds contain two cotyledons, venation reticulate, flowers tetra or pentamerous.

Sub-class: Polypetalae: Petals are free, stamens not epipetalous.

Series: Calyciflorae: Sepals united to form a prominent calyx.

Order: Rosales: Leaves stipulate and alternate.

Family: Leguminosae: Fruit a legume, placentation marginal.

Sub-family: **B) Caesalpinae** (calyx petaloid, petals clawed, stamens 10)

Genus: ***Caesalpinia***

Species: ***pulcherrima*** **(Eng- peacock plant)**

Habit: A shrub or a small tree.

Leaves: Alternate, exstipulate, bipinnately compound; leaflet, Elliptic-oblong with rounded apex, venation reticulate unicostate.

Inflorescence: Terminal or axillary raceme.

Flower: Slightly zygomorphic (sub-regular), bisexual, hypogynous, bracteate.

Calyx: Sepals 5, gamosepalous, petaloid, deeply cleft and free, posterior sepal

is largest and outermost. One of the sepals (anterior one) is different from the rest of them & is boat-shaped.

Corolla: Petals 5, polypetalous, posterior petal smallest, innermost, rest are of the same size. Aestivation imbricate.

Androecium: Stamens 10 free with long petaloid filament, anthers 2-celled dorsifixed.

Gynoecium: Ovary monocarpellary superior, unilocular, placentation marginal style long, petaloid; stigma inconspicuous.

Fruit: A long and flat legume.

Economic importance- Plant cultivated in gardens as it flowers throughout the year. Seed of C.bonducella are used in making rosaries.

Other examples of economic importance:-

1. ***Saraca indica*:** Commonly known as Ashok tree sacred tree of Hindus. The bark decoction is used in uterine ailments.
2. ***Cassia tora*** - Common weed; tender leaves are used as vegetables. Seeds used medicinally against ring worm.

CLASSIFICATION:

Division: Spermatophyta: Seed bearing plants.

Sub-Division: Angiospermae: Ovules enclosed in an ovary.

Class: Dicotyledonae: Seeds contain two cotyledons, venation reticulate, flws 4or5merous.

Sub-class: Polypetalae: Petals are free, stamens not epipetalous.

Series: Calyciflorae: Sepals united to form a prominent calyx.

Order: Rosales: Leaves stipulate and alternate.

Family: Leguminosae: Fruit a legume, placentation marginal.

Sub-family: C) Mimosae (stamens indefinite)

Genus: ***Mimosa***

Species: ***pudica***

Habit: An undershrub.

Leaves: Alternate, bipinnately compound sensitive and stipulate

modified into spines; leaflets 10-20 pairs oblong, acute; venation reticulate unicostate.

Inflorescence: A globose head with prickly peduncles.

Flower: Regular, bisexual, hypogynous, sessile, tetramerous.

Calyx: Sepals 4, gamosepalous, companulate.

Corolla: Petals 4, gamopetalous, aestivation valvate.

Androecium: Stamens 4, exserted petaloid, anthers 2 celled.

Gynoecium: carpel 1 (monocarpellary) superior, unilocular marginal placentation; style filiform; stigma minute.

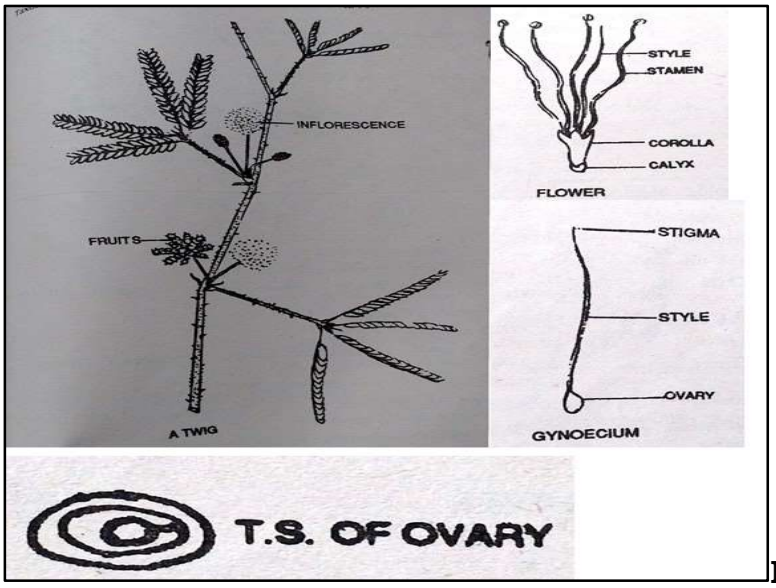
Friut: Flat jointed pod.

Economic importance- A common cultivated plant known as touch-me-not plant (ver-lajjavati).

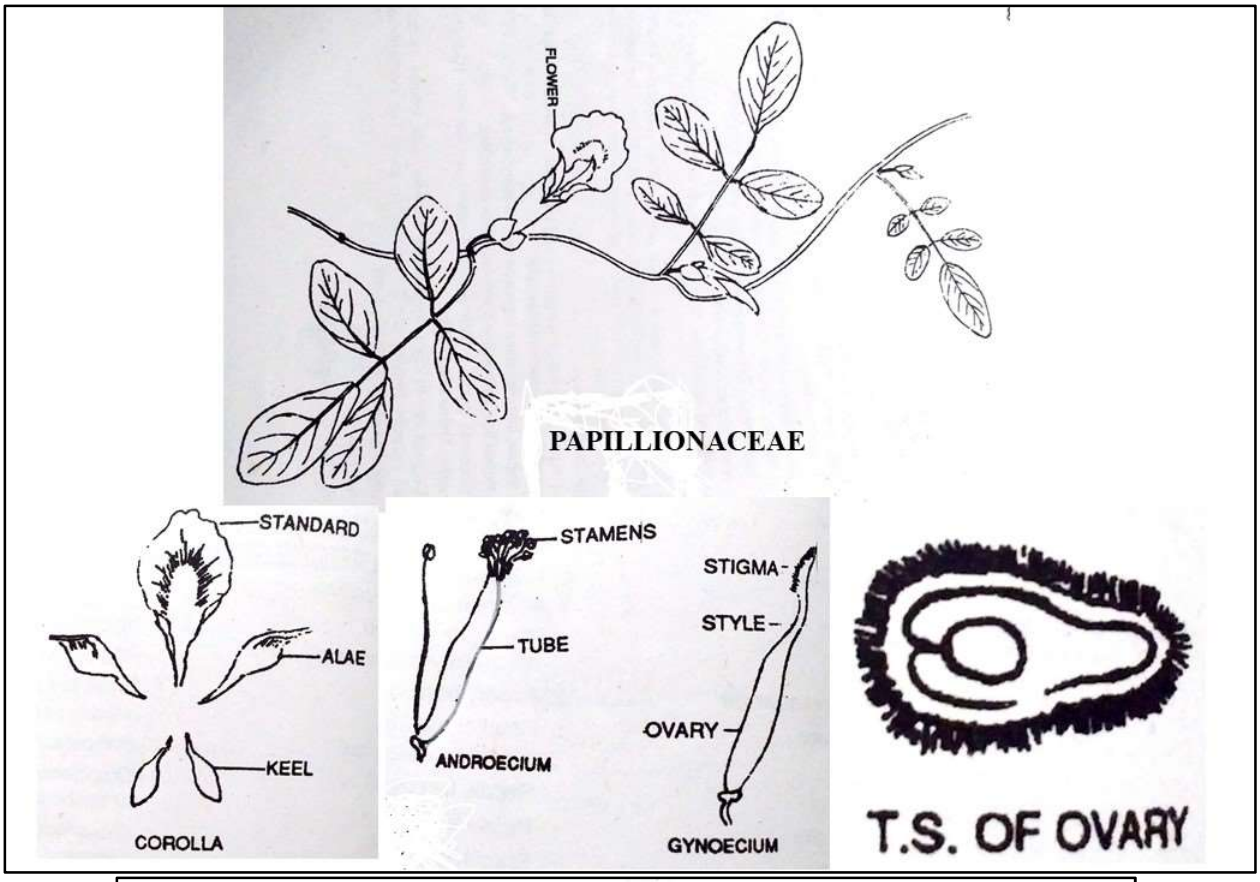
Other examples of economic importance:-

1. ***Acacia catechu***: commonly known as kattha. Obtained from bark is used in 'Pan'. It is also used as adhesive for plywood.

2. *Samanea saman* (*Pithecolobium saman*): commonly known as the rain tree. The leaves are bipinnately compound; leaflets are oval, oblique. The plants are grown on roadsides as shade tree.



MIMOSAE


FLOWER
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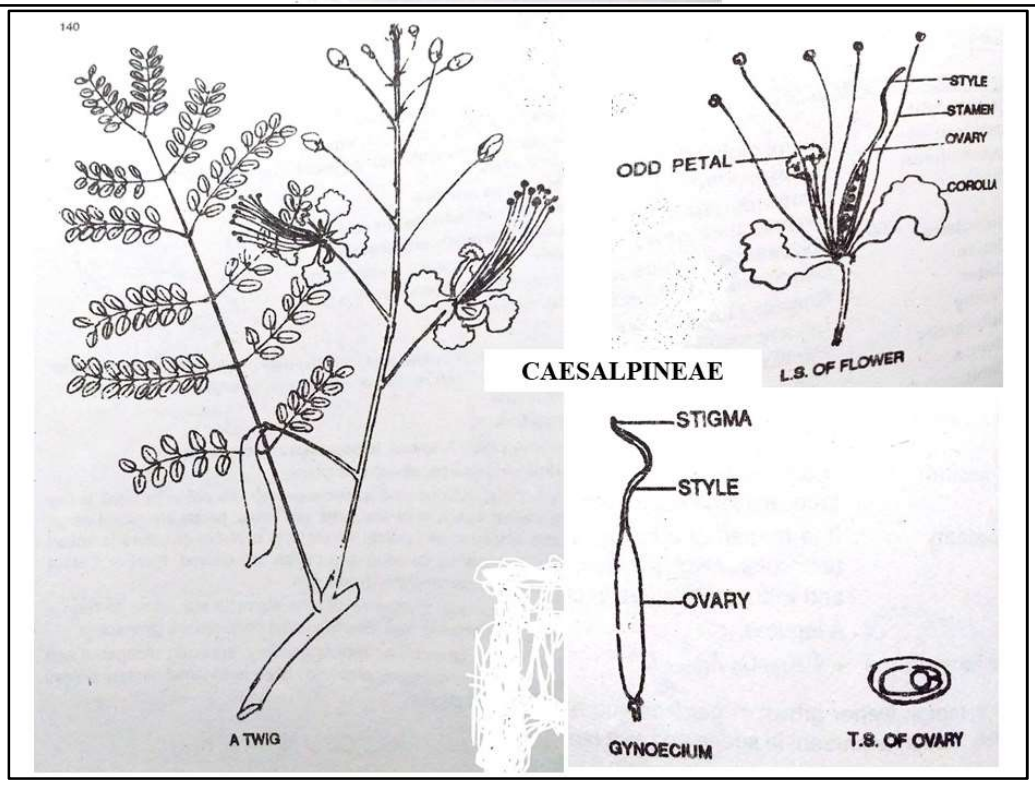
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T.S. OF OVARY

Asteraceae

Classification

Division: Spermatophyta (Seed bearing plants)

Class: Dicotyledoneae (Reticulate venation in leaves, tetra or pentamerous symmetry of flowers)

Sub-class: Gamopetalae (petals united, stamens epipetalous)

Series: Inferae (flowers epigynous, ovary inferior)

Cohort: Asterales (inflorescence capitulum or head)

Family: Asteraceae (Compositae)

Genus: ***Tridax***

Species: ***procumbens***

Distinguishing characters:

Habit: A small, procumbent, perennial herb.

Leaves: simple, alternate, exstipulate, eliptic with incised margin. Venation reticulate.

Inflorescence: long, peduncled, solitary Capitulum

Florets: 2 types of florets are present on the inflorescence namely ray florets and disc florets. Epigynous, bracteate. Involucre bracts five in number and appear like calyx cup. Ray florets are female flowers while disc florets are bisexual.

Ray florets: zygomorphic, unisexual, situated towards periphery of capitulum, incomplete, epigynous.

Calyx: sepals modified into Pappus calyx.

Corolla: petals 5, united to form strap shaped or ligulate corolla.

Androecium: absent

Gynoecium: bicarpellary, syncarpous, ovary inferior, one celled, single ovule on basal placenta. style single, stigma bifid.

Disc florets: situated in the centre of the inflorescence, actinomorphic, bisexual, fertile, epigynous, centripetal arrangement.

Calyx: sepals modified into Pappus calyx.

Corolla: petals 5, gamopetalous, tubular.

Androecium: stamens 5, epipetalous, syngenecious-anthers united but filaments free

Gynoecium: bicarpellary, syncarpous, ovary inferior, one celled, single ovule on basal placentation, style single stima bifid.

Fruit: Cypsela

Plants of economic importance:

1) ***Chrysanthemum indicum*** (Shevanti): ornamental herbaceous flowering plant. It is used as insecticide plant as well as for CO_2 trapping

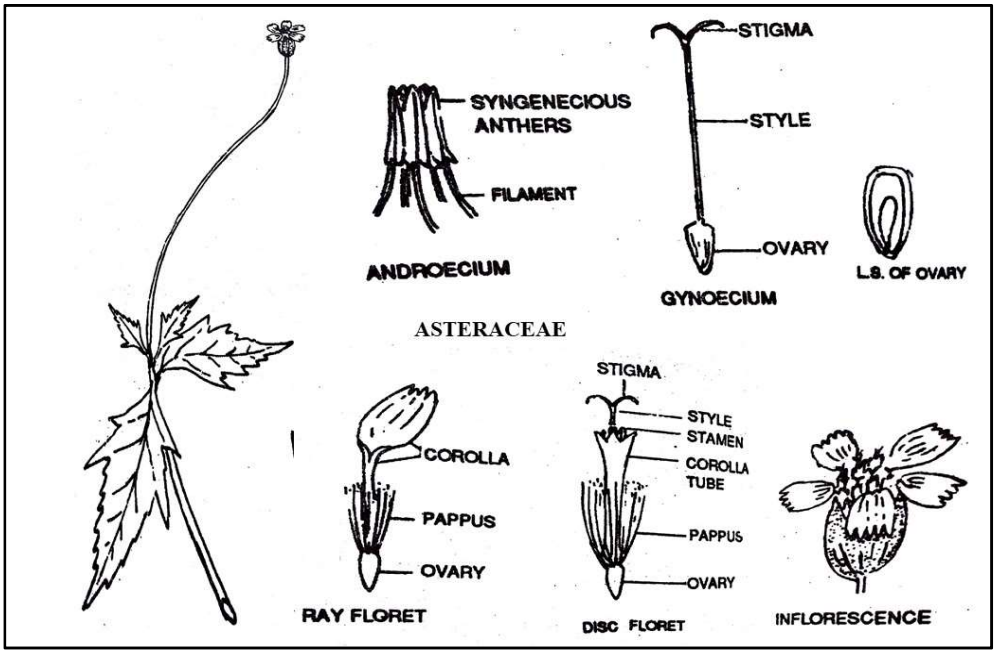
2) ***Tridax procumbens****:* common herbaceous weed

3) ***Eclipta alba*** (Maaka, Bhringaraj): extract of leaves mixed with coconut oil keeps hair black. It is a good tonic for spleen also cures leucoderma.

4) ***Helianthus annus*** (sunflower): seeds yield edible sunflower oil used in cooking.

5) ***Carthamus tinctorius*** – (safflower or kardai) seeds yield edible oil, flowers are tonic for liver and also yield red dye.

6) *Dahlia*, *Zinnia*, *Tagetes*, *Gallardia*, *Cosmos*, *Gerbera* etc. are annual flowering ornamentals



Amaranthaceae

Classification:

Division: Spermatophyta (seed bearing plants)

Class: Dicotyledoneae (tap root system, reticulate venation, tetra or pentamerous symmetry, 2 - cotyledons)

Sub-class: Apetalae/Monoclamydeae (flowers often unisexual with or without perianth, perianth with only one whorl, usually corolla absent)

Series: Curvembryeae(flowers regular, ovary superior,embryo curved)

Order: Chenopodiales (mostly herbs, ovary superior, monocarpellary with one ovule)

Family: Amaranthaceae

Genus: ***Gomphrena***

Species: ***globosa***

Distinguishing characters:

Habit: small herb.

Leaves: simple, opposite, exstipulate, sessile.

Inflorescence: globose cyme.

Flower: Actinomorphic, bisexual, presence of dry bracts and bracteoles, bracts green, bracteoles scarious (thin, dry, membranous), hypogynous.

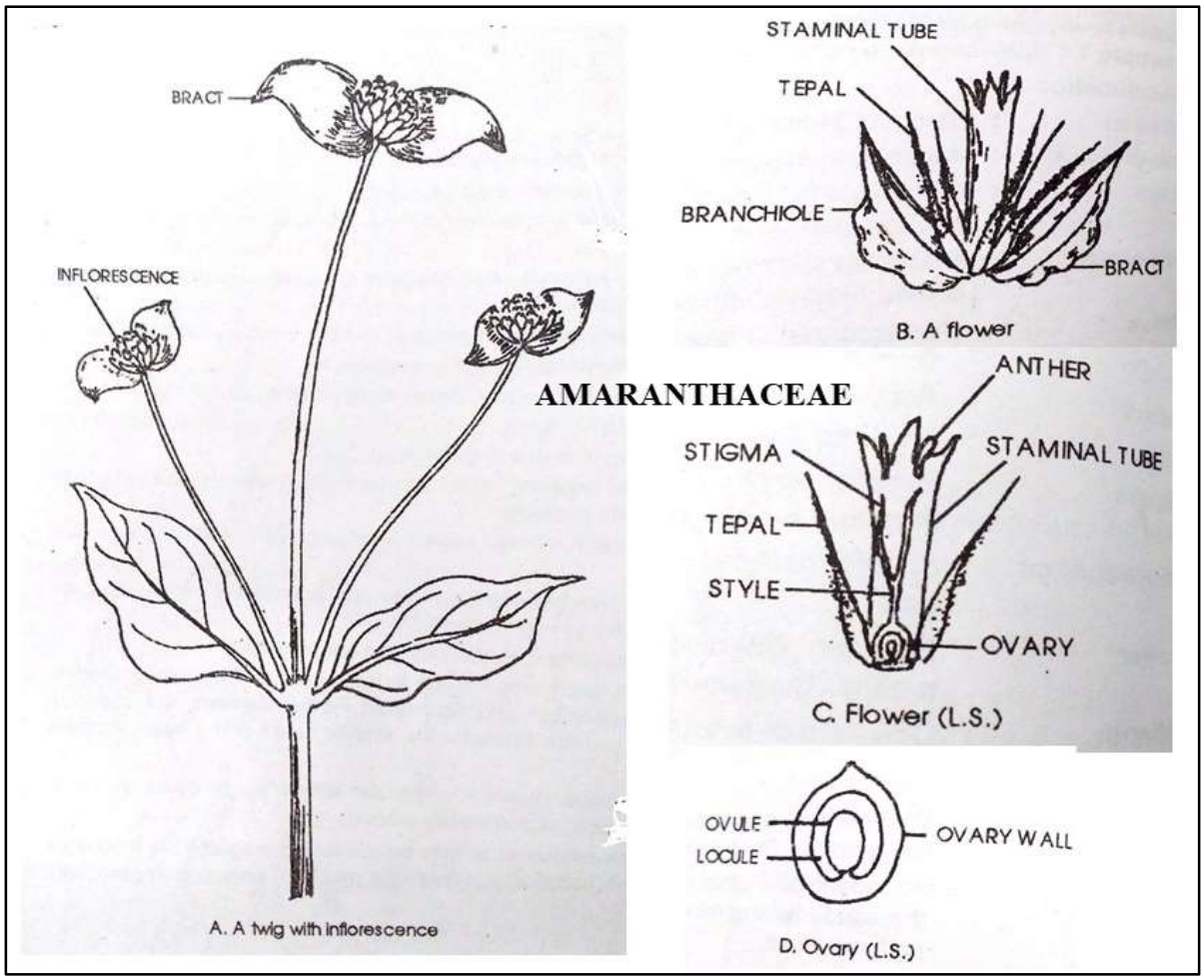
Perianth: Tepals 5, in a single whorl, free or connate, dry, brightly coloured.

Androecium: Stamens: 5, united to form staminal tube, stamens opposite the tepals.

Gynoecium: bicarpellary, syncarpous, one celled, superior, single ovule on basal placenta, style slender, stigma bifid.

Plants of economic importance:

1. *Gomphrena globosa*: Gentleman's button, ornamental plant.
2. *Amaranthus paniculatus*: seeds edible (Rajgira), leaves used for purifying blood.
3. *Amaranthus polygamous*: Chavli, lal math, leaves used as vegetable.
4. *Celosia cristata*: Ornamental plant cock's comb, in yellow, orange & red in colour.
5. *Celosia argentea*: common weed after rains, seeds useful in diarrhea.
6. *Achyranthus aspera*: Aghada, common weed, useful in dropsy, piles, boils and colic. Also used as a cure for cough, hydrophobia.



Palmae

Classification:

Division: Spermatophyta (seed bearing plants)

Class: Monocotyledoneae (leaves with parallel venation, seeds with one cotyledon, fibrous roots, trimerous symmetry)

Series: Calycinae (unbranched trees with palmate or pinnately compound leaves, inflorescence compound spadix, flowers cyclic, hypogynous, 3 carpels)

Family: Palmae

Genus: ***Cocus***

Species: ***nucifera***

Distinguishing characters:

Habit: An unbranched erect tree.

Leaves: Bipinnately compound forming a crown at the apex, petiole long, sheathing leaf-base. Lower leaflets maybe smaller and modified into spines.

Inflorescence: A compound spadix enclosed in a boat-shaped spathe.

Male Flower

Flower: Regular, actinomorphic, sessile, dioecious, hypogynous.

Perianth: In two whorls of three members each, leathery and greenish in colour, maybe separate or slightly connate.

Androecium: Stamens 6 in 2 whorls of 3 each, anthers 2-celled.

Gynoecium: Absent in male flowers or rudimentary.

Female Flower

Flower: One or a few flowers develop at the base of the branch on which a number of male flowers develop. The fower is supported by bracteoles.

Perianth: Same as in male flower

Androecium: Absent in female flowers.

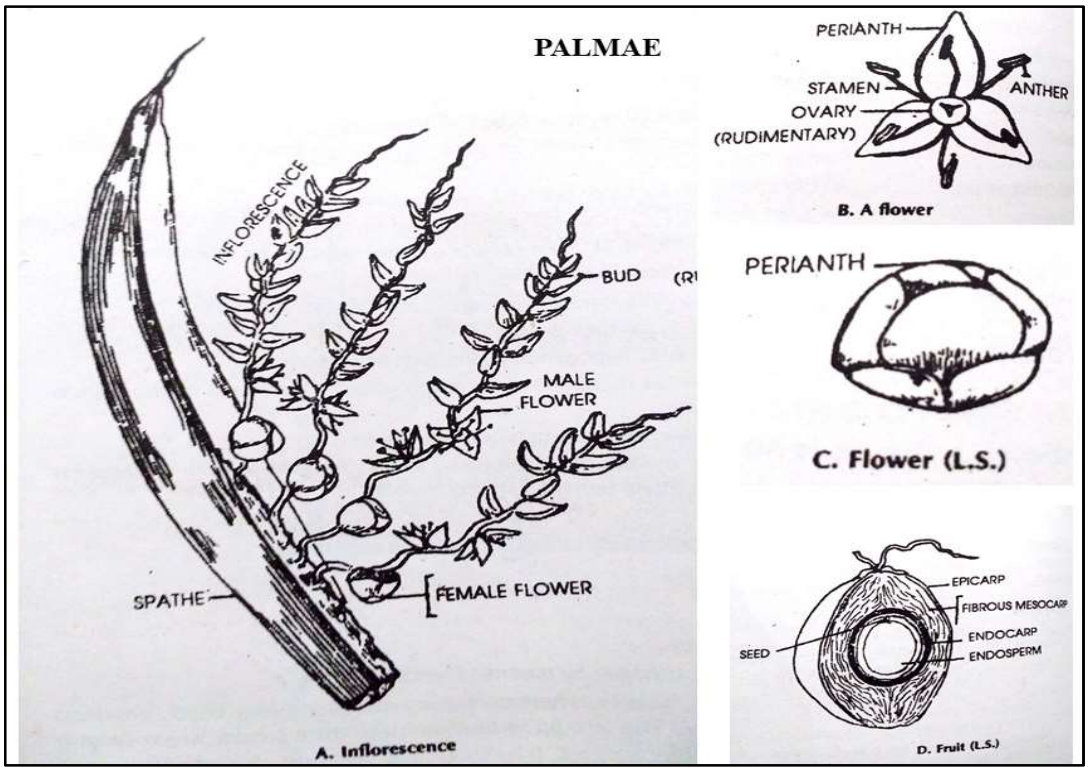
Gynoecium: tricarpellary, syncarpous, superior, 3 celled but only one carpel develops into a fruit.

Fruit: A fibrous drupe

Seed: Testa brown and endosperm white (transparent liquid when fruit is young – coconut milk)

Plants of economic importance:

1. ***Cocos nucifera*:** coconut (Kalpavriksha): leaves used for thatching, making baskets, hats & brooms. Coconut water is the liquid endosperm, very nutritious, also used in tissue culture. The solid endosperm is eaten raw or cooked, dry coconut yields oil which is edible. The fibrous husk (mesocarp) is used in making coir which is used in making mats and rugs etc.
2. ***Borassus flabellifer*:** Taad, sap is tapped and fermented (taadi) or consumed fresh (nira), stem used for making poles, pillars, rafters.
3. ***Caryota urens:*** leaf fibre is known as kittul fibre, sap is tapped for preparing toddy.
4. ***Pheonix dactylifera*** (s khajur): Fruits rich in calcium
5. ***Areca catechu*** (supari): grown commercially in India, used as masticatory & chewed along with Paan, powdered nut is useful in checking diarrhea & urinary disorders. An alkaloid, arecoline is used in ophthalmic treatment.



HERBARIUM AND WET PRESERVATION TECHNIQUE

Aim: To study herbarium and wet preservation technique.

HERBARIUM

A collection of dried plant specimens, mounted on sheets is known as herbarium. Freshly-picked specimens are dried and pasted on mounting paper of regulation-sized herbarium sheets. The purpose of such a collection is to study the vegetation of a locality and maintain its record.

Preparation of herbarium sheets

Equipments - On excursion, for collection of plants, several items are required like trowel - to collect the entire plant, if possible, vasculum - to bring few numbers of plants to the lab. Along with this, newspapers or blotting papers are required to press the plants on the field itself to avoid loss of natural colour of some of the plants.

1. Collection: Collected plants are placed in the collecting sheets. The most practical size is **16.5 x 23 inches** and when folded, **16.5 x 11.5 inches**. A specimen collected should represent the root, stem, leaves and flowers. The plants are placed between the sheets or newspapers. Herbaceous plants, 2 feet or less higher, may be collected entire. The most desirable is to collect a branch, about one foot high, containing leaves and flowers. After a specimen has been collected and placed in a collecting sheet, it is kept in a plant press. This collecting sheet is placed in between blotting papers, one on either side. While on collection, it is important to note the , locality, habitat, height, method of branching, colour of reproductive parts, common name etc. This should be noted separately in a field-book.

2. Pressing: The collecting sheets should be transferred to a heavy laboratory press. The press is securely tightened. The press should be placed in a warm, well-aired place to dry. After 24 hours, the press is taken out and opened. The old newspapers and blotting Sheets are replaced by new unused ones. At least such 3 – 4 changes are given at an interval of 2 – 3 days. An average specimen takes about a week for complete drying. Sometimes to hasten the process of drying, plant press may be placed near an appropriate heat source.

3. Mounting: The specimen are ready for mounting once they are completely dry. The standard size of the sheet is 16.5 x 11.5 inches. The paper should be of good weight and not thin and flexible. The quality should be so that it does not turn yellow even with a considerable lapse of time. To mount the specimen, one of the following methods can be used. The gum is spread on a glass plate and the specimen is laid on it. As soon as all the parts come in contact with the gum, it is lifted and then placed in position on a mounting sheet. The specimen is inverted and painted with gum by a brush and then transferred to a mounting sheet. The specimen is placed on a herbarium sheet and small strips of gummed tape or cellulose tape are pasted at suitable places, so that most of the part remains loose. After mounting the specimen, a label is pasted in the right hand lower corner of the sheet. This carries information regarding the botanical name of the plant, common name, , collector's name, place of collection etc.

4. Arrangements of sheets – The sheets are finally arranged in accordance with a standard classification system (preferably Bentham and Hooker's classification for Angiosperms).

The sheets are arranged into groups according to species, genera, families, classes, orders, series and sub-divisions etc. Each group is placed in a separate envelop slightly larger than the herbarium sheets.

5. Care of sheets – Herbarium sheets are often attacked by museum pests, fungi etc. To guard them against pests, specimens are fumed with carbon bisulphide, 3 – 4 times a year. Mounted specimens may also be treated with mercuric bichloride or copper sulphate. To prevent them from being attacked, powdered naphthalene balls orgamaxene powder should also be spread or dust from time to time.

WET PRESERVATION:

While on a collection trip, local or outstation, following things are to be carried along.

Containers - For packing the collected material, preferably unbreakable plastic containers or polythene bags.

Preservatives - Formalin-Acetic-Alcohol (FAA) or Alcohol 70 % or Alcohol 90 %, and/or Formalin 4 %-10 %.

Other requirements - Scalpel, knife, blade, forceps, pencil, paper, a hand lens, a bag or vasculum for keeping plants or a plant press with many newspapers or blotting papers.

After collecting the plant, it should be immediately killed and preserved or pressed to avoid its rotting and dehydration. On return to the laboratory, collected material should be transferred to new and suitable containers with fresh preservative. A few plants e.g. filamentous algae, fungi, reproductive parts of bryophytes, fertile parts of pteridophytes and different parts of gymnosperms, if collected in large quantities, are preserved in containers. Even if large quantities of such plants are available, one plant with fertile parts should be preserved in the form of a herbarium sheet, while others should be packed in a container. Every tube should be labeled. It is important to write the name of the specimen, place and of collection. The place of collection and should also be written on a small piece of white card paper with a pencil, on the spot and inserted in the container. On return to the laboratory, the material is identified with the help of standard books. A label bearing the name of the division and class to which the material belongs, the name of the

material, and place of collection and also the name of student should be pasted on the container. All the containers should be of uniform size as far as possible. Generally for cryptogamic and gymnospermic material, 4% formalin or formaldehyde is preferred. But occasionally after a period lapse, because of formalin the specimen may get bleached and lose their original colours. Some of the fungi can grow even in this condition and contaminate the preservation. To avoid this 1 % copper sulphate solution is used. When specimens are preserved (anthers, root tips etc.) for cytological studies, they are pretreated with fixative agents like FAA and then preserved in 70% alcohol. For VAM studies roots are preserved in 70 % alcohol before staining whereas after staining procedure, they are preserved in lacto phenol.

PREPARATION OF A REPORT ON VISIT TO ANY HERBARIUM CENTRE.

Guidelines for preparation of report –
Name and address of the herbarium –
Brief history of the herbarium –
Year of establishment
Founder/s name/s and contribution / relevant data
Other contributors
Present size of the herbarium –
Administration and staff –
Herbarium as a reference centre –
Documentation
Pattern / Special methods of preservation if any
Important user instructions
Handling specimens
Remark –
Condition
Maintenance
Usefulness
Personal remarks

SEPARATION OF AMINO ACIDS BY CIRCULAR PAPER CHROMATOGRAPHY

Aim: To study the separation of Amino acids by circular paper chromatography

Requirements: Whatman No. 1 Chromatography paper, big petridish, Solvent system (Butanol: Acetic Acid: Distilled water; 4 : 1 : 5), microcapillaries, wick, known amino acids and unknown amino acids (Leucine, Proline, Lysine), Ninhydrin.

Procedure: Chromatography papers are generally 5% moist and thus should be dried in an over before use.

Take a petridish, add a little solvent and cover with a bowl. Take Whatman No. 1 filter paper and mark the center. Mark a small circle with pencil on the paper. Mark different point A, B, C and D little away from eachother. Make a small slit at the center and insert a small wick in it. At points A, B, C and D put very small drops of amino acids with help of microcapillaries. Use a different microcapillary for each different amino acid. Keep the chromatography paper in a petridish in such a way that only the wick attached at the center of the chromatography paper touches the solvent. Cover the petridsh with a bowl and allow the chromatogram to run. When the solvent reaches the periphery remove the paper and dry it at 80 deg C in an oven. After 2 to 3 mins remove the paper from the oven and spray it with ninhydrin. After spraying dry the paper once again. The colour develops after drying. Find out the distance travelled by the solvent and the amino acids from the center. Identify the amino acids by their colour and Rf value.

$$Rf\ value = \frac{Distance\ travlled\ by\ the\ amino\ acid}{Distance\ travelled\ by\ the\ solvent}$$

From the standard chart of Rf for that particular solvent identfy the amino acid present.

R*f* value: Leucine = 0.01
Proline = 0.51
Lysine = 0.29

R*f* value will change according to the solvent system.

Result: The unknown amino acid is _____

SEPARATION OF CAROTENOIDS BY THIN LAYER CHROMATOGRAPHY

Aim: Separation of carotenoids by thin layer chromatography

Requirements:

Glassware and apparatus: Pre-coated silica gel plates, chromatography chamber, mortar & pestle, muslin cloth, glass rod, separating funnel etc.

Chemicals: Acetone, Petroleum ether, benzene, 5% sodium sulphate solution, methanolic KOH, silica gel powder, distilled water, solvent system – Petroleum ether: benzene (1:9)

Plant material: Tomato fruit/ carrot root

Principle: Carotenoids are tetraterpenoid (C_{40}) compounds existing as hydrocarbons to plant pats, flowers and fruits. The total carotenoids are extracted and separated on the basis of differential absorption between stationary phase and mobile solvent phase. The solutes compete with the solvent for surface sites of the absorbent. Depending on the distribution co-efficient on the surface of the absorbent.

Procedure: Weigh 5-10 gm of tomato pulp or carrot tissue and extract it with 10 ml acetone in mortar and pestle. Filter it and take the filtrate ub a separating funnel containing 20 ml petroleum ether. Add 20 ml of 5% sodium sulphate solution and shake it gently. Two separate layers are obtained. Collect the upper petroleum ether layer in a brown bottle containing 10 gm anhydrous sodium sulphate. Keep it aside for 30 minutes. Decant the extract in separating funnel and add 60% methanolic KOH, distilled water and more ether. Two layers are obtained.

The upper layer containing yellow carotenoid pigments is collected for separation.

Separtion of carotenoids:

Take the pre-coated TLC plates and load the caroteinoid extract on activated TLC plate and place it in pre-saturated chromatography chamber. Keep it covered. Allow the solvent to run upto 2/3rd of the plate, remove the plate and mark the solvent front. Let it dry and mark the spots of carotenoids to calculate the Rf value using following formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Observations –

Distance travelled by solvent - _____ cm

Distance travelled by solute (1) - _____ cm

Distance travelled by solute (2) - _____ cm

Calculations:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Rf of first spot =

Rf of second spot =

Result

Rf of carotene - _____ (standard Rf 0.74 – 0.80)

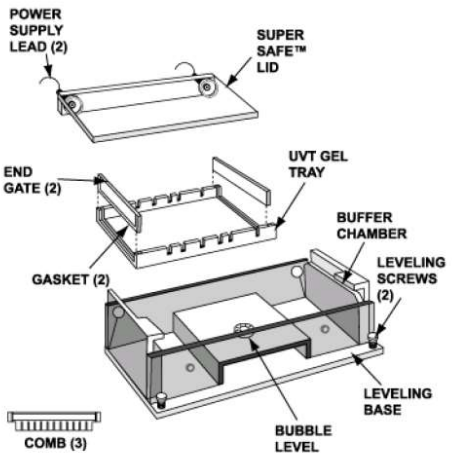
Rf of Lycopene - _____ (standard Rf 0.13)

GEL ELECTROPHORESIS

Electrophoresis is a technique for separation of components on the basis of charge and mass ratio.Agarose gel electrophoresis is the standard method for the separation, identification and purification of DNA and RNA fragments. The location of DNA within the gel can be determined directly by staining with low concentrations of intercalating fluorescent dye 'Ethidium bromide' under U.V. light.

HORIZONTAL GEL ELECTROPHORESIS

The apparatus consists of buffer tank, gel casting unit, comb and lid. Agarose gels are cast by melting the agarose in the presence of suitable buffer. DNA samples are mixed with glycerol and bromophenol blue (tracking dye) is loaded in wells specially made in the gel with the help of the comb. When electric field is applied across the gel, the DNA, which is negatively charged at neutral pH, migrates towards the anode. TAE (Tris- Acetate EDTA) buffer is the commonly used buffer for the DNA samples. DNA fragments migrate at different rate under the influence of electric field (due to their mass difference) and separate as bands.



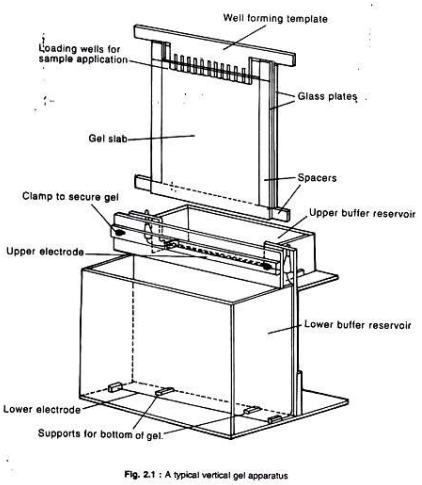


Fig. 2.1 : A typical vertical gel apparatus

VERTICAL GEL ELECTROPHORESIS

The apparatus consists of buffer tank, glass plates, spacers, comb, clips, clamps, petroleum jelly, lid, silica plate etc. After cleaning and drying the glass plates and spacers, assemble them properly. Hold the assembly together with the help of bulldog clips. White petroleum jelly or 2% melted agar is then applied around the edges of the spacers to hold them in place and seal the chamber between glass plate and silica plate. Prepare a sufficient volume of gel mixture (agarose or polyacrylamide gel) and pour the gel solution in the chamber between silica plate and glass plate and place the comb in the upper end of gel and allow the gel to set (30-60 min.)

After setting, remove the comb carefully. Then install the gel after removing the clips, agar etc. in electrophoresis apparatus. Fill it with electrode buffer Prepare sample for electrophoresis. Mark the well properly with permanent marker for correct and easy sample loading in sample well. Sample is loaded with the help of micropipettes. Cover the apparatus properly with lid. Apply electric field for separation. Then continue the separation until the bromophenol blue (tracking dye) reaches the bottom of the gel. After the run is complete, carefully remove the gel from between the plates.

INDEX

SEMESTER- III

PRACTICAL: II (Form and Function - II)

CELL ORGANELLES

The cell is regarded as a structural and functional unit of living organisms. A typical eukaryotic cell has a well-defined nucleus surrounded by a nuclear envelope. The cells are characterized by a complex structural organization in which various cell organelles carry out different functions.

Mitochondria:

Mitochondria are present in all plant and animal cells.

They may be oval, rod shaped, club shaped etc.

Oval mitochondria range from 0.5 -1.0 μ in diameter and length may be twice the diameter.

It is surrounded by two membranes.

The outer membrane is stretched and the inner membrane shows invagination known as Cristae.

The inner membrane is associated with small stalked particles referred to as F_1 particles or elementary particles.

Each F_1 particle shows a base piece, a stalk and a head piece.

The mitochondria is filled with a jelly like matrix which also contains ribosomes, mitochondrial DNA, sugars and salts.

Matrix is site for Kreb's cycle and inner membrane for the ETS. Mitochondrion is called the power house of the cell.

Microbodies:

In addition to lysosomes the cytoplasm contains other membrane bound vesicles called microbodies.

Two common microbodies are Peroxisome and Glyoxysome.

Microbodies contain catalase and oxidase enzymes.

Peroxisome

It is having potential peroxidase activity.

It is oblate or spherical organelle containing granular and amorphous matrix.

It is bounded by single unit membrane which arises from Endoplasmic Reticulum.

The membrane permits the substrates like amino acids, α – hydroxyl acids and uric acid.

The peroxisome oxidizes a variety of substrates in to two step reaction.

In first step, substrates (like uric acid, amino acids and lactic acid) are oxidized by molecular Oxygen to form H_2O_2 by oxidase enzymes. H_2O_2 is destructive to cells and has to be removed. In second step, H_2O_2 is broken down into water molecule and the process is catalyzed by Catalase enzymes.

Peroxisome involves in photorespiration process occurring in C_3 plants.

Glyoxysome

It is oblate or spherical organelle containing granular and amorphous matrix.

It is bounded by single unit membrane which arises from Endoplasmic Reticulum.

In Glyoxysomes, the glyoxylate cycle converts two acetyl co.A into succinic acid i.e. four Carbon compound for gluconeogenesis.

Glyoxylate cycle enzymes have been found in the Protozoa, some Fungi and the Metazoa.

Glyoxysomes also contain the enzymes for β – oxidation of fatty acids.

Seeds rich in fat convert storage fats to carbohydrates during germination.

Conversion of storage fat to carbohydrates in germinating seed via glyoxylate cycle involves three organelles – Oleosome, Glyoxysome and mitochondria.

The four carbon organic acid – Succinic acid which form inside glyoxysome converts into hexose sugar inside mitochondria.

Ribosomes:

Palade, 1955, revealed the presence of dense granules in cytoplasm. Later on it was termed as Ribosomes.

These granules may be found in the free-state (either monosomes or associated with m-RNA i.e. polysome) or attached to membranes of organelles.

It is made up of protein and RNA and having small and large two subunits.

Ribosomes are of two basic types, 70S and 80S ribosome.

The 'S' refers to Svedberg Unit. This is a Sedimentation Coefficient and it is not additive.

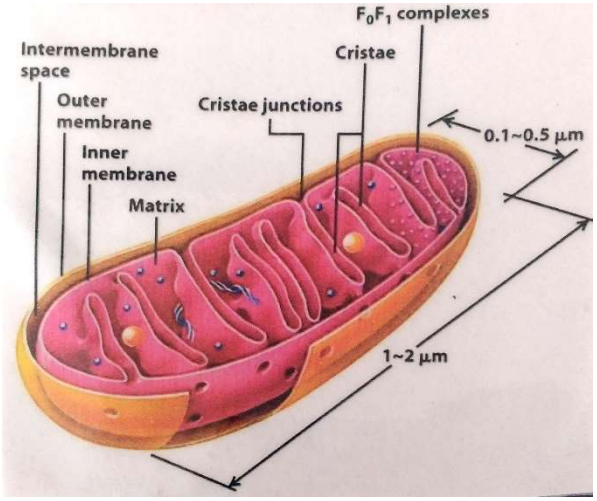
70S ribosomes are relatively smaller and found in prokaryotes e.g. bacteria. The 70S ribosome consists of a large 50S subunit and a small 30S subunit.

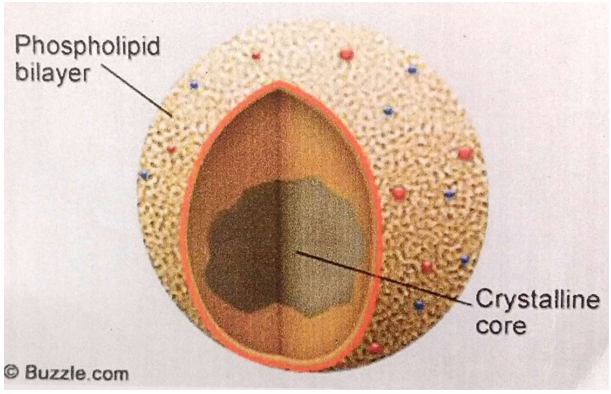
80S ribosomes are found in eukaryotes e.g. algae, fungi, higher plants and animals. The 80S ribosome consists of a large 60S subunit and a small 40S subunit.

Ribosomes found in mitochondria and chloroplasts of eukaryotes are closer to prokaryotic ribosomes.

Metal ions, mainly Magnesium ion, play an important role in holding the two subunits together.

Ribosomes take part in protein synthesis.


F_0F_1 complexes
Intermembrane space
Cristae
Outer membrane
Cristae junctions
0.1~0.5 µm
Inner membrane
Matrix
1~2 µm


Phospholipid bilayer
Crystalline core
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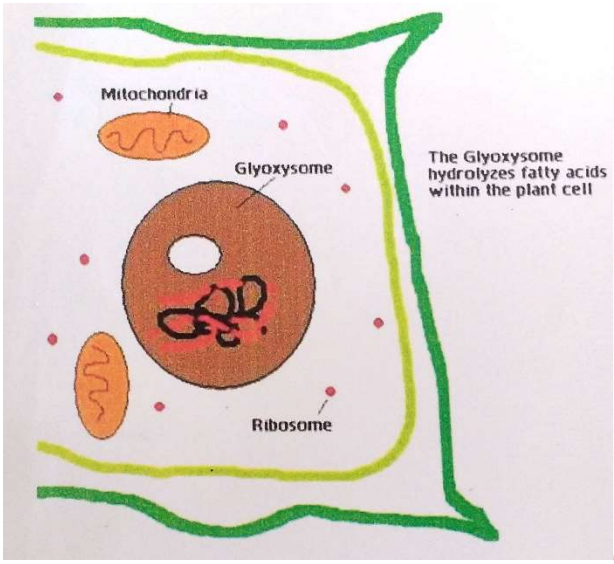
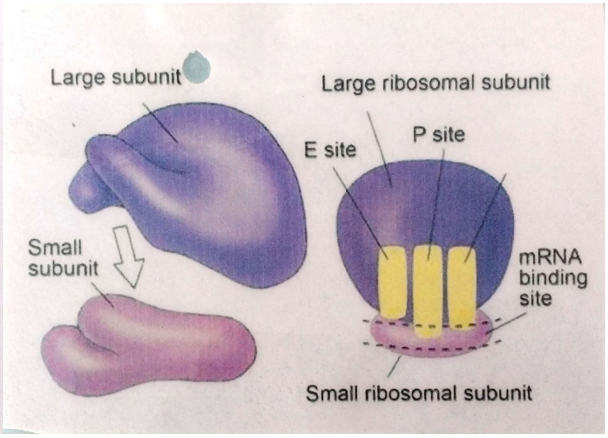

Mitochondria
Glyoxysome
The Glyoxysome hydrolyzes fatty acids within the plant cell
Ribosome


Large subunit
Large ribosomal subunit
E site
P site
Small subunit
mRNA binding site
Small ribosomal subunit

ESTIMATION OF DNA

Aim: Estimation of DNA by Diphenylamine reagent.

Principle: Since the sugar moiety of DNA consists exclusively of 2-deoxyribose in all of its nucleotides, a color reaction with this sugar would serve for the determination of DNA. Free as well as bound deoxyribose sugar of the DNA reacts with diphenylamine in a mixture of Glacial Acetic Acid and sulphuric acid at 100 degree Celsius. In the solution at low pH, the straight chain form of deoxypentose is converted to highly reactive β-hydroxylevulin-aldehyde that reacts with diphenylamine giving blue color which is stable and measured at 600 nm against a blank (the sharp absorption maxima is at 595 nm).

Requirements –

Glassware and apparatus – pestle and mortar, test tubes, pipettes, colorimeter, centrifuge, water bath etc.

Chemicals- SDS-Sodium dodecyl sulphate, Chilled ethanol, diphenylamine reagent, Standard DNA0.4 mg/ml in 5mM NaOH.

Plant Material: Onion

Procedure:

ISOLATION OF DNA

1) Take 1 gm onion and cut it to fine pieces and coarsely grind it in mortar and pestle.
2) Take it in a test tube and add 6ml of SDS to it and keep it in water bath at 50^0C for 20 mins.
3) Cool the tube and take 3 ml of the supernatant and add 3 ml of chilled ethanol slowly from the sides of the test tube.
4) A ring of DNA is formed at the junction of the two liquids.

ESTIMATION OF DNA

1) Dissolve the ring of DNA by shaking the tube and take 0.2 ml of the supernatant and add 0.8 ml of water.
2) Add 2ml of Diphenylamine reagent and mix well. Keep at 70^0C for 15 mins. Cool the tube and read O.D. at 600nm.
3) Simultaneously prepare a tube for blank with 1ml water and 2ml Diphenylamine reagent and mix well. Keep at 70^0C for 15 mins. Cool the tube and read O.D. at 600 nm.
4) Also prepare a tube for Standard DNA by taking 0.2ml of the Standard DNA and add 0.5ml of water. Add 2ml of Diphenylamine reagent and mix well. Keep at 70^0C for 15 mins. Cool the tube and read O.D. at 600 nm.

OBSERVATIONS:

TUBE	Supernatant for Estimation	DNA Standard Solution (0.4mg/ml)	Water	Diphenylamine reagent	Keep at 70^0 C for 15 mins	
Blank			1ml	2ml		
Standard		0.2ml	0.8ml	2ml		O.D.
Unknown	0.2ml		0.8ml	2ml		

CALCULATIONS:

X= O.D. of Standard

Y= O.D. of Unknown

Z= Concentration of standard = 0.08mg

Amount of DNA in 0.2ml of extract= $\frac{Y \times Z}{X}$ = D mg

This 0.2ml was taken from 3ml of supernatant.

0.2ml supernatant contains D mg DNA =

3ml supernatant contains mg DNA

1 g onion contains mg DNA

SDS: 2g SDS + 1.5 g NaCl + 100 ml d/w
DPA: 1.5 g DPA + 100 ml acetic acid + 1.5 ml Conc. H_2SO_4. (Just 30 minutes before use :20 ml DPA + 0.1 ml of acetaldehyde)[16 mg/ml acetaldehyde]

ESTIMATION OF RNA

Aim – To estimate RNA from the plant material

Requirements –

Glassware and apparatus – Pestle and mortar, test tubes, pipettes, colorimeter, centrifuge, water bath etc.

Chemicals – 10% trichloroacetic acid (TCA), ethanol ether, 0.5 N NaOH, RNA standard, orcinol reagent, ferric chloride, conc. HCl, distilled water etc.

Plant material – Cauliflower

Principle – The nucleic acids are usually present in the cell in combination with proteins and are called nucleoproteins. RNA contains the bases adenine, guanine, uracil and cytosine. Acid hydrolysis of RNA releases ribose and this in presence of strong acid, dehydrates to yield furfural. Orcinol reacts with furfural in the presence of ferric chloride to give a green colour.

Procedure –

I. Isolation of RNA -

1. 1 gm of plant tissue (cauliflower head) is homogenized with 10 ml of ice cold 10 % TCA.
2. Centifuge at 3000 rpm for 10 minutes and discard the supernatant.
3. Suspend the precipitate in 5 ml ice cold 10 % TCA and centrifuge at 3000 rpm for 10 minutes.
4. Discard the supernatant, suspend precipitate in 5 ml ethanol – ether (3:1) mixture and centrifuge at 3000 rpm for 10 minutes.
5. Discard the supernatant, add 5 ml of 0.5 N NaOH to the precipitate, mix well and leave at 37^0 C for 18 hours.
6. Centrifuge at 3000 rpm for 10 minutes. The supernatant contains RNA in the hydrolysed form along with the proteins.
7. Add equal volume of 10 % TCA, centrifuge at 3000 rpm for 10 minutes.

8. Discard the precipitate (proteins) and use the supernatant for estimation of RNA.

II. Estimation of RNA –

1. RNA solution is prepared by dissolving 0.4 mg of pure yeast RNA /ml in 5mM NaOH.
2. Prepare a tube for blank by using 2 ml of distilled water and one tube for reaction mixture by talking 1 ml of RNA isolated from plant material in a test tube, make up the volume to 2 ml by adding distilled water.
3. Prepare a third tube for standard by using 2 ml of std RNA solution.
4. Add 3 ml of orcinol reagent to each test tube and mix well.
5. Keep the test tubes in boiling water bath for 15 minutes, after which cool them to room temperature and measure the O. D. at 660 nm.
6. Calculate the amount of RNA in the plant tissue.

Observation Table –

TUBE	Supernatant for Estimation	RNA Standard Solution (0.4mg/ml)	Water	Orcinol reagent (ml)	Keep the tubes in boiling water bath for 15 minutes	O.D.
Blank	-------	----	2	3ml		
Standard	-------	1 ml	1 ml	3ml		
Unknown	2ml	------	----	3ml		

CALCULATIONS:

X= O.D. of Standard

Y= O.D. of Unknown

Z= Concentration of standard = 0.4mg

Amount of RNA in 2ml of extract = $\frac{Y \times Z}{X}$ = R mg

This 2ml was taken from 10ml of supernatant.

2ml supernatant contains R mg RNA =

10ml supernatant contains 5 x R mg RNA

10 ml of supernatant contains 1 g of plant material

1 g of plant material contains 5 x R mg RNA

10 ml supernatant contains 5 x 10 x R mg of RNA

Amount of RNA = $\frac{\text{O.D. of Unknown x Cone. Of Std x 50}}{\text{O.D. of Standard}}$ mg/g of plant tissue

In 2ml of extract

Orcinol Reagent: 210 mg of Orcinol & 1gm of Ferric chloride dissolved in 100 ml of Conc. HCl

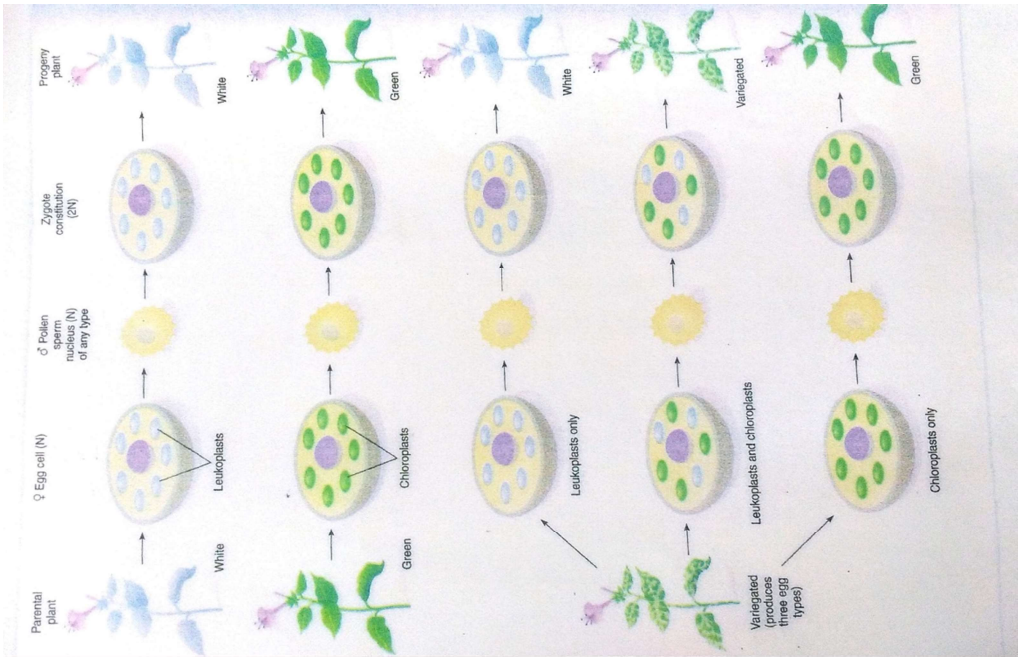
Std RNA: 0.4mg /ml in 5mM NaOH. (Store in freezer)

STUDY OF INHERITANCE PATTERN WITH REFERENCE TO PLASTID INHERITANCE

Inheritance due to factors present in the cytoplasm (non- nuclear) and does not follow Mendelian inheritance is called cytoplasmic inheritance. They show maternal effects.

Example: Inheritance of plastid transmission.

In certain variety of *Mirabilis jalapa* (4 o'clock plant), leaves show green, white or mixed (variegated) colour pattern. In various crosses conducted for the above said trait it was observed that the progeny inherits the character of the female parent plant irrespective of the male parent plant. This trait is due to the type of plastids inherited through the cytoplasm.The phenotype of the progeny depends upon a factor present within the cytoplasm of the egg. The plant leaves show green colour due to chloroplast, white colour due to leucoplasts while variegated variety shows mixed plastids of both types in the cytoplasm of the egg.



STUDY OF CYTOLOGICAL CONSEQUENCES OF CHROMOSOMAL ABERRATIONS (LAGGARDS, CHROMOSOMAL BRIDGE, RING CHROMOSOME, CHROMOSOMAL RING) FROM PERMANENT SLIDES/ PHOTOMICROGRAPHS

Aim: To study of cytological consequences of chromosomal aberrations (Laggards, Chromosomal bridge, Ring chromosome, Chromosomal ring) from permanent slides/ photomicrographs)

A chromosome is an assemblage of genes grouped in a linear order. The number of genes and the position of each gene on the chromosome is always fixed. Deviation from normal or the change from the normal is called aberration. Chromosomal aberrations are important in evolutionary changes of the species or because they produce phenotypic changes like those to simple mutation.

The chromosomal aberrations are of two types namely:

1. Aberration seen in structure of chromosomes.
2. Aberration seen in the number of chromosomes.

Aberration seen in structure is further categorized:

I. LAGGARD CHROMOSOME:

Breakage and reunion of homologous chromosomes may result into deletion of chromosomal segment. The segment deleted may lose its centromere and form acentric chromosome. Such acentric chromosome is unable to get attached to the spindle fibers. Therefore it is unable to move to either poles during anaphasic separation and remain at equatorial plane that is it lags behind.

In anaphase due to the contraction of the spindle fibers chromosomes are separated to form two groups which are pulled towards the different poles. Sometimes one of the chromosomes from either of the group is not pulled towards the respective pole. Such chromosome lies in equatorial region and is called 'lagging' chromosome and the phenomenon the LAGGARD.

II. CHROMOSOMAL BRIDGE:

The aberrations observed were anaphase bridges. An anaphase bridge forms because of the dicentric chromosomes, since this was mitotic divisions. The formation of Anaphase Bridge can be attributed only to translocation.

Translocation involves the breakage of two chromosome and the joining of non-homologous chromosomes. The occurrence of dicentric bridge can be attributed only to the two joining fragments being such that both contain the centromere. The accentric fragments should be visible as laggards. However since they were not seen, it possibly means that they were low by exonuclease activity. The frequency of aberrations is found externally low.

III. RING CHROMOSOME:

Chromosomes are not always rod shaped. Occasionally ring chromosomes is encountered in higher organisms. Sometimes break occur at each end of the chromosomes and broken ends are joined to form a ring chromosome. Crossing over between ring chromosomes can lead to bizarre anaphase figures.

IV. CHROMOSOMAL RING:

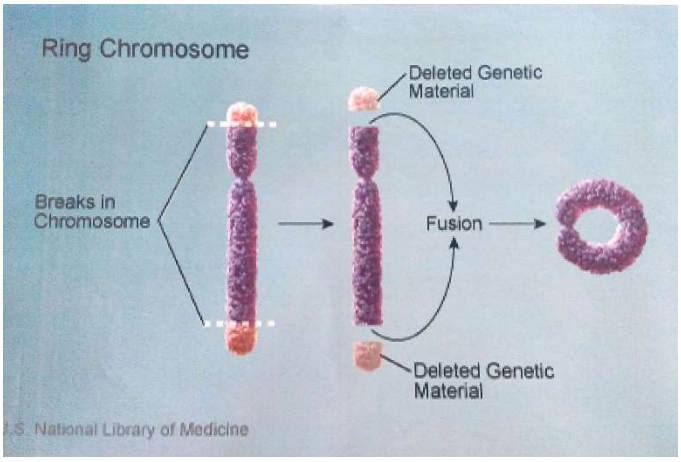
Translocation are usually reciprocal and take place due to interchange of segments between two non-homologous chromosomes. Reciprocal translocations can be homozygotic and heterozygotic.

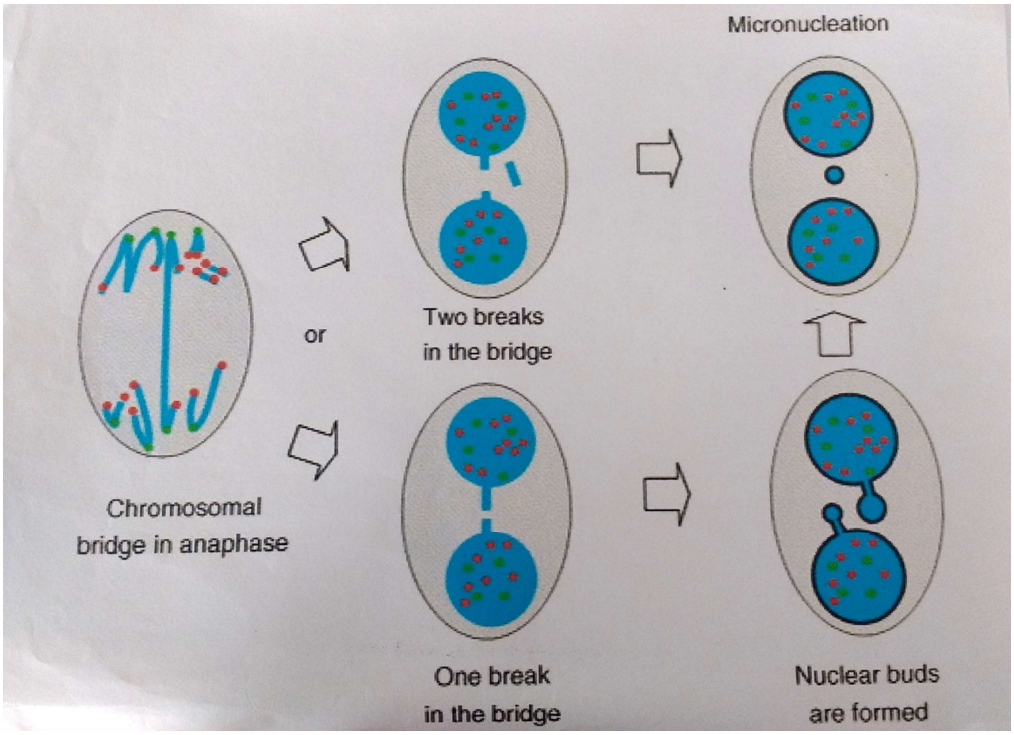
In heterozygotic translocation only one of the chromatids from a chromosome exchanges its segment with a segment from chromatid of a non-homologous chromosome. During synapsis, a cross shaped figure is formed which later opens out in the form of a ring. Thus chromosomal rings are formed. All chromosomes may or may not be involved in the ring formation.

V. CHROMOSOMAL BRIDGE:

Breakage and reunion of homologous chromosomes may result in addition of chromosomal segment in one of the chromosomes (duplication).

If duplication results into formation of chromosome with two centromeres, then, during anaphasic separation of such dicentric chromosome, the two centromeres will move in opposite direction extending a portion of chromosome from one pole to the other. Thus a chromosomal bridge is formed between two sets of separated chromosomes.





TO STUDY THE DIFFERENT STAGES OF MITOSIS BY TEMPORARY MICRO – PREPARATIONS IN ONION ROOT TIP CELLS USING SQUASH TECHNIQUE

Aim: To study different stages of mitosis in onion root tips.

Requirements: Onion root tips fixed in carnoy's fluid, dil. HCl, acetocarmine stain, distilled water, watch glass, slide and cover slip.

Procedure:

Take two to three fixed root tips and wash them thoroughly in a watch glass containing water to remove the fixative. Take a watch glass and put a few drops of dil. HCl. Transfer the root tips in a watch glass containing HCl. (HCl hydrolyses the material, i.e., it dissolves the cementing material between the cells so that they can be easily separated). After hydrolyzing the material, wash the tips once again in the watch glass containing distilled water to remove the traces of HCl. Take a drop of acetocarmine (nuclear stain) on a slide and transfer the root tips to acetocarmine. Warm the slide containing root tip in acetocarmine gently on a burner. Keep the root tip in the stain for five minutes. (warming intensifies the reaction.) Put a cover slip on the slide slowly so that there are no air bubbles. After putting the cover slip, tap the cover slip gently (with the blunt end of the needle) so as to separate the cells. Mount the slide and observe under low magnification first, and the change to high magnification to observe the different stages of Mitosis.

Observation:

Different stages of Mitosis: To observe the different stages move the slide as all the stages may not be seen at one time.

The stages are:

Prophase: In prophase chromosomes appear to be spirally coiled, thread like structure, but later become thick due to duplication of DNA. Each chromosome is now formed of two chromatids. The nucleolus disappears and the nuclear membrane disintegrates.

Metaphase: The chromatids become short and thick. Spindle fibres are formed and chromosomes get attached to the spindle fibres. The chromosomes are arranged on the equatorial plane.

Anaphase: The chromatid of each chromosome is separated into two identical chromosomes. Due to the contraction of the spindle fibres, the daughter chromosomes move towards the opposite poles. The poles in which chromosomes are very near the poles are called late anaphase.

Telophase: The chromosomes at the two poles get elongated and form two spiral thread – like structures, one at each pole. Nuclear membrane is formed round each nucleus. A cell plate is formed between the two nuclei in the equatorial plane.

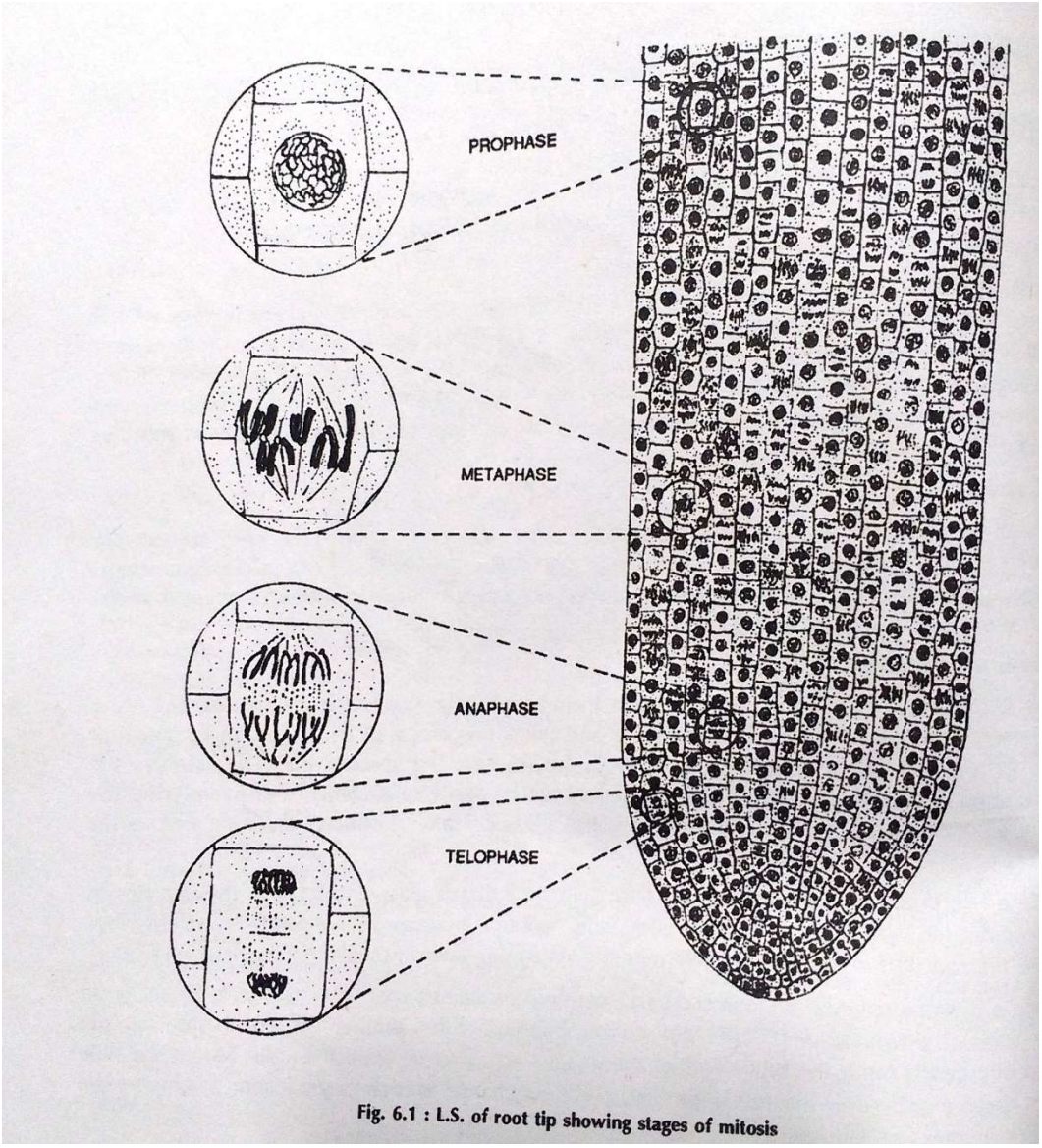


Fig. 6.1 : L.S. of root tip showing stages of mitosis

TO STUDY THE DIFFERENT STAGES OF MEIOSIS BY TEMPORARY MICRO – PREPARATIONS IN *TRADESCANTIA* FLOWER USING SMEAR TECHNIQUE

Aim: To study meiosis from *Tradescantia* buds or any other suitable material by smear technique.

Procedure:

1. Take a preserved and fixed flower bud.
2. Select the smallest flower bud on a clean dry slide.
3. Dissect it open and isolate the anthers with the help of a needle under the dissecting microscope.
4. Tap the anthers to liberate the fluid smear containing pollen mother cells.
5. Discard away the anther wall cells.
6. Add a drop of acetocarmine onto the smear.
7. Pass the slide over the burner few times and wait for 3-5 min., till the pollen mother cells turn dark red in colour.
8. Don't allow the slide to dry. Place a coverslip and press it gently.
9. Blot away excess stain, if any.
10. First observe under low power and then under high power of the microscope.

Theory: Meiosis is also called reductional division. It consists of Meiosis I and Meiosis II. Meiosis I is actually the reductional division in which the chromosome number reduces from diploid to haploid, while Meiosis II is equational division and results in the separation of sister chromatids.
Meiosis I consists of 4 stages: Prophase I, Metaphase I, Anaphase I and Telophase I.
PROPHASE I: It is the longest stage and is further subdivided into 5 substages.
Leptotene or Leptonema: The extended chromosomes begin to coil and become visible as long, thin thread like structures.

Zygotene or Zygonema: Pairing of homologous chromosomes takes place called synapsis. This results in the formation of zipper like structure called synaptonemal complex.

Pachytene or Pachynema: Crossing over occurs between two non-sister chromatids of homologous chromosomes. Each set of homologous chromosomes consists of 4 chromatids and is called a bivalent or tetrad.

Diplotene or Diplonema: The result of crossing over becomes visible as a cross shaped structure called chiasma. The homologous chromosomes begin to move apart, but are still held at the points of chiasma.

Diakinesis (late prophase I): The nucleolus and nuclear envelope breakdown. Simultaneously, the spindle starts appearing. Bivalents show maximum condensation.

METAPHASE I: The nuclear envelope completely breaks down. Bivalents are arranged on the equatorial plane of the cell. Spindle is completely formed.

ANAPHASE I: The homologous chromosomes in each bivalent separate and move towards opposite poles, resulting in dyads.

TELOPHASE I: Nuclear membrane and nucleolus reappear. Cytokinesis follows, producing two haploid cells.

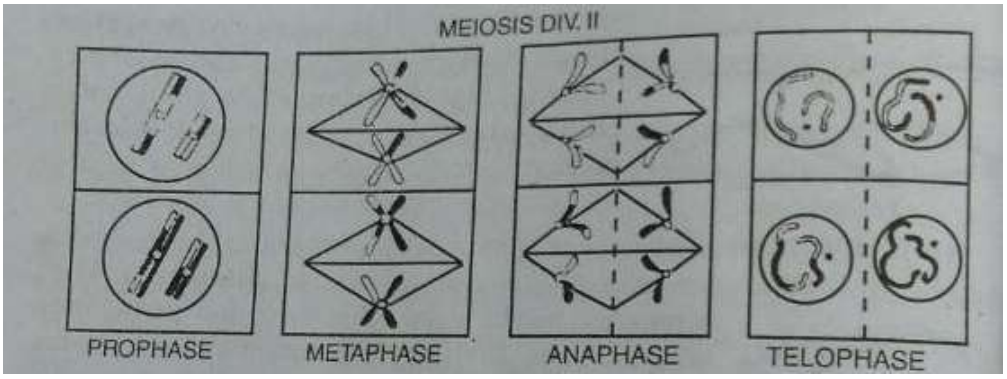
MEIOSIS II is similar to mitosis.

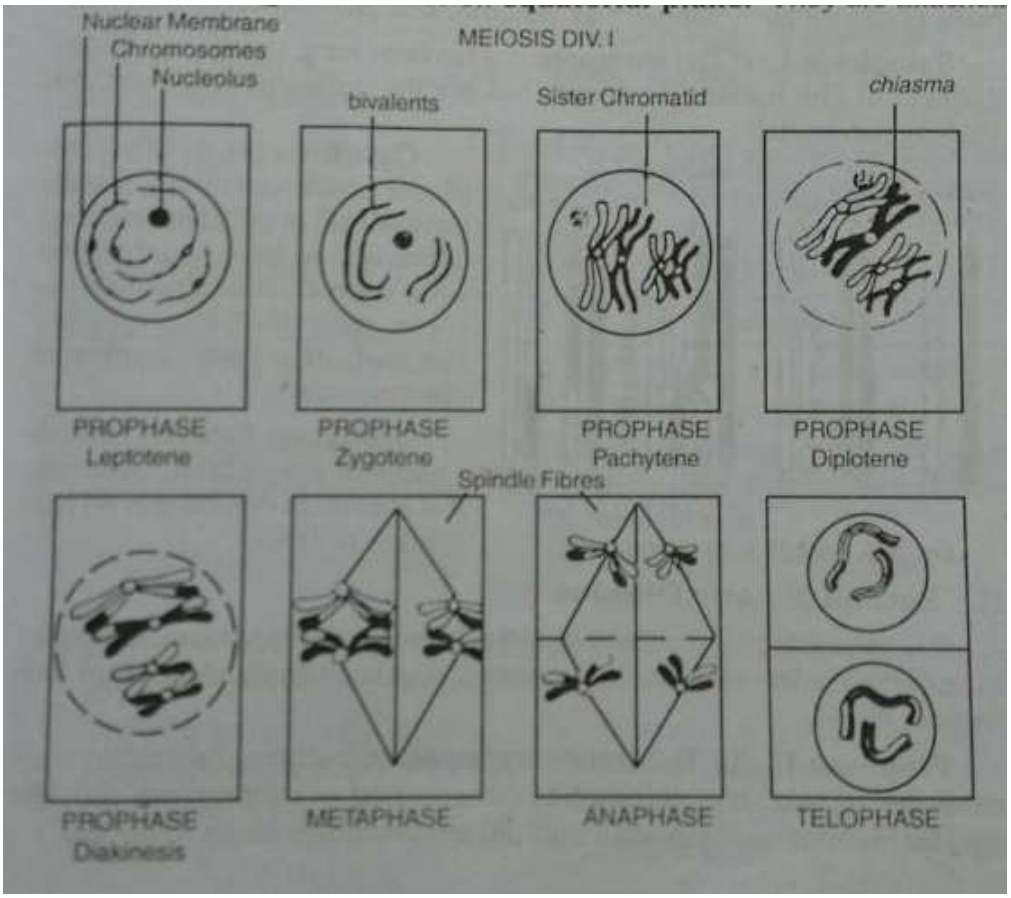
PROPHASE II: The chromosomes condense.

METAPHASE II: Chromosomes move towards equatorial plate which is at right angles to the equatorial plate of the 1st division.

ANAPHASE II: The centromeres split and the sister chromatids are pulled up to the opp. poles of the spindle.

TELOPHASE II: Chromosomes uncoil, nuclear membrane reappears around each set of chromosomes and cytokinesis takes place. This results in the formation of 4 cells.





N.B.

1. Carnoy's fluid: alcohol: chloroform : acetic acid in 6:3:1

2. The best results are obtained if the bud is fixed between 7.00 am and 8.00 am

DNA SEQUENCING – SANGER'S METHOD

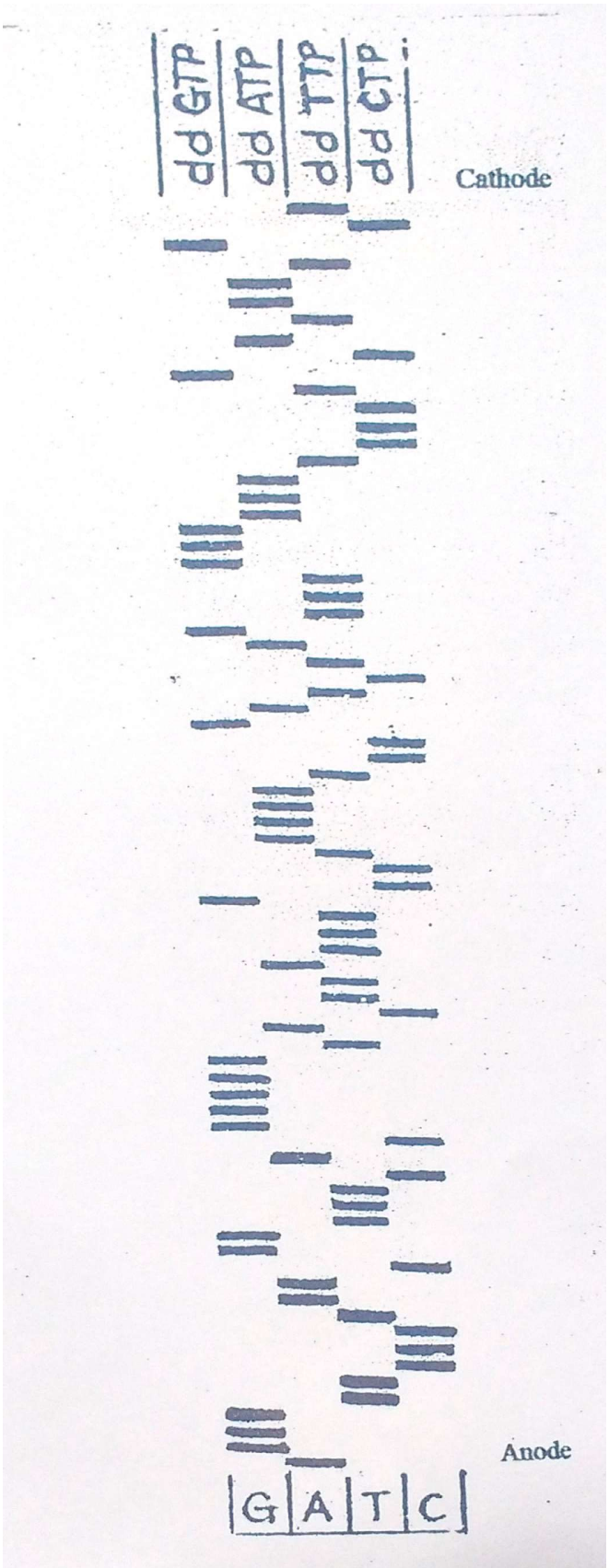
Aim: To read the DNA sequence from the given autoradiogram.

Principle: DNA sequencing can be carried out by two methods.
Maxum and Gilbert's chemical degradation method
Sanger and Coulson's enzymatic method.

Theory: In this method, a single stranded DNA is used as a template for DNA synthesis. 2′ 3′ Dideoxynucleotides (ddNTP) are incorporated leading to termination of synthesis. These ddNTPs are used as triphosphates and they can be incorporated into the growing chain. The respective ddNTPs terminate the synthesis since they cannot form a phosphodiester bond with the incoming nucleotide.

Procedure: This method consists of following steps:
Four reaction tubes are set up each containing single stranded DNA sample to be sequenced cloned in M13 phage.
The DNA synthesis is terminated by the incorporation of ddNTP to terminate any one of the four bases.
The reaction mixtures are then electrophoresed.
It is exposed to X ray photographic plates which gives the autoradiogram.

Reading autoradiogram: The autoradiogram is always read from the anode to the cathode. The sequence is that of the nascent strand which is in 5'→ 3' direction. The complemetary strand can be deduced from this in 3'→ 5' direction


dd GTP
dd ATP
dd TTP
dd CTP
Cathode
Anode
G
A
T
C

AMINO ACIDS SEQUENCING IN THE PROTEIN MOLECULE SYNTHESISED FROM THE GIVEN m – RNA STRAND

Aim: To determine the sequence of amino acid in the protein molecule synthesized from the given m – RNA strand.

Theory: Proteins are polymers of amino acids linked by polypeptide bonds. It starts from the amino group which begins as N- terminal and ends with either C- terminal or the carboxy end. Protein exhibit the phenotypic character of an organism. It is synthesized as a result of transcription and translation.

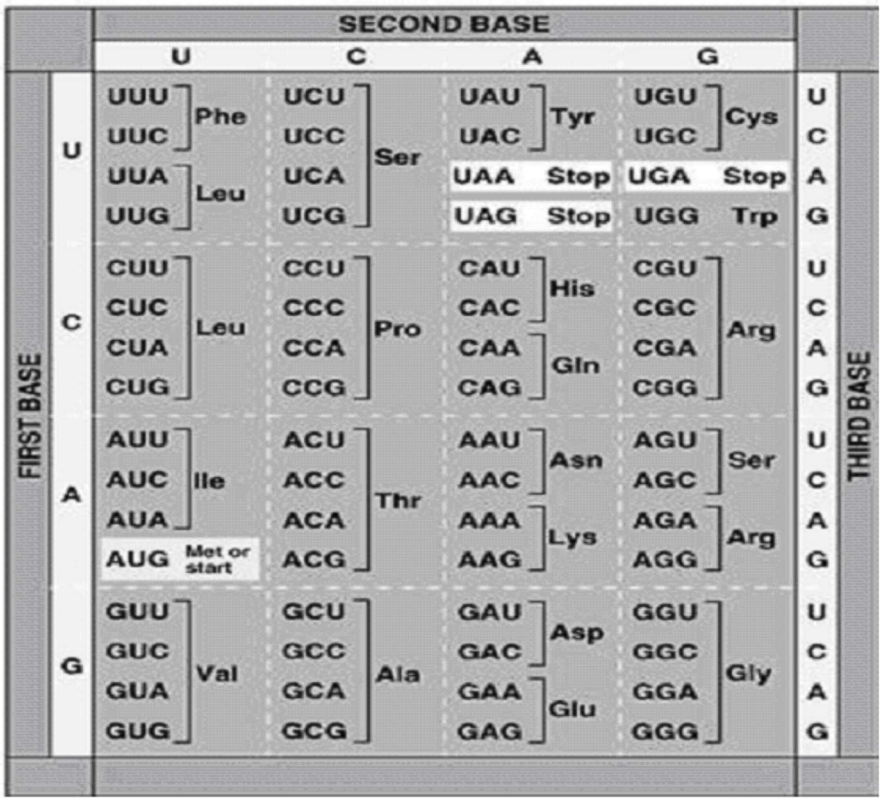
Transcription: This involves the synthesis of complementary single stranded m – RNA from the double stranded DNA template. The RNA based sequences are read in 5'→ 3' direction.

Translation: It involves the formation of protein molecules. It can be divided into three steps namely initiation, elongation and termination of polypeptide chain.

Reading m – RNA strands of PROKARYOTES: The given m – RNA strand does not show 7 methylated C cap (7 mc) at the 5' end. Therefore it is inferred that it is prokaryotic m – RNA. In the prokaryotes, **30S** ribosomal unit always gets attached to the ribosome binding site called "The Leader sequence" or **"Shine – Delgarno sequence (SD)"**. It is a purine rich sequence (5' – AGGAGGU – 3'). It is 8 – 13 bases upstream of initiation codon AUG or GUG. After the identification of SD, AUG or GUG is identified as initiating codon both of which could code for formylated Methionine (f - met). The m- RNA is now divided into codons of 3 nucleotides each. The amino acid sequence is read with the help of genetic code dictionary. The m – RNA of prokaryotes is said to be polycistronic and hence more than 2 polypeptide chains can be synthesized.

Reading m – RNA strands of EUKARYOTES: The eukaryotic strands are always having 7 mc cap at the 5' end. The end of the tail is always made up of poly A base. The initiating codon AUG or GUG is identified and the amino acid sequence is read till a terminating codon is reached. The m – RNA of eukaryotes is monocistronic therefore only one type of polypeptide chain can be syntheised.

AMINO ACID DICTIONARY



Phe– Phenalamine	Leu – Leucine	Ser – Serine	Tyr – Tyrosine
Cys – Cysteine	Trp – Tryptophan	Pro – Proline	His – Histidine
Gln – Glutamine	Arg – Arginine	Ile – Isoleucine	Thr – Threonine
Asn – Asperagine	Lys – Lysine	Ser – Serine	Arg – Arginine
Val – Valine	Ala – Alanine	Asp– Aspartic acid	Glu – Glutamine
Gly – Glycine	Met – Methionine (Initiator)	UAA /UGA – Ochre (Stop)	UAG – Amber (Stop)

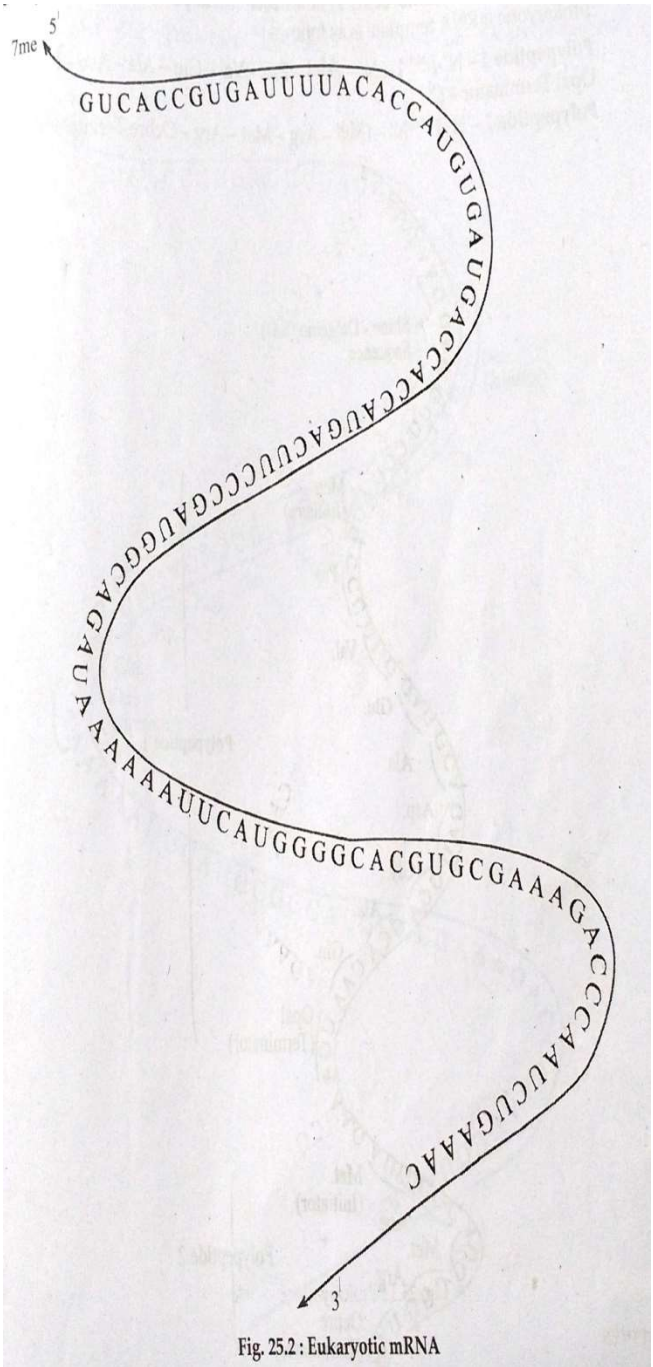


Fig. 25.2 : Eukaryotic mRNA

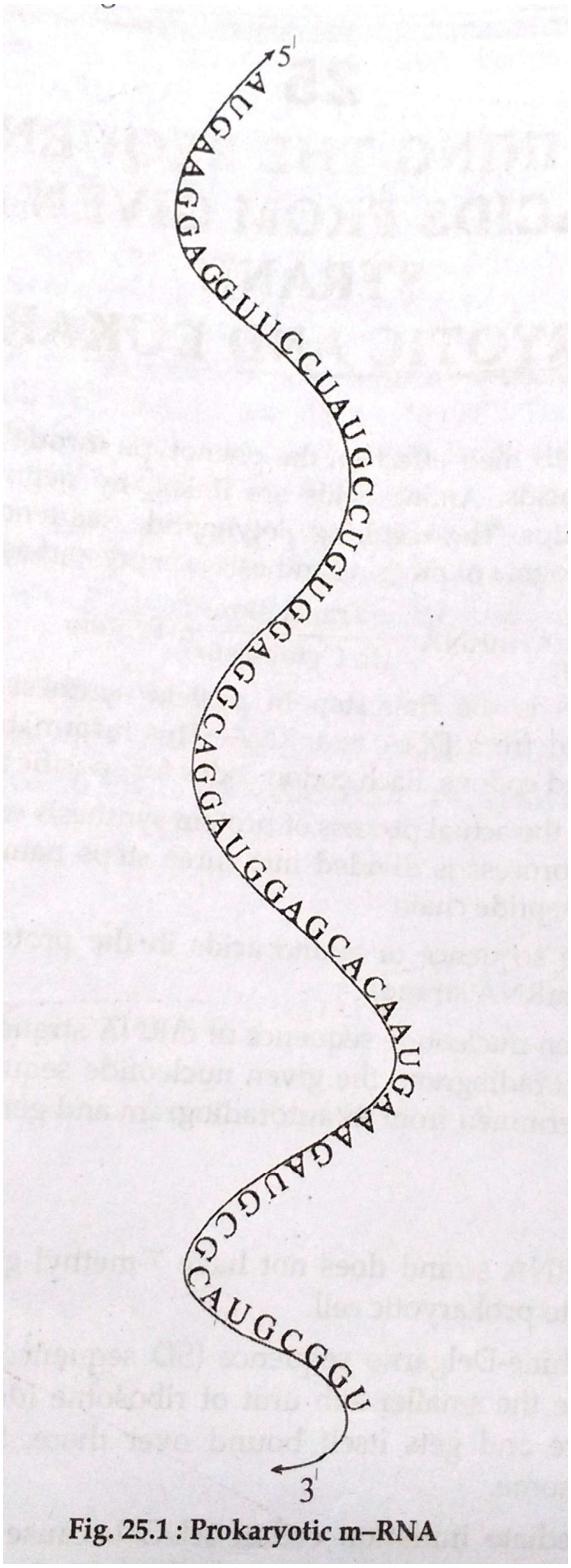


Fig. 25.1 : Prokaryotic m–RNA

INDEX

SEMESTER- III

PRACTICAL: III (Current Trends in Plant Sciences)

STUDY OF *PHYLLANTHUS AMARUS*, *SARACA ASOKA*, *BACOPA MONIERI*

Eclipta alba: Bhringraj consists of the dried whole plant of *Eclipta alba* (L.) Hassk. (Fam. Asteraceae). Bhringraj contains not less than 0.1 per cent of wedelolcatone, calculated on the dried basis.

Description. A green to greenish brown colour when completely dry.

Macroscopic characters — Root. Well developed, a number of secondary branches arise from main root up to about 7 mm in dia, cylindrical, greyish. Stem. Herbaceous, branched occasionally rooting at nodes, cylindrical or flat, rough due to oppressed white hairs, node distinct, greenish, occasionally brownish. Leaf. Opposite, sessile to sub sessile, usually oblong, lanceolate, sub-entire, sub -acute or acute, strigose with appressed hairs on both surfaces. Flower - Solitary or 2, together on unequal axillary peduncles, involucral bracts about 8, ovate, obtuse or acute, herbaceous, strigose with oppressed hairs; ray flowers ligulate, ligule small, spreading, scarcely as long as bracts, not toothed; white disc flowers tubular, corolla often 4 toothed; pappus absent, except occasionally very minute teeth on the top of achene; stamen 5, filaments epipetalous, free, anthers united into a tube with base obtuse; pistill bicarpellary; ovary inferior; unilocular with one basal ovule. Fruit - Achenial cypsella, one seeded, cuneate, with a narrow wing, covered with warty excrescences, brown. Seed-Dark brown, hairy and non-endospermic.

Microscopic characters — Powder Dark green in colour; shows vessels in large groups or single broken pieces with pitted walls, numerous fibres entire or in pieces, trichomes entire or in pieces, warty, a few attached with epidermal and subsidiary cells, anomocytic and anisocytic stomata.

Root: The cells of outer one or two rows of secondary cortex, elongated or rounded with air cavities, while cells of inner secondary cortex, elongated to irregular in shape. Stone cells scattered in secondary cortex. Phloem rays broader towards the periphery, cells rounded. Xylem rays distinct, run straight in tangential section, rarely uniseriate and biseriate, cells pitted.

Stem: A few epidermal cells elongate to form characteristic non-glandular trichomes. Secondary cortex composed of large, rounded parenchymatous cells having wide air space. Vascular bundle in a ring, collateral, endarch, of varying size. Vessels barrel-shaped, some elongated with simple perforations, pitted with spiral thickening. A few xylem fibres bifurcate. Xylem rays uniseriate or biseriate. Leaf. Anomocytic and anisocytic stomata and non-glandular hairs are present on both surface, more abundant on lower side. Vascular bundle, fine in mid rib, central one largest while four other small flanking either side of central bundle.

Determine by thin-layer chromatography:

Stationary phase: pre-coated silica gel plates

Mobile phase: toluene: acetone: formic acid. (9:6:1)

Test solution. Reflux 1g of the coarsely powdered substance under examination with 25 ml of methanol for 30 minutes, cool and filter. Reflux the residue further with 3 × 25 ml of methanol, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with anisaldehyde sulphuric acid reagent. Heat the plate at 100º for 5-10 minutes and examine the plate in day light.

Foreign organic matter: Not more than 2 .0%

Ethanol-soluble extractive value: Not less than 5.0%

Water- soluble extractive value: Not less than 15.0%

Total Ash: Not more than 22 %

Acid-insoluble ash: Not more than 11 %

Loss on drying: Not more than 15.0 %

Phyllanthus amarus: Bhuiamla consists of the dried aerial parts of *Phyllanthus amarus* Schum. & Thom. (Fam. Euphorbiaceae). Bhuiamla contains not less than 0.25 per cent of total phyllanthin and hypophyllanthin, calculated on the dried basis.

Description. A green to greenish yellow in colour, taste, slightly bitter.

Macroscopic characters

Stem: erect, 1-4 mm in diameter. Leaves oblong 5 × 3 mm, short stalked, greenish brown in colour.

Microscopic characters

Stem: inner cortex chlorenchymatous; xylem rays 1-2-seriate.

Leaf: stomata mostly paracytic; epidermal cell wall markedly sinuous; rosette and prismatic crystals of calcium oxalate along the veins and midrib.

Determine by thin-layer chromatography

coating the plate with silica gel GF254. Mobile phase.

Mobile phase: toluene: ethyl acetate:formic acid:methanol.(6:2:1:0.2)

Test solution: Reflux 2 g of coarsely powdered substance under examination with 50 ml methanol on a boiling water-bath for 30 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of methanol, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with methanolic sulphuric acid (10 per cent, v/v) Heat the plate at 120° for 5-10 minutes and examine in day light.

Foreign organic matter: Not more than 2.0 %.

Ethanol-soluble extractive: Not less than 6.0%

Water-soluble extractive: Not less than 15.0 %

Total Ash: Not more than 8.0 %

Acid-insoluble ash: Not more than 5.0 %.

Loss on drying: Not more than 12.0 %

Bacopa monnieri: Brahmi consists of the dried whole plant, preferably leaves and stem of *Bacopa monnieri* (Linn.) Pennell (Fam. Scrophulariaceae). Brahmi contains not less than 2.5 per cent of bacoside A, calculated on the dried basis.
Description. A brown to reddish brown colour when completely dried or green colour when partially dried with slightly bitter taste.

Macroscopic characters:

Herbaceous comprising of stems, runner stems and leaves. Stems glabrous, leafless towards the base; internodes long. Leaves spatulate-obovate, sessile, and glabrous.

Microscopic characters:

Stem: Cortex in stem composed of parenchyma cells enclosing large air spaces; xylem vessels radially arranged xylem rays uniseriate; pith parenchyma collapsed.

Leaf: midrib indistinct, mesophyll isobilateral of spongy cells, a few prismatic crystals of calcium oxalate in mesophyll; stomata anomocytic on both the surfaces of leaf.

Determine by thin-layer chromatography

Coating the plate with silica gel GF254.

Mobile phase. Chloroform: methanol. (7:3)

Test solution: Reflux 2 g of coarsely powdered substance under examination with 25 ml methanol for 15 minutes, cool and filter. Reflux the residue further with 2 x 25 ml of methanol, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with methanolic sulphuric acid (20 per cent v/v). Heat the plate at 100º for 5-10 minutes and examine in day light.

Foreign organic matter: Not more than 2.0%

Ethanol-soluble extractive value: Not less than 6.0%.

Water-soluble extractive value: Not less than 22 %

Total Ash: Not more than 18 %

Acid-insoluble ash: Not more than 6.0 %.

Loss on drying: Not more than 12.0 %

Saraca asoka: It consists of dried stem bark of *Saraca asoca* (Rose.) De. Willd, (Fam. Leguminosae), collected in spring from mature, wild or cultivated trees, found in Central and Eastern Himalayas, Western Ghats and Deccan.

Macroscopic characters:

Bark: channeled, externally dark green to greenish grey, smooth with circular lenticels and transversely ridged, sometimes cracked, internally reddish-brown with fine longitudinal strands and fibers, fracture splintery exposing striated surface, a thin whitish continuous layer is seen beneath the cork layer, taste, astringent.

Microscopic characters:

Transverse section of stem bark shows periderm consisting of a wide layer of cork, radially flattened narrow cork cambium, secondary cortex wide with one or two continuous layers of stone cells with many patches of sclereids, parenchymatous tissue contains yellow masses and prismatic crystals: secondary phloem consists of phloem parenchyma, sieve tubes with companion cells and phloem fibres occuring in groups, crystal fibres present.

Foreign matter: Not more than 2%

Total Ash: Not more than 11%

Acid-insoluble ash: Not more than 1%

Alcohol (90 per cent) soluble extractive: Not less than 15%

Water-soluble extractive: Not less than 11%

Thin Layer Chromatography:

Five gram fine powder of each of the samples are refluxed with 50 ml petroleum ether overnight. These extracts are cooled to room temperature, filtered, concentrated by evaporation under vacuum and are used for the TLC.

Stationary phase: Pre-coated silica gel plates

Mobile phase: benzene: ethyl acetate (9.7:0.3)

Dry the plate in air and spray with antimony trichloride TLC reagent. After drying in a hot air oven for 5 minutes at 90°C viewed under UV-365 nm.

STUDY OF BIODIVERSITY COMPOSITION OF DIFFERENT TYPES OF FORESTS IN INDIA

Geographically, India is a tropical country but whole of the Gangetic plains lies outside the tropics. It has strong monsoonic climate and differs from other tropical regions of world.

Depending upon the climate, the vegetation of India may be divided into tropical, sub-tropical, temperate and alpine.

The following types of forests in India which cover nearly 17 per cent of the total area of Indian Territory.

I. TROPICAL FOREST: A great majority of these forests found in India are of this type. Tropical forests are of two types viz. Tropical moist forests and Tropical dry forest.

A. Tropical moist forest: These are further classified into the following four types on the basis of relative degree of wetness.

A. i. Tropical moist evergreen forests or Tropical rain forests: found in very wet regions receiving more than 250 cm average annual rainfall. Vegetation - Luxuriantly growing lofty trees more than 45 meters in height, shrubs, lianas (woody climbers) and epiphytes in abundance because of high rainfall (Storied vegetation)

Found in – Andaman and Nicobar Islands, Western coasts and parts of Karnataka, Annamalai hills, Assam and Bengal

Plantation – Belonging to families Leguminoceae, Lauraceae, Myrtaceae, Moraceae

A ii. Tropical moist semi-evergreen forests: found in regions receiving annual rainfall between 200 and 250 cm.

Vegetation – Characterized by giant and luxuriantly growing intermixed deciduous and evergreen species of trees and shrubs such as *Terminalia sp.*,

Bambusa, *Ixora*, *Albizzia*, Orchids, ferns and some grasses and several other herbs are also common.
Found in – Along the western coasts, eastern Orissa and upper Assam

A iii. Tropical moist deciduous forests: Extensive area of country receiving sufficiently high rainfall (100 – 200 cm / annum) spread over most of the year.
Vegetation – *Tectona*, *Terminalia*, *Grewia*, *Dalbergia*, *Lagerstroemia*. These forests produce some of the important timber yielding plants.
The forests are found along the western side of the Deccan plateau i. e. Mumbai, N.E. Andhra, Gangetic plains and in some Himalayan tracts extending from Punjab in west to Assam valley in the east.

A. iv. Littoral and Swamp forests: Includes Beach forests, Tidal forests or Mangrove forests and Fresh water swamp forests

B. Tropical dry forests: These are classified into the following types:
B. i. Tropical dry evergreen forests: Found in areas where rainfall is in plenty but dry season is comparatively longer.
Vegetation The trees are dense, evergreen and short (about 10 to 15 meters high). Species of *Zizyphus*, *Randia*, *Calotropis*, *Feronia*. Bamboos are absent but grasses are common.
Found in eastern parts of Tamil Nadu, in east and west coasts.
B. ii. Tropical dry deciduous forests: Distributed in area where annual rainfall is usually low, ranging between 70 to 100 cm. the largest area of the country's forest land is occupied by Tropical dry deciduous forests. The dry season is long and most of the trees remain leafless during the season. The forest trees are not dense, 10 to 15 meters in height and undergrowth is absent.
Vegetation – Acacia, Terminalia, Moringa, Aegle, Sterculia, Dalbergia
Found in Punjab, U. P., Bihar, Orissa, M. P. and large part of Indian peninsula

B. iii. Tropical thorn forests: These forests occur in the areas where annual rainfall is between 20 and 70 cm, dry season is hot and very long.
Vegetation – The vegetation in these regions are found only along the rivers consisting of small trees (8 to 10 meter high) and thorny and spiny shrubs of stunted growth. Species of *Acacia*, *Cassia*, *Calotropis*, *Zizyphus*, *Aegle*, *Atriplex*, *Asparagus*, *Butea*, *Capparis*, *Salvadora*, *Prosopis*, *Euphorbia*. Found in South Punjab, most of Rajasthan and part of Gujarat.

II SUBTROPICAL MONTANE FORESTS: These forests are found in the region of fairly high rainfall but where temperature between winter and summer are less marked. They are found up to the altitude of about 1500 meter in south and up to 1800 meters in the north. In composition, these forests are almost intermediate between tropical forests and temperate forests.

i. Wet hill broad leaved forests: These are found in Mahabaleshwar, Coorg, Karnataka, parts of Assam, Panchmarhi and other parts of M. P.
Vegetation – *Eugenia*, *Randia*, *Terminalia*, *Elegnus*, *Ficus*, *Lantana*, *Cedrella*, *Garcinia*.

ii. Pine forests: Mostly in Himalayas and in Assam hills.
Vegetation – The forests are dominated by species of *Pinus* (*P. khasya*, *P. roxburghii*). *Quercus*, *Carissa*, *Bauhinia* may also occur rarely in Pine forest.

III. TEMPERATE MONTANE FORESTS: These forests occur in the Himalayas at the altitude from 1800 to 3800 meters where humidity and temperature are comparatively low.
Himalayan Moist temperate forests: Develop in the areas of lesser rainfall.
Vegetation – Oak and conifers, undergrowth is shrubby and consists of deciduous species.

IV SUB-ALPINE FORESTS: Are found throughout Himalayas from Ladakh in the west to Arunachal in the east at the altitude from 2800 to 3800 meters. Annual rainfall is less than 65 cm but snowfall occurs for several weeks in a year.

Vegetation – *Betula*, *Quercus*, *Pyrus*, *Rhododendron*, *Juniperus*, *Salix*, *Abies* are common plants of these forests.

V ALPINE FORESTS: Plants growing at the altitude from 2900 to 6000 meters are called alpine plants. In India, alpine flora occurs in Himalayas between 4500 to 6000 meters.

Vegetation – *Quercus*, *Betula*, *Pinus*, *Abies*, shrubby *Rhododendron*

Report of visit to forest/ garden

Location ----------, Climate ---------- DATE ----------

NAME OF PLANT	*FAMILY*	*LOCAL NAME*	*LOCALITY DETAILS*				*PHENOLOGY*					*Evergreen/deciduous*	*REMARK*
			Rock	*plain*	*slope*	*Water body*	*V*	*FL*	*FR*	*S*	*SD*		

SOURCES, PROPERTIES AND USES OF FIBRES, PAPER AND SPICES AND CONDIMENTS

A) PLANTS YIELDING FIBRES –

1. Cotton (Kapas):

Botanical Name: ***Gossypium hirsutum***
Family: Malvaceae
Source: upper surface of the seed coat

The fibres are of two kinds viz. Lint and Fuzz. Lint fibres are long hairs which are more commercially important. Fuzz are short hairs and not commercially important. The fibres are chiefly composed of cellulose.

Uses:
In Textile industry, stuffing, rubber tyres, fabrics, carpets, blankets, cordage, cellulose industry, paper making, rayon etc.

2. Jute:

Botanical Name: ***Corchorus capsularis***
Family: Tiliaceae
Source: Stem

It is a textile fibre, soft fibre or bast fibre, achieved from the secondary phloem of the stem.

Uses:
This fibre is utilized in various purposes i. e. in preparation of gunny bags, twines, carpets, inferior cloth, curtains, packing cloth, ropes etc.

B) PLANTS USEFUL IN PAPER INDUSTRY:

Plants which are useful in the manufacture of paper are mainly Eucalyptus and Bamboo. (Recently Eicchornia is also used for particular type of paper).

i. Botanical Name: ***Eucalyptus globulus***
Family: Myrtaceae
Plant part used: Bark

ii. Botanical Name: ***Bambusa arundinaceae***
Family: Gramineae (Poaceae)
Plant part used: Stem

C) PLANTS USEFUL IN SPICES AND CONDIMENT–

Spices and Condiments are plant products used to improve the taste, flavour and aroma of food. They also have medicinal properties.

i. Cardamom:

Botanical Name: ***Elettaria cardamomum***
Family: Zingiberaceae
Plant part used: Dried fruits

Uses:
1. Used in curry powder.
2. Flavouring sweetmeats, pastries, cakes etc.
3. Medicinally used in treatment of cold and cough, as carminative, in tea.
4. Eating a cardamom once daily with a tablespoon of honey improves eye-sight, strengthens the nervous system and keeps one healthy.

ii. Saffron:

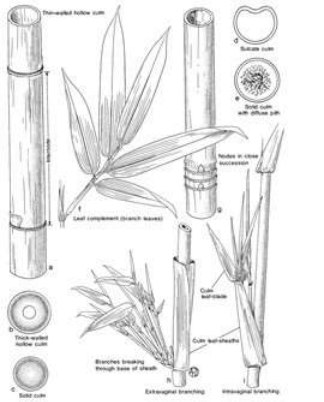
Botanical Name: ***Crocus sativus***

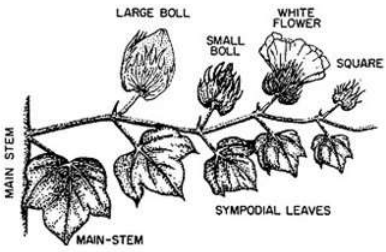
Family: Iridaceae

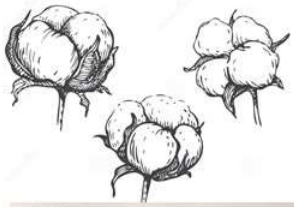
Plant part used: Dried stigma of flowers.

Uses:

1. It is used as colouring, flavouring and garnishing agent in sweetmeats.
2. It is used in treatment of fevers and enlargement of the liver and spleen.
3. It helps in urinary, digestive and uterine troubles.







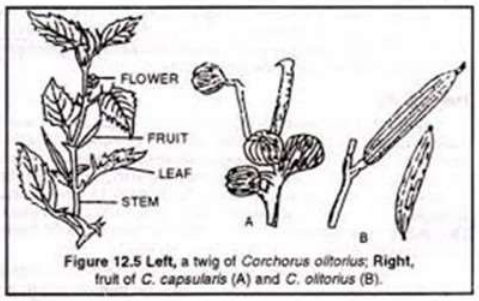


Figure 12.5 Left, a twig of *Corchorus olitorius*; Right, fruit of *C. capsularis* (A) and *C. olitorius* (B).

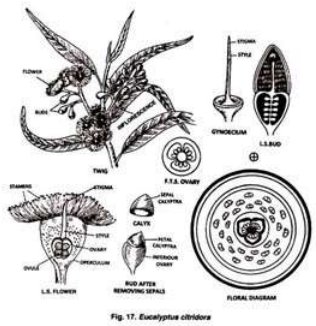


Fig. 17. *Eucalyptus citriodora*

PREPARATION OF HERBAL COSMETICS

Aim: To prepare herbal cosmetics (Face pack/ De-tanning cream**)**

Principle: Acne, black head, pimples, dark circle are common among youngsters and person who suffers from it. According to Ayurveda, Skin problems are normally due to impurities in blood. Accumulated toxins in the blood during improper food and lifestyle are causing skin related diseases. Various herbs, medicines are described in Ayurveda for blood purification. Herbs like Manjistha, Lodhra, Chandana, Haridra etc. are good example of blood purifier. The herbal paste which is applied on face to treat acne, pimple, scars, marks and pigments are known as “mukha lepa” in ayurveda. The process of smearing this herbal mix on face is known as “mukha lepana”. This beauty therapy is popular as facial. The smooth powder which is used for facial application is “face pack”. A good herbal face pack must supply necessary nutrients to skin. It should penetrate the subcutaneous tissues in order to deliver the required nutrients. Different types of skin need different types of herbal face packs.

Benefits of Applying Face Pack

1. Nourishes the skin. Fruit face packs supply essential nutrients to skin.
2. Helps to reduce, acne, pimple, scars and marks depending on its herbal ingredients.
3. Face packs usually remove dead cells of skin.
4. These face masks provide a soothing and relaxing effect on skin.
5. They help to restore the lost shine and glow of skin in short span of time.
6. Regular use of natural face masks bring glow to skin, improve skin texture and complexion.
7. The harmful effects of pollution and harsh climates can be effectively combated with judicial use of face packs.
8. They help to prevent premature aging of skin.
9. Formation of wrinkles, fine lines and sagging of skin can be effectively controlled by using natural face packs.

10. Natural face packs make the skin look young and healthy.

MATERIALS AND METHODS

For preparation of facepack following palnt materials are taken:

Multani Mitti (Calcium bentonite): Multani mitti will remove all the impurities and dead skin cells. Multani mitti will help to make you skin radiant and excellent for aggravated and irritated skin. Its cooling action soothes the skin, relieves the inflammation caused due to aggravated pitta. It removes the dirt and dead skin cells accumulated and replace with fresh, radiant and glowing skin.

Manjistha (*Rubia cordifolia*): Manjistha holds the reputation of a very good skincare herb. Used externally and internally, it helps one to gain lustre and glow (of the skin) and aids to remove pimples, freckles and discoloration. Its paste should be applied in various skin disorders like itching, black spots on the face, pimples, leucoderma etc. According to Charaka, Manjistha is varnya (improving the complexion), jvarahara (febrifuge) and visaghna (detoxifier)

Haridra (*Curcuma longa*): Haridra has anti-inflammatory and anti-allergic activity. It is best blood purifier and helps in wound healing. It possess best blood purification action so it is used in all disease with blood impurities origin. Haridra is rejuvenator of skin and revitalizes skin; delays the signs of aging like wrinkles.

Raktachandan (*Santalum album*): Rakta chandan (Red Sandalwood Powder) has curative value in skin allergies. Raktha Chandan powder cooling and soothing action, protects the skin against the impact of environmental pollution and keep the skin cool, fair and healthy. Sandalwood is helpful Ayurvedic herb with antimicrobial properties is used for healing various skin problems and removes scars.

Lodhra (*Symplocos racemosa*): Its name lodhra in Sanskrit means “that which makes the body firmer.” Lodhra nourishes the skin and benefits in acne, wrinkles and other health issues related with skin. It lightens skin colour, reduces skin irritation and benefits for acne, wrinkles and other skin related issues. Lodhra is useful in skin diseases requiring purification of the skin.

lodhra is shavaro (making the body and skin very attractive) andgalavastatha (helping the whole body to maintain its shape).

Formulation of Face Pack

The powdered ingredients are sieved using a fine mesh, weighed accurately and mixed geometrically for uniform mixing This is then stored in an air tight container for evaluation.

How to use?

- Prepare powder of all herbs- Lodhra, Rakta chandan, Haridra, Manjistha, Multani Mitti (10:20:20:20:30).
- Mix all powder as per formula
- Make the paste mixing final mixture of herbs with rose water (normal or dry skin) or butter milk (oily skin).
- Apply paste locally in face.
- Wash the face with fresh water before it dries up.

Evaluation of Face Pack

Organoleptic Properties: The nature, color, odor, taste and texture of the dried powder of combined form were tested manually.

Physicochemical Evaluation: total ash and acid insoluble ash was performed using incinerator, pH was found by using pH meter and moisture content was also performed. **General powder Characteristics**: The particle size of the dried powder of combined form was tested by microscopy method for presence of lignified fibers, fibers, sclerenchyma cells etc.

Shinoda Test: To the extract add few magnesium turnings and concentrated hydrochloric acid. The appearance of red colour indicates the presence of flavonoids.

RESULTS AND OBSERVATION:

Table: Evaluation of Face Pack

Evaluation parameters	Observation
Organoleptic evaluation 1. Nature (appearance) 2. Color 3. Odor 4. Taste 5. Texture	
Grittiness	
Microscopical features Presence of lignified fibers, fibers, sclerenchyma cells were found	
Chemical test: Shinoda Test Presence of flavonoids.	
Nature of face after wash	

Aim: To prepare a skin de-tanning cream

The application of plants and plant products are widely used for preparation of herbal formulations such as moisturizing, whitening, tanning, colour cosmetic, sunscreens, radical-scavenging, anti-oxidant, immunostimulant, washing, preservatives, ands thickeners. Cosmetic preparations become more advantageous when using antioxidant as an active ingredient. There is growing interest in the natural antioxidants found in
plants. Many acting compounds are isolated from natural herbs and extracts and used as potential antioxidants in cosmetics. The most successful recent and natural skin whitening agents are vitamin C, kojic acid, litoric extract, burnet root extract, scutellaria extract, and mulberry
In fair skin, the keratinocytes would cluster poorly their pigment melanosomes above the nuclei. Meanwhile, in the dark skin, the pigmented melanosomes are individually distributed in the keratinocytes. Environment also can be ones of the factors to the constitutive of melanocytes density which is by chronic ultraviolet (UV) radiation or by toxic compounds such as hydroquinone. These ultraviolet rays may increase the melanocytes density by 3- or 4-fold. The present of the toxic compound may be serious effect to the skin structure where it can be very selectively and permanently destroy the melanocytes in the skin. Increasing melanocytes density such as freckles or decreasing the melanocytes density such as vitiligo in the skin may be caused by the inherited of pigmented disorder.
The skin colour is a function of size, number and the distribution of melanin cells. Melanin cell of darkly pigmented skin have thicker, longer and branched dendrites. Tyrosinase is a rate-limiting enzyme of this biosynthetic pathway. Activities of tyrosinase give effect on the skin colour. Melanin cells are produced from the black pigment due to the increasing of tyrosinase activity. Therefore, this is the main reason why this research is conducted that are to inhibit the tyrosinase activity in order to reduce the synthesis of melanin through the papaya or kojic acid extract.

An extracts of *Glycyrrhiza* and Papaya are rich in natural antioxidants. The best natural antioxidants are glycyrrhizin (glycyrrhizic acid), papin and flavonoids. The role of plant extract on skin is mainly attributed to its antioxidant activity particularly to its potent antioxidants triterpene, saponins and flavonoids. Skin whitening, skin depigmenting, skin lightening, anti-aging, anti-erythemic, emollient, anti-acne and photo protection effects.

Materials for De-tanning cream

The materials which are used to prepare de-tanning cream are as follows:

Olive oil

Cetomacrogol - 1000.

Cetostearyl alcohol.

Beeswax.

Glycerin.

Lemon oil.

Aloe vera gel

Shea Butter or cocoa butter

Extract of *Glycyrrhiza glabra* and papaya (dried in oven for 4 days 40 degrees temperature and powdered) (ethanolic extract) is to be prepared.

Preparation of Base and Formulation: Water in oil (W/O) cream is prepared by the addition of aqueous phase to the oily phase with continuous agitation.

1) To prepare base(placebo); oily phase that consisted of olive oil, beeswax, Shea butter and surfactants (cetomacrogol 1000 and cetosteatyl alcohol), is heated up to 75°C±1°C.
2) Aqueous phase consisting of aloe vera gel, glycerin and water is heated to the same temperature.
3) The formulation was also prepared by same method; the only difference is the addition of Glycyrrhiza glabra and Papaya extract (active drug) that is added in aqueous phase consisting of glycerin and water.

5) Stirring was continued at 2000 rpm by the mechanical mixer for about 10 minutes until complete aqueous phase was added. After the complete

addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization. Then triethanolamine was added and homogenization was carried out for a period of 5 minutes, then the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the formed cream cooled to room temperature.

Table I. Formulation's composition.

Formula	Composition % (W/W)						
	Licorice/ Papaya extract	Glycerin	olive oil	Shea butter	Beeswax	Cetoma-crogol 1000	Cetosteatyl Alcohol
Formulation	1.0	18	20	3.0	5.0	1.0	5.0
placebo	0.0	18	20	1.0	5.0	2.0	6.0

De-tanning Night cream:

2 spoon Aloe vera gel

1/2 spoon olive oil

2-3 threads of Saffron

1 spoon Shea butter

1/2spoon sandal powder

All material are added and heated in a double boiler. After melting of bees wax, mixing is done to avaoid lump formation. The mixture is cooled and refrigerated.

Cream evaluation

Stability Tests, Physical analysis, types of emulsion, pH determination, electrical conductivity and centrifugation tests of creams are analyzed to assure the formulation of desired properties.

Stability tests are performed at different conditions for emulsions to note the effect of these conditions on the storage of creams. These tests are performed on samples kept at 80C ± 0.10C (in refrigerator), 250C ± 0.10C (in oven), 400C ± 0.10C (in oven) and 400C ± 0.10C (in oven) with 75% relative humidity (RH).

Physical characteristic of creams, i.e. color, creaming and liquefaction are noted at various intervals for 5 days intervals.

An observation table is prepared with all the parameters.

Results and observation:

Parameter for evaluation of cream	**Observation and results**
Stability test	
80C ± 0.10C (in refrigerator), 250C ± 0.10C (in oven), 400C ± 0.10C (in oven) and 400C ± 0.10C (in oven) with 75% relative humidity (RH).	
Physical characteristic of creams, i.e. color, creaming and liquefaction	

ESTIMATION OF CRUDE FIBRE IN CEREALS & THEIR PRODUCTS

Aim: To find out the amount of crude fiber in a given food sample.

Theory:

Crude fiber consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. The crude fiber content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

Principle: During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filteration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber content.

Requirements:

1. Sulphuric acid solution (0.255 ±0.005N) : 1.25g concentrated sulphuric acid diluted to 100mL (concentration must be checked by titration)
2. Sodium hydroxide solution (0.313 ±0.005N) : 1.25g sodium hydroxide in 100mL distilled water (concentration must be checed by titration with standard acid)

Procedure:

1. Extract 2g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35 □38°C and final temperature 52°C). if fat content is below 1%, extraction may be omitted.
2. After extraction with ether boil 2g of dried material with 200mL of sulphuric acid for 30min with bumping chips.
3. Filter through muslin and wash with boiling water until washing are no longer aidic.
4. Boil with 200mL of sodium hydroxide solution for 30min.
5. Filter through muslin cloth again and wash with 25mL of boiling 1.25% H2SO4, three 50mL portions of water and 25mL alcohol.
6. Remove the residue and transfer to ashing dish (preweighed dish W1).
7. Dry the residue for 2h at 130 ±2°C. Cool the dish in a desiccator and weigh (W2).
8. Ignite for 30min at 600 ±15°C.
9. Cool in a desiccator and reweigh (W3).

Calculation

% crude fiber in ground sample = Loss in weight on ignition (W2 □ W1) – (W3 □ W1)/Weight of the sample*100

Aim: Preparation & evaluation of probiotic foods

Principle: Probiotic lactic acid bacteria strains are used in various food formulations. The supplementation of lactic acid bacteria in cereals with potential probiotic strain may possibly impart beneficial health effects. Culture viability is a reasonable measure of probiotic activity in the products and the ability of the strain to attain high cell population with rapid growth is very important.

The application of probiotic microbial strains for fermentation of cereal substrates is an approach for the development of functional foods which are a part of the daily food intake. Cereals have proved to be an appropriate base for lactic acid bacteria fermentation. Cereal grains like wheat are consumed as food all over the world. It is widely used for various processed foods. Wheat chapattis are the major food items prepared from wheat widely used in northern India. Ragi (millet) is used in southern region of India, particularly in Karnataka. Ragi ball preparation usually includes boiling, baking and pudding. Wheat and ragi based products constitute the major part of the human diet in many parts of India. With this in mind, a probiotic cereal blend of wheat and ragi is prepared by supplementation of Lactobacillus lactis.

Requirements: Ragi: 100g; Wheat: 100g Sugar: 100g
Chemicals: Lactobacilli lacti tablet
Equipment: Centrifuge, Spectrophotometer, Homogenizer, pH meter

Procedure

Preparation of the cereal blend:

1. The selected cereals (wheat and ragi) are separately cleaned to remove the foreign particles and soaked overnight.
2. The next day water is decanted and is washed twice with tap water followed by distilled water.
3. The soaked cereals are then allowed to malt at room temperature for 48 hours.
4. The malted samples are taken in equal proportion for the preparation of probiotic cereal blend.
5. The mashed cereal blend is boiled with continuous stirring.
6. Sugar (2.0%) is added to the product during stirring.

7. The cereal blend is then pasteurized for 20min in cotton plugged Erlenmeyer flask (250ml) and allowed to cool.

Preparation and supplementation of inoculum to the cereal blend

1. The probiotic isolate Lactobacillus lactis is purchased from market.
2. The tablets are diffused and homogenised in autoclave water.
3. The prepared cereal blend was inoculated (1.0 %) with native Lactococcus lactis probiotic isolate.
4. It is mixed evenly and allowed to ferment at 37 °C for 24 h.
5. The flow chart for the preparation of the probiotic cereal blend is given figure in 1.

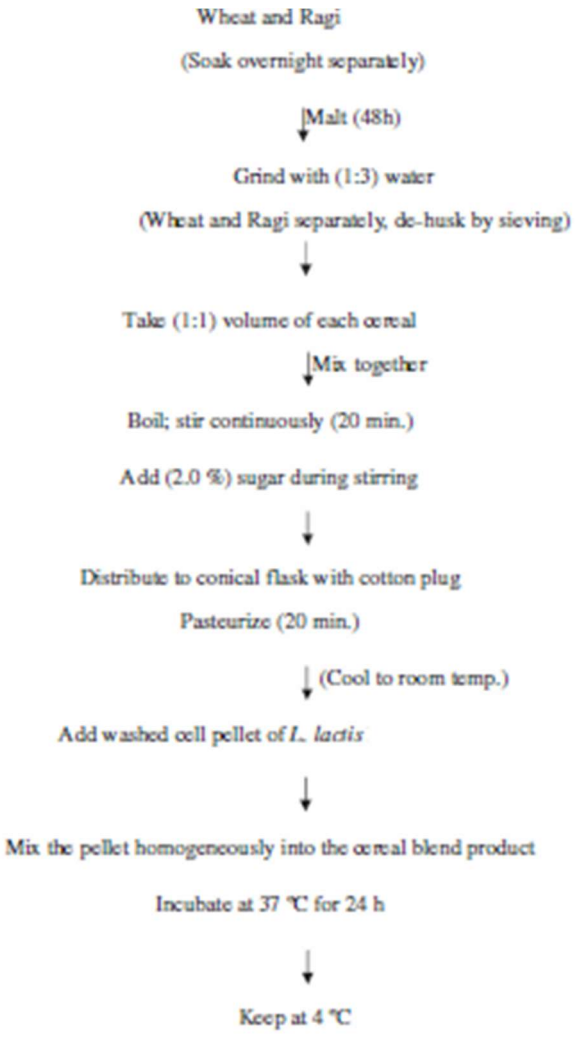


Figure1: Schematic representation of preparation of Probiotic food

Evaluation of the probiotic cereal blend

1. The prepared probiotic cereal blend is subjected to sensory evaluation (Table 1).
2. Products are well coded and evaluated by a selected panel of ten students groups.
3. The evaluation criteria were physical appearance, texture, taste/flavour, pH and overall quality using 5-point scale.
4. fill the observation and calculated result in the observation table.

Result and observations

Table 1. Sensory profile of fermented probiotic cereal blend prepared from ragi and wheat.

S.NO.	WHEAT/RAGI Ration	Ph	Texture	Taste/flavour	Overall quality
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					

(* Sensory panel of 10 individuals were selected from different departments in institution or students groups)

The data are presented as mean ± SD (N=3 experiments)

EVALUATION OF NUTRACEUTICAL VALUE OF MUSHROOM/WHEAT GERM

ESTIMATION OF VITAMIN C

Vitamin C is very common in Citrus and other fruits which are sour like lemon, orange etc. (Vitamin C is ascorbic acid which is strong reducing agent. It is oxidized by the colour dye 2,6 – dichlorophenol indophenol (DCPIP) to Di-hydro ascorbic acid. At the same time the dye is reduced and the colour is changed).

Requirements: 0.01% solution DCPIP, Celin tablets each of 500 mg, lemon or *Emblica* fruit.

Apparatus: Conical flasks, burette, mortar and pestle, muslin cloth.

Procedure: Take Celin tablet containing 500 mg of Vit. C and dissolve in 500 ml of distilled water. 1 ml of this solution contains 1 mg of Vit. C. Take 5 ml of DCPIP in a conical flask. Fill up the burette with the Vit. C solution. Titrate DCPIP against Vit. C solution adding drop by drop till DCPIP colour disappears. Add one more drop from the burette and the solution in flask changes to light pink. Note the reading. This is standard reading.
Take one gram of lemon pulp or *Emblica* pulp and crush it in mortar using pestle. Add 500 ml of D/W and mix well. Filter the solution using a muslin cloth. Take this solution in burette and titrate 5 ml DCPIP against it till the colour changes to light pink. Note the reading.

Readings:

1. _____ ml of Vit. C required to change the colour of 5 ml DCPIP. (Standard)
2. _____ ml of Lemon/ *Emblica* solution (Unknown) required to change the colour of 5 ml DCPIP.

Result: One gm of Lemon/*Emblica* contains _____ mg of Vit. C

Calculations:

Control:

1 ml of Celin tablet ≡ 1 mg of Vit. C

1 ml of DCPIP requires _____ ml solution of Vit. C to change the colour.

∴ 1 ml of DCPIP ≡ _____ mg of Vit. C

Experiment:

1 ml of DCPIP requires _____ ml solution of Lemon/*Emblica* (unknown) solution to change the colour.

∴ _____ ml of unknown contains _____ mg of Vit. C

One gm of Lemon/*Emblica* pulp was diluted to 500 ml.

∴ 1 gm of Lemon/ *Emblica* contains (i.e. 500 ml of unknown)

Contains = _____ mg of Vit. C

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Aim: To estimate the amount of proteins in the given plant material.

Requirements: Standard protein solution, suitable plant material, Reagent C, Folin's reagent, test tubes, pipettes, mortar and pestle, colorimeter.

Preparation of Reagents:

Standard protein solution – 10mg. bovine serum albumin (BSA) in 100 ml. distilled water.

Reagent C: 50 ml. reagent A + 1 ml. reagent B

Reagent A: 200mg NaOH and 1 gm. Na_2CO_3, dissolved separately in small amount of distilled water and make volume 50 ml. with distilled water.

Reagent B: 120mg. $CuSO_4$ and 250 mg Sodium tartarate dissolved separately in small amount of distilled water. Mix and make vol. 25 ml. with distilled water.

Principle: Aromatic amino acids such as Tryptophan and Tyrosine react with Copper in alkaline medium which reduces phosphomolyb (Folin's reagent) to form blue coloured complex. The intensity of colour depends on the amount of these aromatic amino acids present and thus vary for different proteins.

Procedure: Weigh 0.5 gm. of suitable plant material. Homogenize it in a mortar with pestle using 10 ml. of distilled water. Filter with muslin cloth and make the volume of the filtrate 100 ml. with distilled water.

Preparation of Standard Graph:

Prepare a series of tubes containing 0 (blank), 0.1, 0.2,……1.0 ml. of standard protein solution. Make final volume in all tubes to 2 ml. with distilled water.

Estimation:

1. Take 0.1 ml. of the Plant extract in a tube and add 1.9 ml. distilled water.
2. Add 5.0 ml. of Reagent C to all the above test tubes, mix thoroughly and allow it to stand.
3. After 15 minutes add 0.5 ml. of folin's reagent to all the tubes and shake vigorously.
4. Allow it to stand for 20 minutes.
5. Read OD at 625nm.
6. Plot a standard graph for protein using concentration of protein on x-axis and OD on y- axis. Calculate amount of proteins / 100 gm plant tissue.

Result: The amount of protein is _____ gm/ 100 gm. Plant tissue.

INDEX
SEMESTER IV

PRACTICAL: I

FUNGI: *Erysiphe* (Powdery mildew)

Classification:

Division : Eumycophyta
Class : Ascomycetae
Sub –class : Euascomycetae
Series : Plectomycetes
Order : Erysiphales
Family : Erysiphaceae
Genus : ***Erysiphe***

Structure study:

Erysiphe is an obligate parasite causing diseases like mildew of wheat and powdery mildew of gooseberries, roses etc. The fungus is an ectoparasite with white mycelium covering the host surface. The mycelium is branched and septate. At certain places small haustoria are formed. The haustoria may be branched or unbranched. The function of haustoria is to absorb food material from the host plant.

Asexual reproduction:

Asexual reproduction is by the formation of conidia. The conidia are formed in chains on the conidiophores. The conidiophores are unbranched and are formed at right angles to the leaf surface. Each conidium is oval or ellipsoidal, large in size and uninucleate.

Sexual reproduction:

Sexual reproduction takes place after the formation of conidia ceases. It is by formation of antheridia and ascogenous hyphae. After fertilization a number of cleistothecia (ascocarps) are formed. Each cleistothecium is a globose structure surrounded by a protective covering called peridium, which is formed of six to seven layers. Some superficial cells of the peridium develop into elongated, unbranched appendages characteristic to the species. A number of

asci are present in a cleistothecium. Each ascus gives rise to eight ascospores. Each ascospore is one celled, uninucleate, and elliptic in shape.

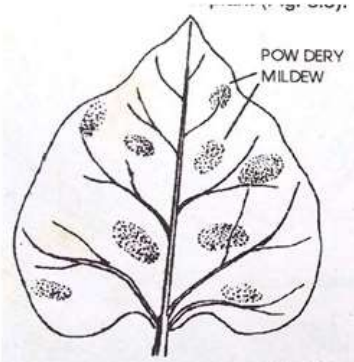


Fig. 3.4 *Erysiphe* : Infected plant

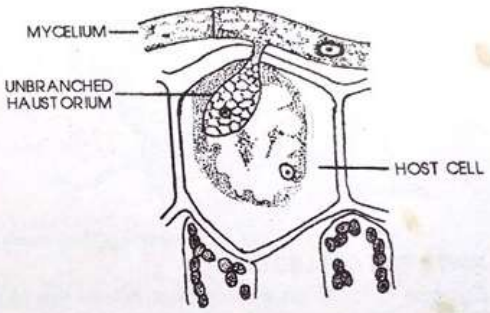


Fig. 3.5 *Erysiphe* : Mycelium and haustoria

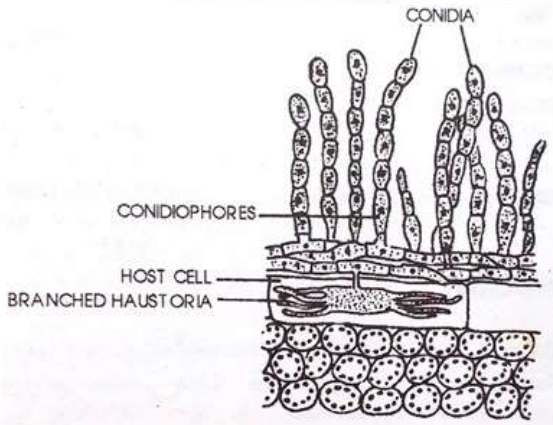


Fig. 3.6 *Erysiphe* : Section of host leaf showing conidia

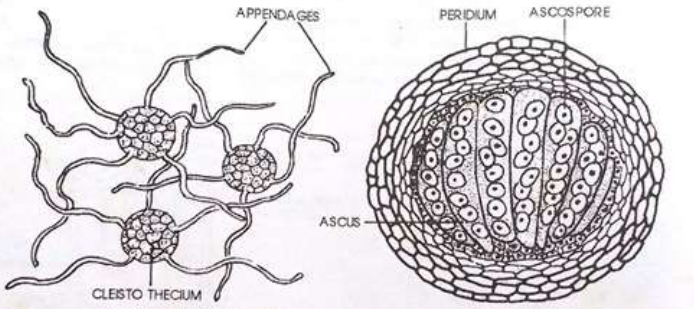


Fig. 3.7 : *Erysiphe* : Cleistothecia and section of cleistothecium

TS FOR COLLECTION :

B. *Xylaria*

Classification:

Division	:	Eumycophyta
Class	:	Ascomycetae
Sub –class	:	Euascomycetae
Series	:	Pyrenomycetes
Order	:	Polyporales
Family	:	Xylariaceae
Genus	:	***Xylaria***

Structure of Stroma:

- It grows on dead wood.
- Some species are known as “Dead man’s finger”.
- The main body is known as stroma.
- The stroma is composed entirely of fungal mycelium in which perithecia with definite cell wall are embedded.
- The perithecia are flask shaped cavities with their neck protruding out of stroma and have a small opening called ostiole. Each perithecium has many asci & paraphyses.
- The ascus ia long one celled dark brown to black in color, slightly bent and contain 8 ascospores.
- Ascospores are haploid and produced inside the ascus after meiosis.

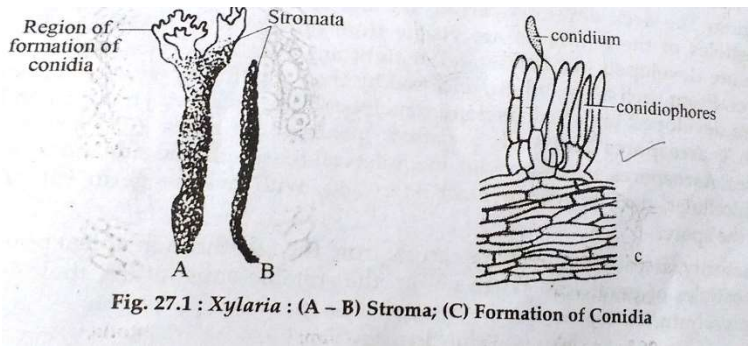


Fig. 27.1 : *Xylaria* : (A – B) Stroma; (C) Formation of Conidia

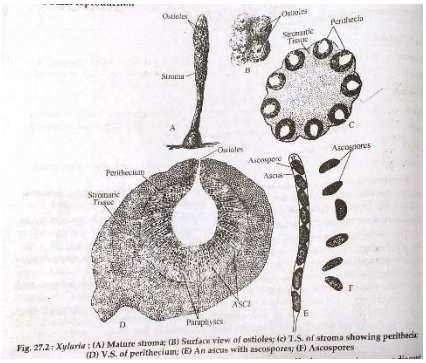


Fig. 27.2 : *Xylaria* : (A) Mature stroma; (B) Surface view of ostioles; (C) T.S. of stroma showing perithecia; (D) V.S. of perithecium; (E) An ascus with ascospores; (F) Ascospores

FUNGAL DISEASES

Powdery Mildew

It is one of the most common disease infecting economically important plants.

Causative organism: *Erysiphe sp.*

It generally causes powdery mildew disease. Other genera like Sphaerotheca and Oidium also cause powdery mildew.

Symptoms: Symptoms are mostly confined to leaves but sometimes, even extend to leaf sheaths, stem, glumes and even the fruit walls. The upper surface of the leaf is more severely attacked. It can be recognized as white floury mass spread in circular manner. The adjoining spots get united to form larger spots. At initial stage, the colour of the spot is white to dirty white which turns reddish brown later.

Effects:

In severe infection, the colour of the leaves is affected. Even leaves get wrinkled, spirally twisted and deformed. Photosynthetic surface is affected and reduced but the rate of respiration and transpiration is increased. The major crops affected by this disease are cereals, pea, cucurbits, grape vine, apple, rose, mango, sissam, raspberry etc.

Control measures:

1. Crop rotation.
2. Use of disease resistant varieties.
3. Dusting of host/affected plant with sulphur.

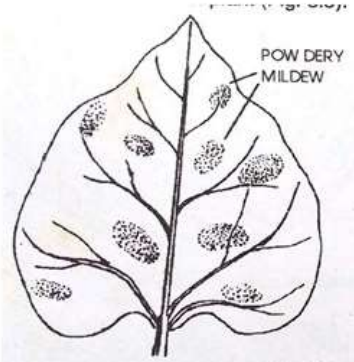


Fig. 3.4 *Erysiphe* : Infected plant

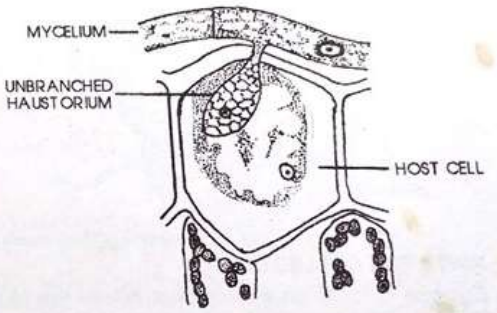


Fig. 3.5 *Erysiphe* : Mycelium and haustoria

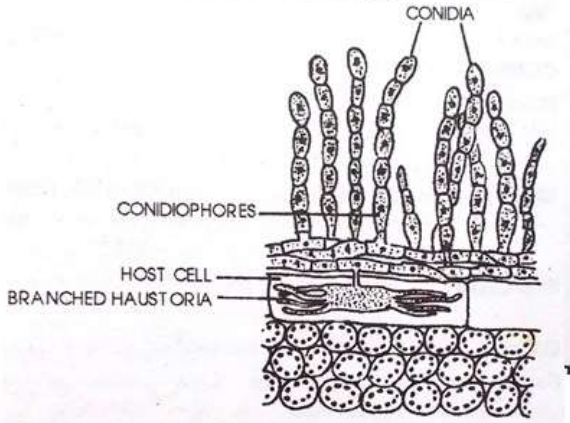


Fig. 3.6 *Erysiphe* : Section of host leaf showing conidia

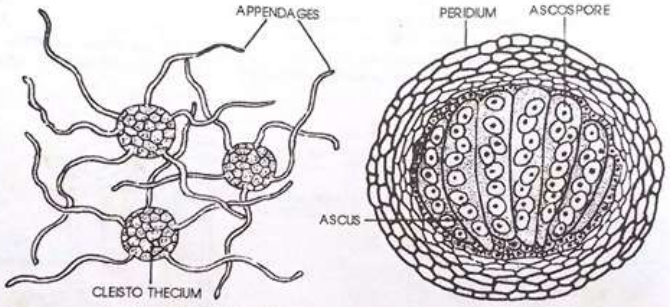


Fig. 3.7 : *Erysiphe* : Cleistothecia and section of cleistothecium

Late Blight of Potato

Host: *Solanum tuberosum*

Pathogen: *Phytophthora infestans*

The Late Blight of Potato is a serious fungal disease. It occurs worldwide in all Potato growing areas .Due to disease the tubers are reduced in size, numbers and weights.

Symptoms of the disease: The disease first appears on the top of the plant and generally after the blossoming period. It shows small, dead and purplish to black areas i.e. lesions on leaflets, rachis petiole and on the stem. The blighted leaves curled and shriveled in dry weather. A rapid blightnings on foliage occurs at low temperature and high humidity causing severe damage to the crop. Potato tubers are also infected in the field after the tops have been blighted and show brownish discoloration.

Control Methods

(i) The best method of control of the disease is timely and repeated foliage spray of Copper fungicides like Fytolan and Dithane Z-78

(ii) Dusting of Copper and Lime dust is also an effective control method.

(iii) Sanitation and use of disease resistant varieties are also very effective

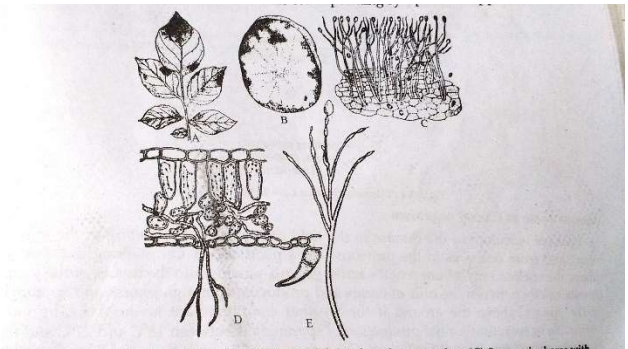


Fig. 28.3 : Late blight of potato : (A) Infected leaf; (B) Infected potato tuber; (C) Sporangiophores with sporangia passing through stomata; (D) T.S. of infected leaf showing sporangiophores passing through stomata; (E) A sporangiophore

STUDY OF LICHENS

Aim: To study of Lichens from the given preserved plant material/ permanent slides.

External Thallus Structure of Lichens/ Types of Lichens

The plant body is thalloidal having irregular shape and greenish colour. Some of the thalli are strongly pigmented and appear red, yellow.

There are three types of Lichen thalli a) Crustose, b) Foliose c) Fruticose

a) Crustose Lichen:

The thallus of crustose lichen is flat, thin and of insignificant size. It is just a crust closed attached to rocks or barks by its entire lower surface. The surface of thallus is divided into hexagonal areas called the areolae. e.g. Graphis

b) Foliose Lichen:

The thallus is leaf-like, flat, much lobed. It has distinct upper and lower surfaces. The lower surface is sooty or white in colour. The thallus is attached to rocks and twigs by rhizoid like outgrowths called the Rhizinae. The free ends of rhizinae often broaden to form disc-like structure which secrete mucilage. e.g. Physcia.

c) Fruticose Lichen:

The thallus is conspicuous, complex and much branched. The branches are slender, cylindrical and ribbon-like. The thallus is attached to the substratum only by a flattened-disc. The thallus shows no differentiation in the upper and the lower surfaces. e.g. *Usnea*

Internal thallus structure of Lichen:

The structure of lichen thallus is divided into two groups i) Homoiomerous ii) Heteromerous

i) Homoiomerous thallus: The thallus of fruticose lichen exhibit a simple structure with little differentiation. It consists of loosely arranged fungal hyphae in which algal cells are equally distributed throughout. e.g. *Collema*.

ii) Heteromerous thallus: The thallus belonging to this category exhibits considerable differentiation. The algal component is restricted to a specific region. If the vertical section is observed, the thallus can be distinguished into four zones. They are a) Upper cortex, b) Algal zone, c) Medulla and d) Lower cortex.

a) Upper cortex: It forms the upper surface of the thallus. It is thick and protective in nature and consists of fungal hyphae. The fungal hyphae are compactly interwoven to produce a tissue-like layer without any intercellular spaces.

b) Algal zone: It is a zone beneath the upper cortex and is formed of algal cells belonging to Cyanophyceae intermingled with the fungi known as gonidial layer. In some species the fungal hyphae send haustoria into the algal cells.

c) Medulla: It is the central core of the thallus consisting of loosely arranged fungal hyphae with intercellular spaces. The hyphae run parallel to the long axis. The walls of hyphae are strong and thick.

d) Lower cortex: It is formed of densely grouped hyphae which run perpendicular to the surface. The bundles of hyphae arise from the lower surface and penetrate the substratum functioning as anchoraging and absorbing organs. In some lichens the lower cortex is absent and its place is taken by a sheet of hyphae forming hypothalamus.

V.S. of apothecium:

As a result of fertilization, a fruiting body or ascocarp is formed which may be cup-shaped or flask shaped. Present in the fruitifications are a number of asci each possessing eight ascospores. Asci are mixed with paraphyses. In the upper and lower cortex there is an algal zone which is formed of algal cells intermingled with the fungal hyphae. The central core called medulla which consists of loosely arranged fungal hyphae with intercellular spaces. The hyphae run parallel to the long axis. The walls of hyphae are strong and thick.

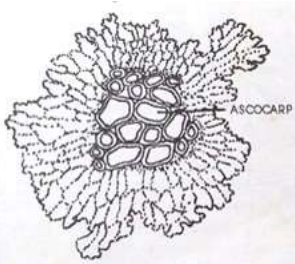


Fig. 4.1 *Lichens* : Crustose

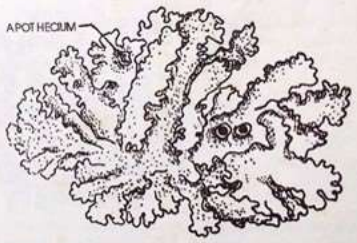


Fig.4.2 *Lichens* : Foliose

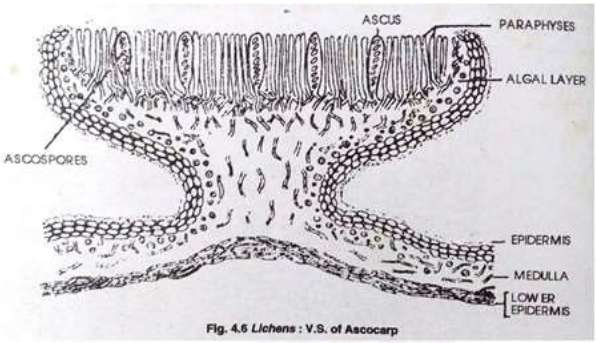


Fig. 4.6 *Lichens* : V.S. of Ascocarp

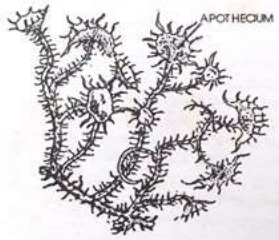


Fig. 4.3 *Lichens* : Fruticose

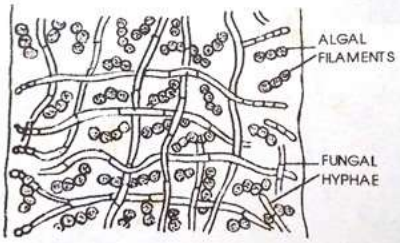


Fig. 4.4 *Lichens* : Homoiomerous thallus (V.S.)

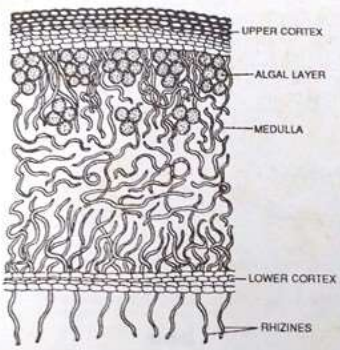


Fig. 4.5 *Lichens* : Heteromerous thallus (V.S.)

PTERIDOPHYTA

Selaginella

Classification:
Division: Lepidophyta
Class: Lycopodinae
Sub-class: Ligulatae
Order: *Selaginella*les
Family: *Selaginella*ceae
Genus: ***Selaginella***.

Structure study

Selaginella has prostrate stem with erect branches bearing two types of leaves. The stem is dichotomously branched. At the junction or angles of branching are present leafless structures called rhizophores. Rhizophores are dichotomously branched, leafless and positively geotropic. At the end of the rhizophore are number of adventitious roots. The roots are adventitious and formed on either prostrate stem or at the end of the rhizophores.

The leaves are of two types (dimorphic); bigger and smaller. The bigger leaf alternates with a bigger leaf and the smaller leaf with a smaller leaf, but the big and small leaves are opposite each other at each node. Each leaf is sessile and microphyllous. The leaf bears on its upper (adaxial) surface laminar (leaf-like) outgrowth called the ligule.

Internal structure (anatomy):

T.S. of Stem:

The stem section shows three distinct regions namely epidermis, cortex and stele Epidermis is the outermost layer formed of tabular cells having cuticle on their exposed side.

The cortex is heterogenous consisting of a few layers of sclerenchymatous cells below the epidermis forming hypodermis and the rest of parenchymatous cells

with little or no intercellular spaces form inner cortex. The outer few layers of parenchymatous cortex may possess starch grains.

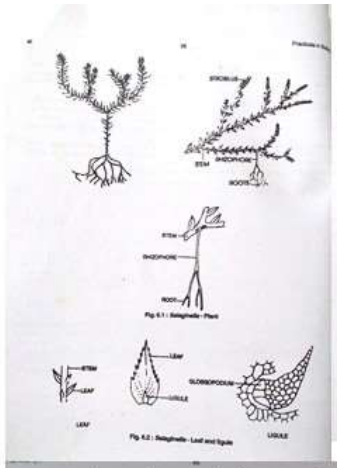
The stem of *Selaginella* is polystelic, having two to three steles, rarely one. Stele is a protostele. The steles, except in a few species, are separated from the cortex by conspicuous intercellular spaces or lacunae. The lacunae are traversed by trabeculae. The trabecular cells show presence of casparian strips indicating its endodermal nature. The stele is protostele enclosing single hadrocentric vascular bundle with exarch origin of xylem.

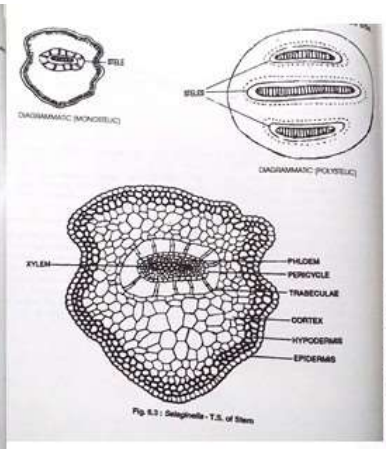
Strobilus:

The strobilus is the aggregation of sporophylls found at the apex of the branch. In some species, strobilli are in between the leaves. *Selaginella* is heterosporous and possesses two types of spores, the microspores and the megaspores. The two types of spores are formed in different sporangia present on different sporophylls. The ligule is present between the sporangium and the upturned part of the sporophyll. The strobilus shows a central axis bearing sporophylls which are spirally arranged. In most species the strobilli bear both, the microsporophylls and megasporophylls. The microsporophylls are either restricted to the upper side of the strobilus or to one side of the strobilus. The megasporophylls are either situated towards the base of the strobilus or on the opposite side of the microsporophylls

Sporangia:

The sporangia are present on the adaxial side of sporophylls. Each sporangium has two-layered wall, of which the cells of the outer layer are thick walled and the inner thin. The microsporangium is slightly smaller in size and contains many microspores. The mega-sporangium (macrosporangium) is bigger in size and is four-lobed. The megasporangium possesses two to four megaspores. The spore possesses two coverings, inner endosporium and the outer, sculputed one called exosporium.





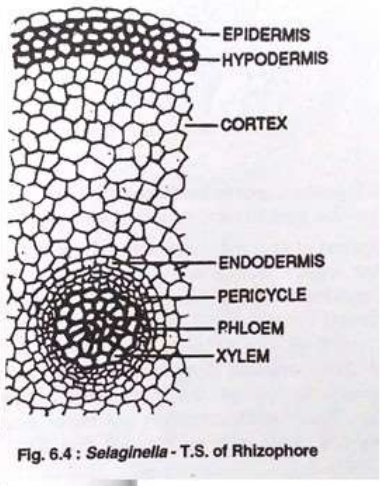
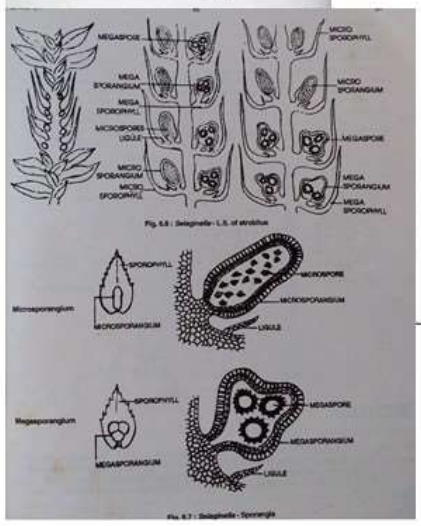
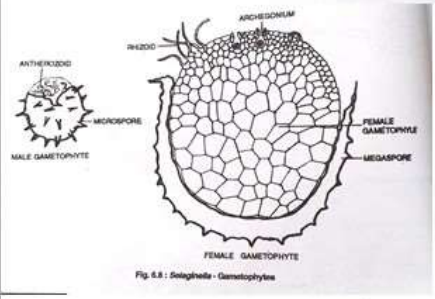


Fig. 6.4 : *Selaginella* - T.S. of Rhizophore





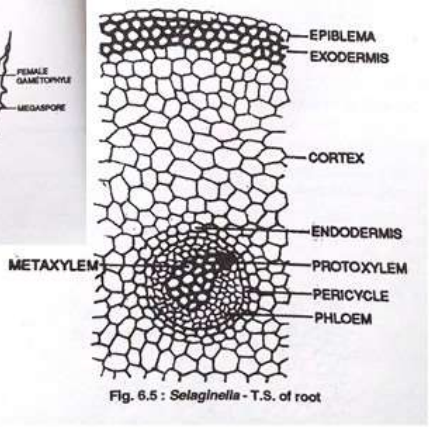


Fig. 6.5 : *Selaginella* - T.S. of root

PALAEOBOTANY

Rhynia

Rhynia is an extinct genus found in the form of fossils. It represents Psilophyta class of Pteridophytes. The group Psilophyta constitutes the earliest known terrestrial plants.

Class: Psilophytopsida
Order: Psilophytales
Family: *Rhynia*ceae
Genus: ***Rhynia***

Internal structure (of stem):

Rhynia exhibits many primitive features in it's internal structure also. A cross section of stem shows a smooth vascular cylinder occupying the central position in a wide cortex. Stele consists of annular tracheids. Surrounding xylem is a tissue of delicate tubular cells (resembles phloem) which together with xylem forms the conducting tissue. Outside conducting tissue is wide cortex differentiated into broad inner cortex and narrow outer cortex. Inner cortical cells have large intercellular spaces. Outer cortex has hypodermal arrangements. Outermost layer of cortex (fusiform cells) forms epidermis, whose outer cell walls are cutinized. Continuity of the surface layer is interrupted by stomata.

Reproduction:

In *Rhynia*, the sporangia are borne distally on certain branches of stem without any relation to leaf like organ. Sporangia produce spores. Within sporangium are number of spores of the same size and kind (homosporous) often found in tetrads.

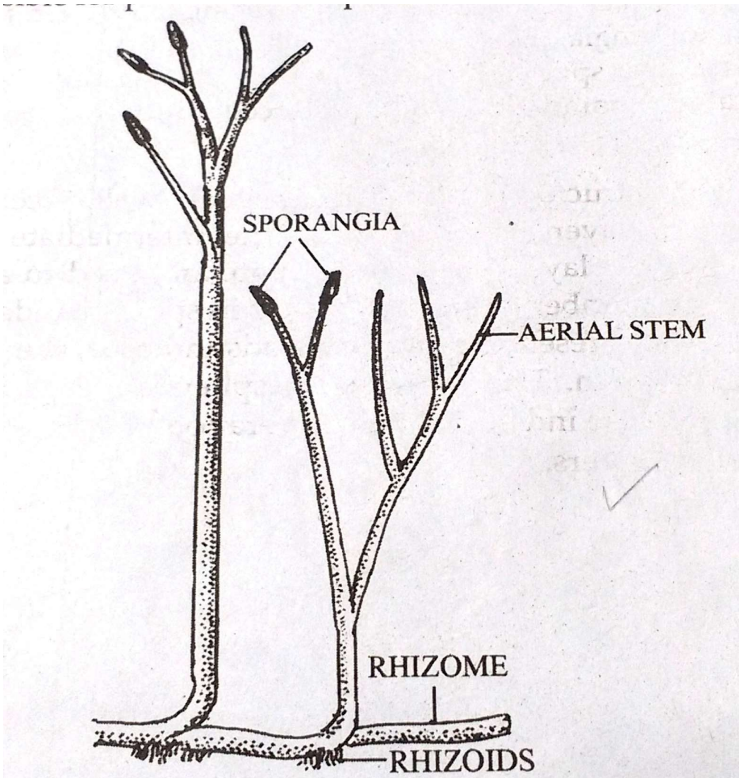


Fig. 31.1 : *Rhynia* : A plant (Sporophyte)

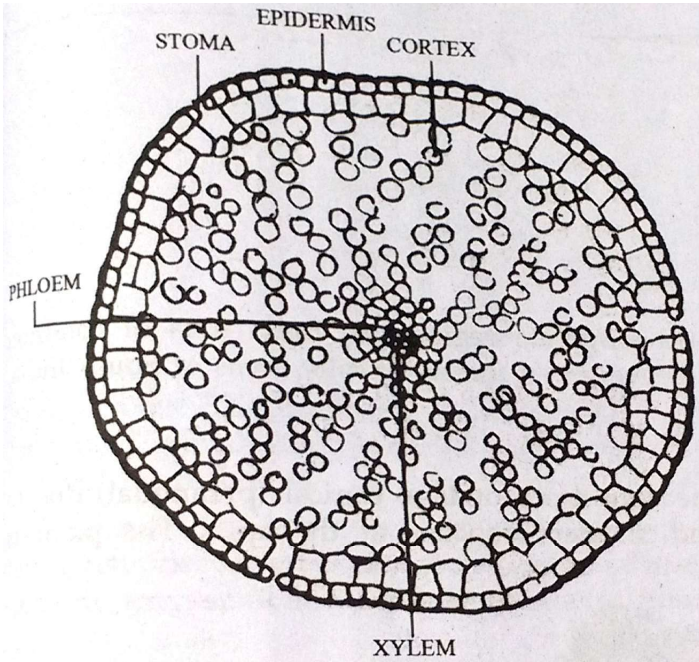


Fig. 31.2 : *Rhynia* : T. S. of stem

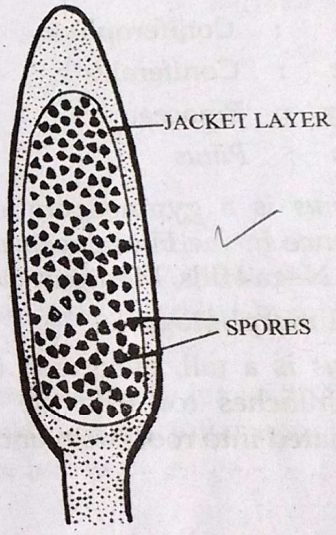


Fig. 31.3 : *Rhynia* : L. S. of Sporangium

Sporangium

GYMNOSPERM

Classification:
Division: Coniferophyta
Order: Coniferales
Family: Pinaceae
Genus: ***Pinus***

External morphology:

Pinus is a huge tree showing single, stout and cylindrical stem covered by scaly bracts. There are two types of branches - long branches (long shoots) and dwarf branches (dwarf shoots).

Long shoots: They are also known as branches of unlimited growth. Long branches or shoots develop on central stem at regular intervals. There are lateral branches. They are covered by scaly leaves and show leaf scars.

Dwarf shoots: They are also called branches of limited growth, spur branches or foliar shoots. They develop on long branches and in axil of scaly leaves. The dwarf shoot possesses a number of needle-like green foliar leaves. The number of acicular (needle-like) leaves vary from species to species but is constant for species. It ranges from one to five.

Scaly leaves: They are present on long as well as on dwarf shoots. They are non chlorophyllous and sessile. Their main function is protection.

Anatomy (internal structure):

***Pinus*: T.S. of young stem**

The young stem shows three distinct regions viz., epidermis, cortex and stele.

Epidermis: The epidermis is outer, single layered and formed of highly thickened and cuticularised cells.

Cortex: The outer few layers of cortex are formed of highly thick-walled sclerenchymatous cells known as hypodermis. The inner cortex is formed of thin-walled parenchymatous cells possessing chloroplasts. In the cortex, resin ducts are found. The endodermis is present but is undistinguishable. Endodermis is followed by a few layered pericycle.

Stele: The stele is eustele. The vascular bundles are arranged in a ring and are separated from one another by broad medullary rays. Vascular bundles are conjoint, collateral, open with origin of xylem as endarch. The xylem consists of tracheids, parenchyma & fibres. Vessels (characteristic of Angiosperms) are absent. Resin ducts are present in the xylem tissue. Phloem is formed of sieve cells, phloem parenchyma & fibres. Companion cells are absent. The centre is occupied by a pith formed of parenchymatous cells.

***Pinus*: T.S. of old stem:**

The old stem shows presence of secondary growth which is similar to that of Dicot stems. It shows the following structures.

Periderm: It consists of cork, cork cambium and secondary cortex.

Cortex: The primary cortex gets mixed with secondary cortex. It is parenchymatous. Resin ducts are irregularly distributed in primary cortex. The endodermis and pericycle are crushed.

Phloem: Primary phloem is crushed. The secondary phloem is in the form of a narrow, continuous ring. It consists of sieve cells and phloem parenchyma.

Cambium: Cambium consists of a few layers of thin-walled rectangular cells. It separates the secondary phloem from the secondary xylem.

Secondary Xylem: Xylem is clearly demarcated into many annual rings. The large sized and less lignified xylem forms a spring wood, while the more lignified and small xylem form an autumn wood. Spring and autumn wood together form a single annual ring. The wood is pycno-xylic (compact). Secondary xylem is formed of tracheids and xylem parenchyma. Medullary rays are primary and secondary. The rays are uniseriate. Resin ducts are scattered in secondary xylem.

Pith: Pith is parenchymatous and many cells contain tannin.

***Pinus*: L.S. of wood:**

L.S. of wood can be taken in two ways namely radially and tangentially and called Radial Longitudinal Section (**RLS**) and Tangential Longitudinal Section (**TLS**).

RLS of wood (Stem):

Cut the stem longitudinally into two halves from the centre. Take one of the two halves and cut longitudinal sections from the cut side. Stain one section with saffranin, mount and observe.

RLS is radial longitudinal section. It shows presence of secondary xylem, rays and cortex. The xylem is formed of tracheids with bordered pits on their radial walls which are seen in surface view. The bordered pits are surrounded by a special cellulose thickening called bars of Sanio (Crassulae).

TLS of wood (Stem):

TLS is tangential longitudinal section. In TLS, the tracheids and medullary rays are cut in transverse planes. The bordered pits show overarching borders of the pits. Between the two overarched borders is a small disc-like structure called torus. The medullary rays are uniseriate. The ray is three to four celled in height. On either side of the ray are tracheid cells in the xylem and

albuminous cells in the phloem. From the TLS, the height of the medullary rays can be found out.

***Pinus*: T.S. of leaf (Needle)**

The T.S. of leaf is triangular or semicircular depending upon the number of leaves present on a single spur.The section is differentiated into epidermis, mesophyll and stele.

Epidermis:

The epidermis consists of one layer of very thick-walled cells. It shows a number of depressions, in which are present sunken stomata. On the outer side of the epidermis is present a cuticle layer. Below the epidermis is sclerenchymatous hypodermis which is interrupted at many places because of the sub stomatal air chambers of the sunken stomata.

Mesophyll:

The mesophyll is not differentiated into palisade and spongy tissues. It consists of thin-walled parenchymatous cells containing chloroplasts. These chlorenchymatous cells are peculiar in the sense that they show numerous peg-like infoldings from the wall which project into the cell cavities. The infolding of walls increases the surface area. Such cells are called armpalisade. Resin ducts are present at angles in the mesophyll tissue. The central region (stele) is surrounded by a conspicuous endodermis.The endodermal cells are barrel-shaped and tangentially thickened.

Stele:

Next to the endodermis is the multi-layered, thin-walled pericycle. Generally there are two vascular bundles in the centre. The vascular bundles are separated by a transfusion tissue. Each vascular bundle is conjoint, collateral and open but the cambium is non-functional. Phloem is present towards the lower, convex surface, while xylem lies towards the upper surface.

Reproduction:

Pinus reproduces by formation of spores. Spores are of two types, viz., microspores (pollengrains) and megaspores. Spores are formed in different sporangia which are present on different sporophylls. The sporophylls aggregate to form cones. The cone is either a male cone or a female one. Male and female cones are found on the same plant, and hence *Pinus* is a monoecious plant.

Male Cone and L.S. of male cone:

The male cone replaces the dwarf shoot and is found in the axil of the scaly leaf. The male cones are produced in clusters.

Each cone has a centrally placed axis around which a number of microsporophylls are arranged spirally. The microsporophyll is scaly and consists of a stalk and an expanded triangular apex. On the lower side are present two sporangia (pollen sacs). Each microsporangium is sessile having a thick jacket layer. Present in the sporangia are a number of microspores

The **microspore** is globular having a three layered wall. The outer layer is called exine. Exine is cuticularised and found on one side of spore, i.e., it does not cover the spore completely. The middle layer is called exo-intine which covers the spore completely forming two conspicuous balloon-like expansions on either side containing air. The inner layer or intine is thin and delicate.

Female cone:

The female cone develops in the place of branch of unlimited growth. Each female cone consists of a central axis on which are number of spirally arranged sporophylls. Each sporophyll is formed of two types of scales - (1) bract scale and (2) ovuliferous scale. The bract scales develop directly on the central axis. They are arranged spirally. The large ovuliferous scales develop from the bract scales on their upper surface. The ovuliferous scales are woody and each scale bears two ovules on its upper surface. The terminal point of the ovuliferous scale is broad.

Ovule:

The ovule is elongated and is enveloped by a single integument. The integument surrounds the central nucellus except at the tip. This opening is called micropyle and is directed towards the cone axis. The integument is three layered. The outer and the inner layers are fleshy, while the middle layer is stony. The outermost layer often disappears during the later stage of growth. Opposite the micropyle in the nucellar region lies a small cavity called pollen chamber. The archegonia develop towards the periphery of the female gametophyte below the micropyle. About two to eight archegonia develop in a single ovule.

Each **archegonium** consists of neck and venter. The neck consists of four rows of cells one cell in height. Neck canal cells are absent. The sterile ventral wall is absent. Present in the ventral cavity are a large egg cell and a small ventral canal cell.

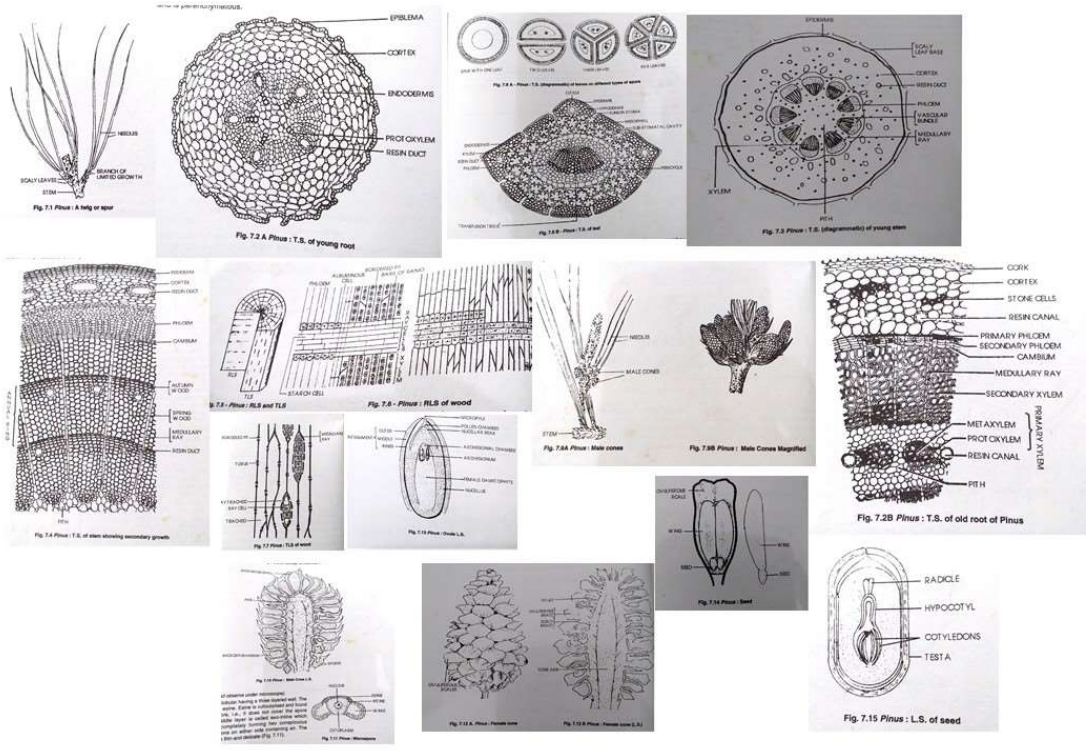


Fig. 7.1 *Pinus* : A twig or spur

Fig. 7.2 A *Pinus* : T.S. of young root

Fig. 7.3 *Pinus* : T.S. (diagrammatic) of young stem

Fig. 7.4 *Pinus* : T.S. of stem showing secondary growth

Fig. 7.5 - *Pinus* : RLS and TLS

Fig. 7.6 - *Pinus* : RLS of wood

Fig. 7.7 *Pinus* : TLS of wood

Fig. 7.8A *Pinus* : Male cones

Fig. 7.8B *Pinus* : Male Cones Magnified

Fig. 7.2B *Pinus* : T.S. of old root of Pinus

Fig. 7.14 *Pinus* : Seed

Fig. 7.15 *Pinus* : L.S. of seed

CORDAITES

Classification –

Division: Spermatophyta
Sub-division: Gymnospermae
Class: Coniferophyta
Order: Cordaitales
Family: Cordaitaceae
Genus: ***Cordaites***

Characteristics –

Cordaites is the main genus of family Cordaitaceae.

The name Cordaites was at first used only for the leaves but gradually it has been applied to the whole plant.

T.S. of Stem -

It shows presence of periderm and wood with usually large discoid pith.

The cortex is large and shows the presence of scattered patches of sclerenchyma in the parenchyma. The cortex also shows presence of leaf traces.

The zone of primary wood is very thin.

The secondary wood is well developed, thick and coniferous.

L.S. of Stem –

It shows the pith which is 1 to 100 cm. or more in diameter. It is entirely parenchymatous but in course of growth it is cracked transversely giving the appearance of a pile of concave discs.

T.S. of Leaf –

The leaves show characteristic xerophytic internal structure.

The epidermis shows cutinized thick walled cells and is present on both upper and lower surface (bifacial).

The sclerenchymatous hypodermis is present in the form of distinct patches on both the sides which later on expands near the vascular bundles to reach them..

Bundle sheath is present surrounding the vascular bundles.

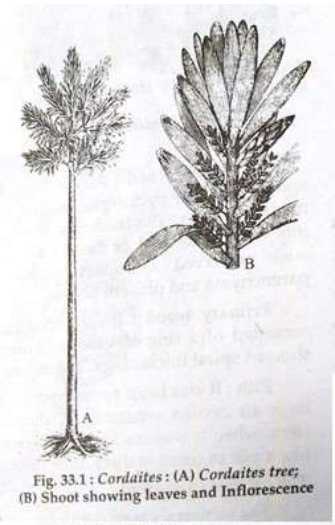


Fig. 33.1 : *Cordaites* : (A) *Cordaites tree;* (B) Shoot showing leaves and Inflorescence

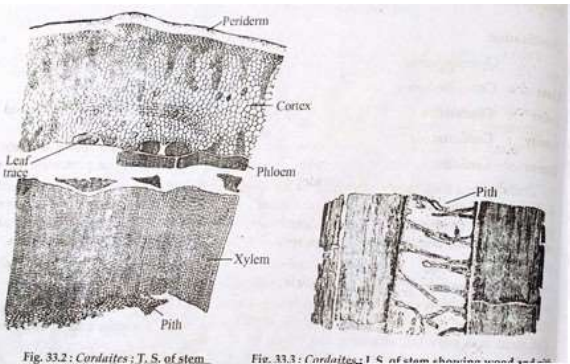


Fig. 33.2 : *Cordaites* ; T. S. of stem

Fig. 33.3 : *Cordaites* ; L. S. of stem showing wood

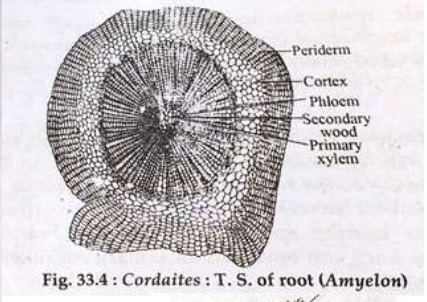


Fig. 33.4 : *Cordaites* : T. S. of root (*Amyelon*)

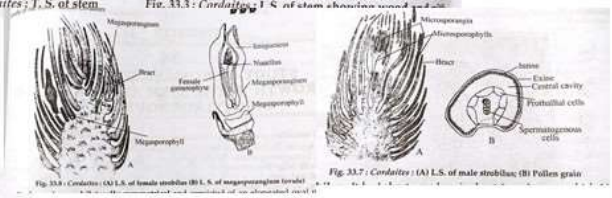


Fig. 33.7 : *Cordaites* : (A) L.S. of male strobilus; (B) Pollen grain

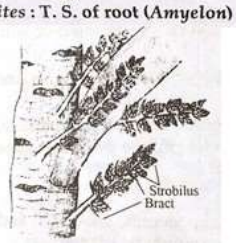


Fig. 33.6 : *Cordaites* : Strobilus

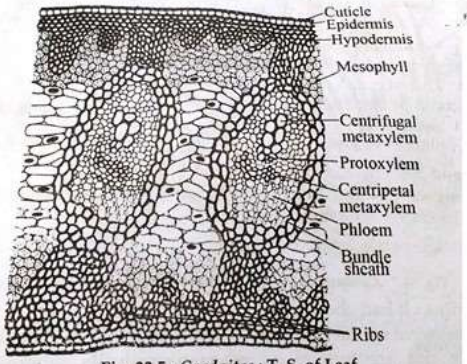


Fig. 33.5 : *Cordaites* : T. S. of Leaf

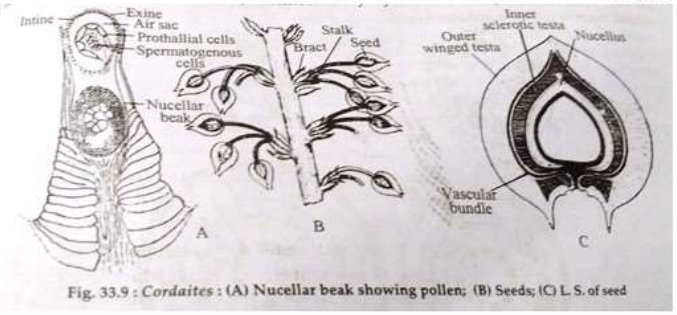


Fig. 33.9 : *Cordaites* : (A) Nucellar beak showing pollen; (B) Seeds; (C) L.S. of seed

PRACTICAL: II

ANATOMY

Normal Secondary Growth In Dicot Stem

T.S. of old stem of Sunflower shows the following regions –

- **Epidermis**: it is a single layered with a thick cuticle and multicellular trichomes.
- **Cortex**: it is divided into outer cortex and inner cortex. Outer cortex is collenchymatous. Inner cortex is parenchymatous with resin ducts.
- **Endodermis**: inner most layer of cortex is the endodermis with large rectangular cells with casparian thickenings.
- **Stele**: A number of vascular bundles are arranged in a ring around the central pith.

In old stem:

- Pith cells may become opaque and white.
- Primary vascular bundles are conjoint, collateral and open with endarch xylem, and the vascular bundles are separated by parenchymatous medullary rays.
- After secondary growth, primary xylem gets pushed towards the pith.
- Secondary xylem made of large metaxylem vessels and fibres.
- The interfasicular cambium also produces secondary xylem towards inside and secondary phloem outside.
- The primary phloem gets pushed towards the hard bast. Secondary phloem is present between cambium and primary phloem.
- Hard bast are sclerenchymatous patches outside the phloem.

Normal Secondary Growth In Dicot Root

T.S. of old dicot root:-

Periderm: In old root epiblema is replaced by periderm or cork. Periderm consists of three layers; cork cambium or phellogen, phelloderm towards inside and phellem towards outside.

Cortex: It is parenchymatous but the cells are stretched laterally.

Endodermis is not prominent.

Stele: The arrangement of vascular tissue in young root is radial with xylem groups alternating with phloem. Xylem is exarch and tetrarch. Cambium is absent.

During secondary growth, parenchymatous cells opposite the primary xylem and towards the inner side of phloem become meristematic to form an irregular ring of secondary cambium. The primary xylem is surrounded by secondary xylem made up of large metaxylem vessels, xylem fibres and xylem parenchyma. Secondary phloem is seen as a continuous ring outside the cambium. Primary phloem can be observed as patches of crushed cells outside the secondary phloem. Meristematic cells opposite the primary xylem form broad conical medullary rays.

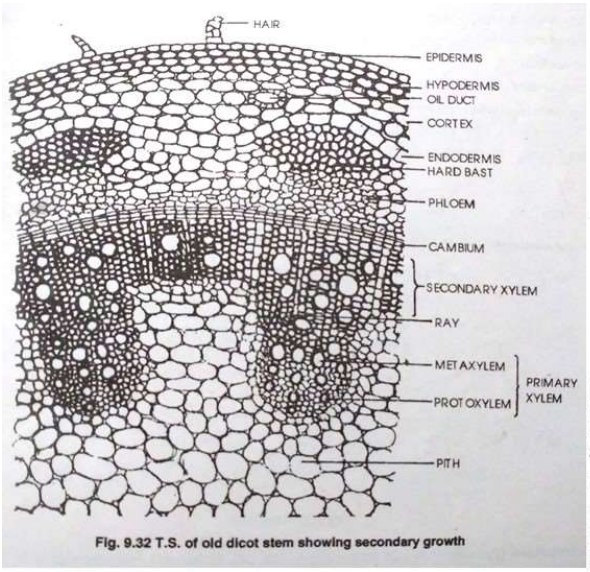


Fig. 9.32 T.S. of old dicot stem showing secondary growth

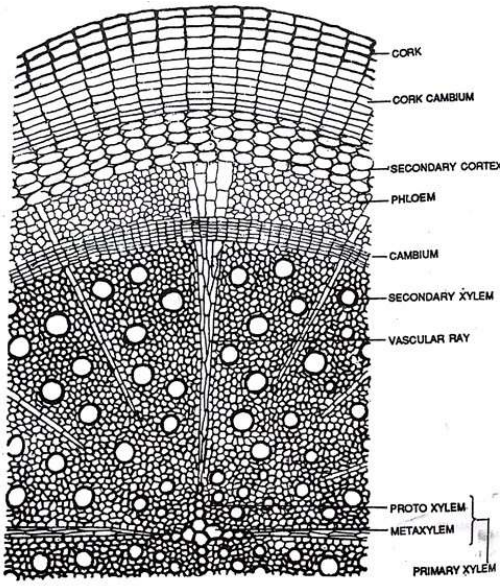


Fig. 9.31 T.S. of old dicot root showing secondary growth

TYPES OF MECHANICAL TISSUES

Mechanical tissues are also termed as supporting tissues as they provide mechanical strength / support to various plant organs. These plant organs are subjected to various types of strains and stresses – e.g. Bending and shearing stresses, longitudinal pulls of tension, compression, various environmental conditions etc.

Parenchyma, Collenchyma, Sclerenchyma and xylem tracheids and vessels may function as mechanical tissues.

Mechanical tissue system in Aerial Organs -

1. **T.S. of Coleus stem**: The stem is square. It is exposed to wind which exerts bending pressure (Tension on the side where there is force and compression on the other side). For the stem to remain erect, it should exhibit the property of inflexibility. This is reflected in the four patches of collenchyma that occur at the four corners of the stem, forming diagonally placed 'I' girders. These corners constitute the phlanges of the I-girdle while the web is formed of parenchyma and sclerenchyma. The mechanical tissue at the angles gives the stem sufficient strength to withstand the bending. Thus, inflexibility (property by which the body offers resistance to bending) is exhibited by the respective mechanical tissues.

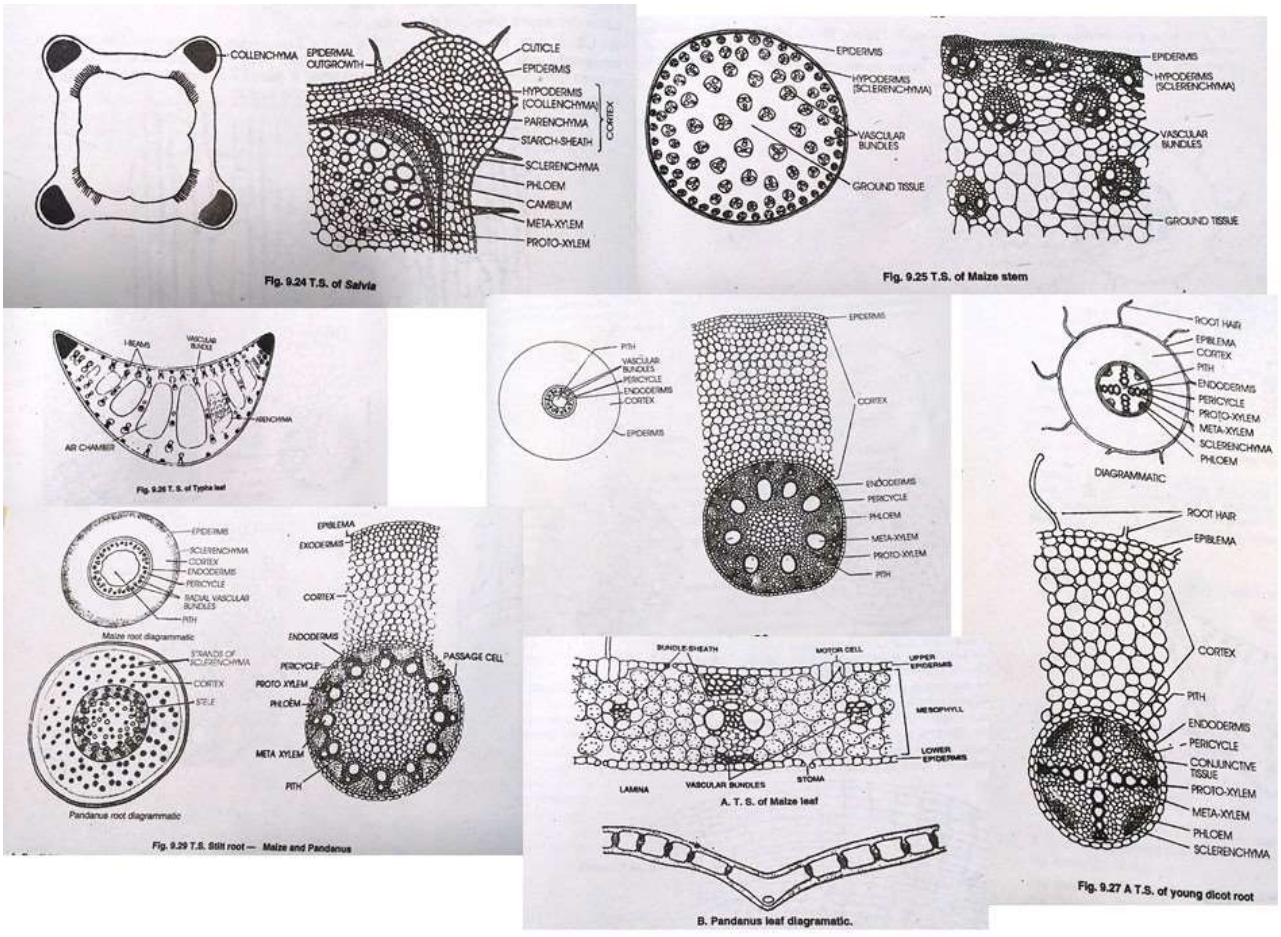
2. **T.S. of Maize stem**: Here, inflexibility is achieved by sub-epidermal girders or in other words by the sclerenchymatous hypodermis. Besides, lying scattered in the ground tissues are the vascular bundles which constitute theI girders. The Sclerenchyma patches above each vascular bundle form the phlanges while the web is constituted by the vascular tissue.

3. **T.S. of Typha leaf**: The almost crescent shaped section shows sclerenchyma tissue at the corners. I girders are formed by the sub-

epidermal sclerenchyma patches while the webs are formed of parenchyma cells and secondary I girders. There are large air chambers which support the leaf and check it from collapsing.

Mechanical tissue system in Underground organs -

T.S. of Maize root: Again, the stereome or mechanical tissue is the sub-epidermal exodermis and the sclerenchymatous tissue which comprises the conductive tissue. This, of course is supported by the lignified polyarch xylem. Endodermal walls too, are thickened. All these factors contribute towards inextensibility of the root.



STUDY OF CONDUCTING TISSUES – XYLEM AND PHLOEM ELEMENTS THROUGH MACERATION TECHNIQUE.

In plants the conducting tissues are Xylem and Phloem. Xylem is water conducting tissue while Phloem is food conducting tissue. They are produced by meristematic tissue, the cambium.

Maceration Technique – Take **angiospermic** stem (Michelia, Anona). Cut them vertically into small segments. Put into corning test tube with 1:3 Concentrated Nitric acid: Water. Boil it on the flame till wood is not getting separated into small fibres. Add a pinch of Potassium chromate for good separation. Allow it to cool completely and then transfer it in watch glass. Wash it with water, and then keep it in saffranin for 10 – 15 minutes. Transfer the same on the slide and mount in dilute glycerin. Observe under compound microscope.

1. Xylem consists of vessels or vessel elements which are dead cells to conduct water and dissolved mineral salts from root to leaves. Vessels are rows of elongated dead elements placed end to end with oblique to transverse perforated end plates.
2. Vessels present show spiral, annular, scalariform and pitted wall thickenings.
3. They show simple or bordered wall pitting.
4. Tracheids are associated with fibres. They have large lumen for conduction of water.
5. They are thick walled lignified; as such they serve as mechanical support to plant body.
6. Fibres are sclerenchymatous dead cells, thick walled, lignified with narrow lumen, and long pointed elements. Fibres are mechanical elements for support and give rigidity to stem. They may be septate or non-septate.

7. Xylem parenchyma associated with xylem is thin walled and stores water.
8. Phloem is living tissue consists of long sieve tubes placed end to end. They are thin walled, perforated with pores. Perforated area looks like sieve and is called Sieve plate.
9. Sieve tubes are associated with companion cells.

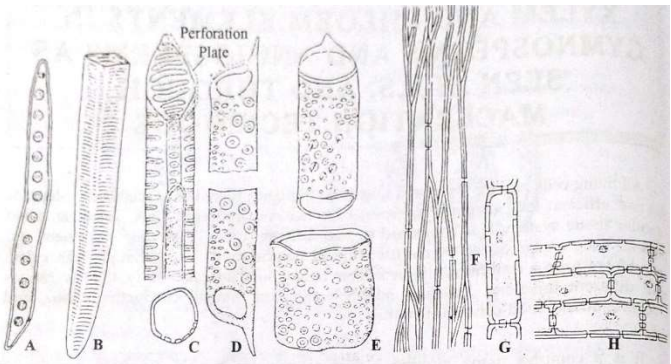


Fig. 37.1 : Components of Xylem

(A) Xylem tracheid with bordered pitted thickenings
(B) Xylem tracheid with scalariform thickenings
(C) Xylem vessel with multiple perforation plate
(D & E) Xylem vessel with simple perforation plate
(F) Xylem sclerenchyma
(G) Xylem axial parenchyma cell
(H) Ray parenchyma cells

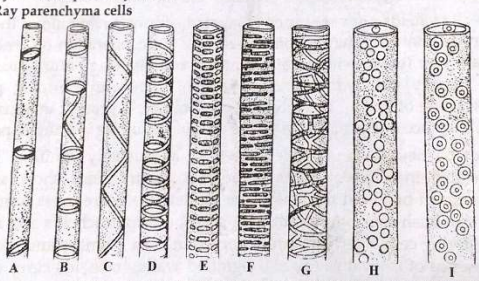
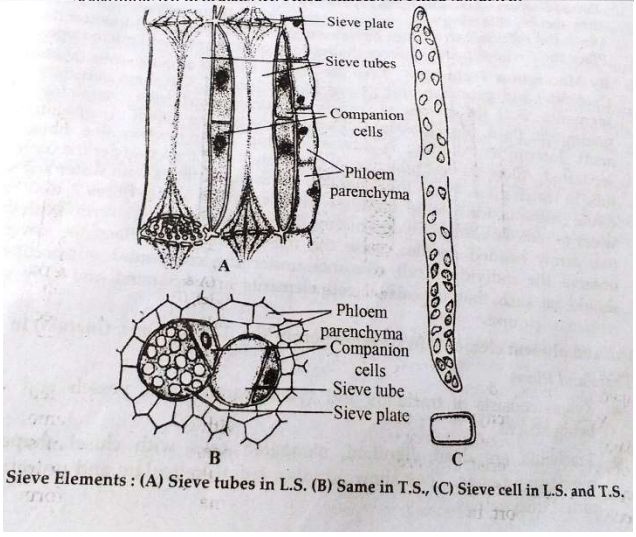


Fig. 37.2 : Secondary wall thickenings in longitudinal view : (A & B) Annular, (C & D) Spiral, (E & F) Scalariform, (G) Reticulate, (H) Pitted (simple), (I) Pitted (bordered)



Sieve Elements : (A) Sieve tubes in L.S. (B) Same in T.S., (C) Sieve cell in L.S. and T.S.

TYPES OF VASCULAR BUNDLES

Aim: To study different types of vascular bundles in plants.

Conjoint, Collateral, and Open – e.g. – Sunflower stem- Vascular bundles are arranged in ring. Each vascular bundle is conjoint (Xylem and Phloem on same axis), collateral (phloem on the outer side of the xylem towards the periphery), open (cambium is present). Origin of xylem is endarch (protoxylem towards the centre and metaxylem towards the periphery).

Conjoint, Collateral, and Closed – e.g. – Maize stem- Each vascular bundle is conjoint, collateral, and closed (cambium absent), xylem is endarch. It is 'Y' shaped and consists of two large metaxylem vessels and protoxylem consisting of annular and spiral vessels.

1. **Bicollateral** – e.g. – Cucurbita stem - The vascular bundles are conjoint (Xylem and Phloem on same radius) Bi collateral (Phloem on lateral side of xylem i.e. on both sides), open (cambium is present) with endarch xylem (protoxylem towards the centre and metaxylem towards the periphery). Between the outer phloem and xylem is present outer cambium and between the xylem and inner phloem is present inner cambium.

2. **Concentric (Amphicribal or Hadrocentric)** – e.g. – Fern rachis – In rachis, there are 4 to 6 vascular bundles which are arranged in an arc. Some vascular bundles are large and some are small. The vascular bundle is amphicribral or hadrocentric with xylem in the centre and phloem surrounding it. Metaxylem is in the centre with protoxylem towards the periphery. Thus xylem is exarch.

3. **Concentric (Leptocentric or Amphivasal)** – e.g. – Dracaena stem – Vascular bundles are scattered. Each vascular bundle is concentric. Phloem is situated at the centre of the vascular bundle and is surrounded by xylem, hence it is leptocentric or amphivasal.

4. **Radial –** e.g. – Maize root – In the section, number of xylem bundles and phloem bundles are arranged alternately. This arrangement is called radial (i. e. xylem on one radius and phloem on the next radii). In the xylem bundles, the metaxylem is towards the centre and the protoxylem towards the periphery. Thus, origin of xylem is exarch.

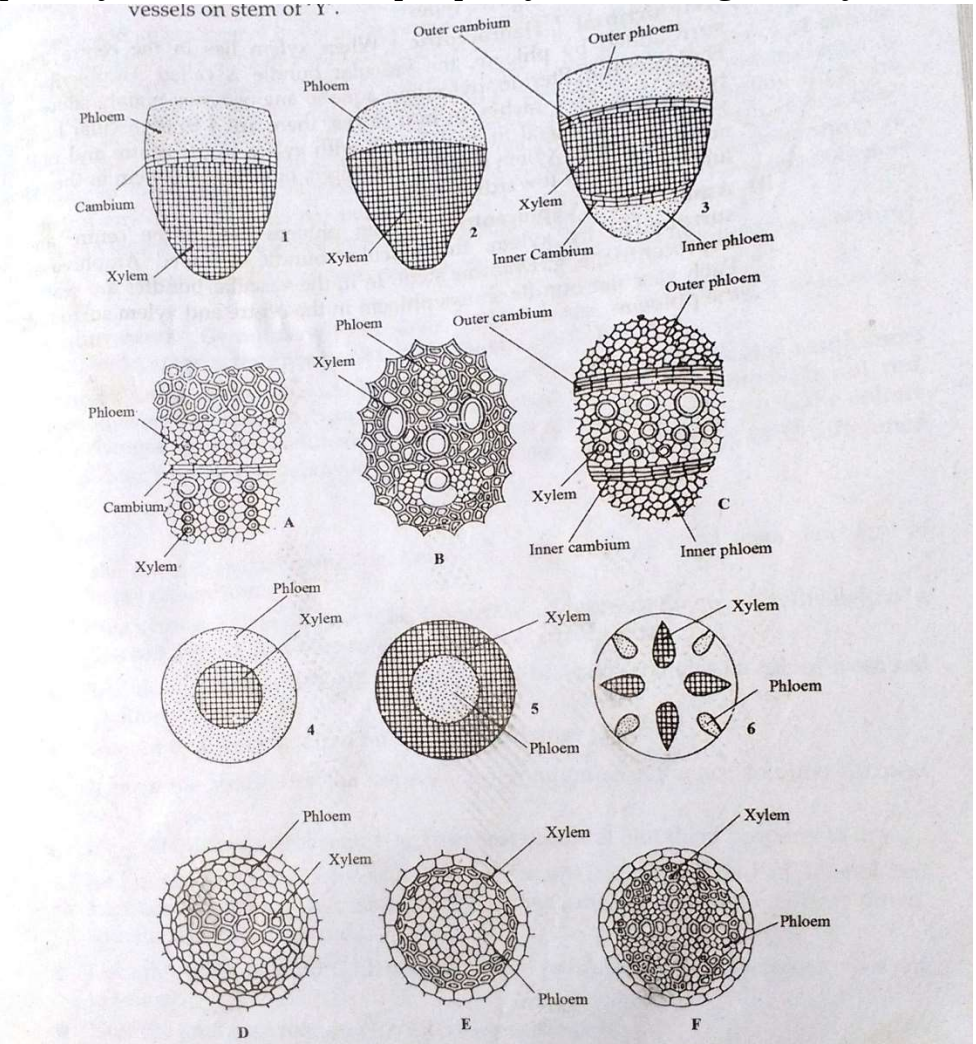


Fig. 38.1 : Various types of vascular bundles (V.B.) : 1 and A–conjoint, collateral and open vascular bundle, 2 and B – conjoint collateral and closed V.B., 3 and C–conjoint, bicollateral and open V.B., 4 and D–concentric and amphicribal V.B., 5 and E–concentric and amphivasal V.B. and 6 and F–radial V.B.

STUDY OF GROWTH RINGS, PERIDERM, LENTICELS, TYLOSES, HEARTWOOD AND SAPWOOD

TYLOSIS: (Cucurbita stem) In many woody trees, the xylem elements are blocked because of the ingrowth of neighbouring parenchymatous cells. These ingrowths are called tyloses (singular tylosis) which are bladder-like. The tyloses may remain thin walled, or later, get lignified. The presence of tyloses in xylem in that portion ceases to function, and the sap wood (laburnum) is transformed to heartwood (duramen). The main function of tylosis probably is mechanical support.

Growth rings: (*Tilia* stem) The old plant axis shows concentric layers of seasonal increments of secondary xylem which appear in rings. These concentric rings are called growth rings. During the growing period, the secondary xylem is well developed. This is because of increased activity of the plants and has more requirements. The xylem vessels are more in number and have large diameters. The xylem sclerenchyma is reduced. This type of woods is called early or spring wood. In autumn the activity of plant is less, the requirements are less and thus secondary xylem vessels are fewer in number and smaller in size. The sclerenchyma is well developed. The spring and the autumn wood together form a single annual ring or growth ring.
The number of annual ring represent the age of the plant, i.e., if two annual rings are there, the plant is two years old. Counting the number of annual rings, one can find out the age of the plant.

Periderm: (old stem/potato tuber) In older plants, due to secondary growth at the centre, the pressure is exerted on the epidermis and because of this it ruptures. To keep pace with the secondary growth in the stellar region and to protect the inner tissue the periderm develops in the extra stellar region. The periderm consists of three regions, namely the outer cork (Phellum), the middle cork- cambium (Phellogen) and the inner secondary cortex (Phelloderm). The Phellum cells are rectangular, compactly arranged and highly suberised. The

cork cambium cells are thin walled and meristematic. The secondary cortex consists of parenchymatous cells. The cork cells are protective in nature.

Lenticels: (Pnematophore) The lenticels are generally formed below the stomata. The activity of cork cambium below the stomata is more and the cells produced by it are loosely arranged with numerous intercellular spaces. This raised structure is called lenticels. This thin walled loosely arranged cells are called the complementary cells. Lenticels allow gaseous exchange.

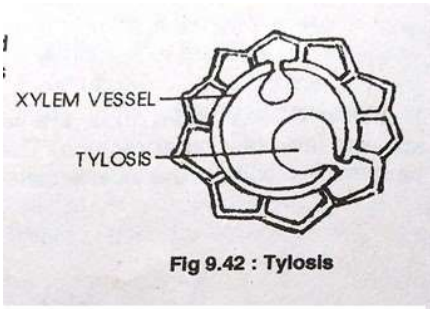


Fig 9.42 : Tylosis

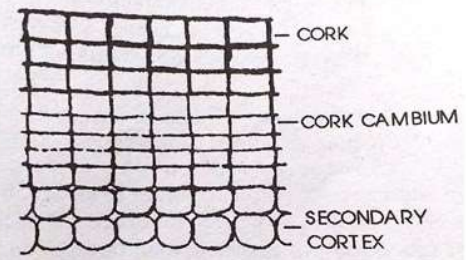


Fig. 9.44 Periderm

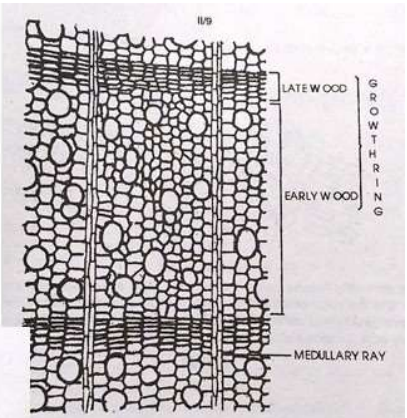


Fig. 9.43 Growth rings (Annual rings)

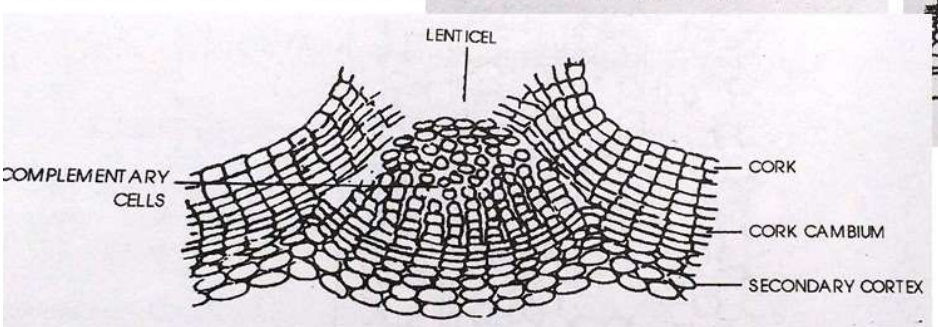


Fig. 9.45 Lenticel

Q_{10} – Germinating Seeds using Phenol Red Indicator

Aim: To study the effect of temperature on the rate of respiration of germinating seeds.

Requirements: 0.01 % phenol red solution, germinating seeds, test tubes, water bath, cotton wool.

Theory: The rate of respiration is affected by temperature and within a certain limit the increase in the rate of respiration due to increase in temperature follows Vant Hoff's rule that is for every 10^0C rise in temperature the rate of respiration gets doubled if any other factor is not limiting. In the temperature range between 0^0C to 45^0C, rise in temperature causes an increase in the rate of respiration.

Principle:

Phenol Red solution is a pH indicator. In the basic range, it is red in colour. During respiration CO_2 is given out which dissolves in water to form a weak acid – carbonic acid which changes the pH of Phenol Red and it turns yellow. The time taken for the change in the colour of the Indicator from red to yellow at two different temperatures with a 10^0C difference indicates **Q_{10}**.

Protocol:

1. Take two germinating bean seeds with their testa removed in a test tube.
2. Add 5 ml. of Phenol Red solution and place the tube at 20^0C.
3. Note the time taken for change in colour to yellow.
4. Transfer this yellow Phenol Red solution to another test tube and use it as reference for next reading.
5. Wash the seeds used earlier and transfer them to a new tube.
6. Add 5 ml. of fresh Phenol Red solution & place the tube at 20^0 C+ 10^0 C temperature in water bath.
7. Note the time taken for change in colour to yellow.

Result:

Nitrate Reductase Activity

Aim: To study the activity of enzyme Nitrate Reductase (NR) in a given plant material.

Requirements: Phosphate buffer (pH – 7.5), 0.02 M KNO_3 (substrate for NR), Sulphanilamide Reagent, 0.02% NEDD (N-1 napthyl ethylene diamine dihydro chloride), Methi leaf discs.

Principle: During assimilation of nitrate in higher plants, the fundamental process is the reduction of nitrates to ammonia. This is achieved by two enzymes Nitrate Reductase (NR) and Nitrite reductase (NiR). In the present study, KNO_3 is used as a substrate for NR activity and its optimum pH – 7.5 and at 37^0 C. The amount of nitrite produced due to NR in leaf disc reacts with Sulphanilamide and NEDD to give pinkish brown colour complex (It is actually due to the formation of azo dye in acidic medium.)

Procedure:

i) Prepare two tubes – one blank and one RM (reaction mixture).

ii) In blank, add 2 ml. phosphate buffer and 0.1 ml. KNO_3.

iii) In RM, add 2 ml. phosphate buffer + 0.1 ml. KNO_3 + 5 leaf discs as enzyme source.

iv) Incubate both the tubes at 37^0 C for 30 minutes.

v) Cool and transfer the contents of RM without leaf discs to another test tubes.

vi) Add 1 ml. each of Sulphanilamide and NEDD to blank and RM tubes.

vii) Read O.D. at 540 nm and find out ΔO.D.

viii) Calculate amount of nitrite formed using multiplication factor (it is derived from Standard Graph of nitrite with varying concentration plotted against Δ OD)

Observation and Calculation

Sr. No.	**Solution**	**OD**	Δ OD(RM – Blank)
1.	**Blank**		
2.	**Reaction Mixture**		

Multiplication factor: 1 Δ O.D. = 0.32 mg of nitrite nitrogen

Result: NR activity = _____ mg of nitrite/ 5 leaf discs/ 30 min. at 37^0C.

Estimation of Protein by Lowry's Method

Aim: To estimate the amount of proteins in the given plant material.

Requirements: Standard protein solution, suitable plant material, Reagent C, Folin's reagent, test tubes, pipettes, mortar and pestle, colorimeter.

Preparation of Reagents:

Standard protein solution – 10mg. bovine serum albumin (BSA) in 100 ml. distilled water.

Reagent C: 50 ml. reagent A + 1 ml. reagent B

Reagent A: 200mg NaOH and 1 gm. Na_2CO_3, dissolved separately in small amount of distilled water and make volume 50 ml. with distilled water.

Reagent B: 120mg. $CuSO_4$ and 250 mg Sodium tartarate dissolved separately in small amount of distilled water. Mix and make vol. 25 ml. with distilled water.

Principle: Aromatic amino acids such as Tryptophan and Tyrosine react with Copper in alkaline medium which reduces phosphomolyb (Folin's reagent) to form blue coloured complex. The intensity of colour depends on the amount of these aromatic amino acids present and thus vary for different proteins.

Procedure: Weigh 0.5 gm. of suitable plant material. Homogenize it in a mortar with pestle using 10 ml. of distilled water. Filter with muslin cloth and make the volume of the filtrate 100 ml. with distilled water.

Preparation of Standard Graph:

Prepare a series of tubes containing 0 (blank), 0.1, 0.2,……1.0 ml. of standard protein solution. Make final volume in all tubes to 2 ml. with distilled water.

Estimation:

7. Take 0.1 ml. of the Plant extract in a tube and add 1.9 ml. distilled water.
8. Add 5.0 ml. of Reagent C to all the above test tubes , mix thoroughly and allow it to stand.
9. After 15 minutes add 0.5 ml. of folin's reagent to all the tubes and shake vigorously.
10. Allow it to stand for 20 minutes.
11. Read OD at 625nm.
12. Plot a standard graph for protein using concentration of protein on x- axis and OD on y- axis. Calculate amount of proteins / 100 gm plant tissue.

Result: The amount of protein is _____ gm/ 100 gm. Plant tissue.

STUDY OF THE WORKING OF THE ECOLOGICAL INSTRUMENTS.

1. **Soil thermometer:**

   Soil temperature is measured by a special type of thermometer – Soil thermometer, which has a vertical arm with bulb at one end and a dial with deflection needle on the other end. The bulb of thermometer is buried at different depths i. e. 1", 6", 12", 18" of the soil. The temperature may be read on the dial in ^{0}C values.

2. **Soil testing kit –** A number of soil testing kits are available, complete with reagent and apparatus for making certain soil tests in the field. Soil testing kit is a compact, mobile, user friendly and fastest way to analyze your soil yourself. Soil testing kit is developed for extension purpose. The results can be used advantageously for soil fertility evaluation and fertilizer recommendation. The whole idea of Soil Testing is based on the efficient use of fertilizers and adopting farming practices. Since the efficient use of fertilizers is directly related to the knowledge about what nutrients the soil lacks.

The soil testing kit analyzes the following parameters.

1. Soil pH
2. Organic carbon
3. Nitrate Nitrogen
4. Ammonical Nitrogen
5. Phosphorous
6. Potassium
7. Calcium
8. Magnesium
9. Soil Moisture

3. Anemometer:

This instrument measures the wind velocity of a place. The wind velocity is measured in miles or kilometers per hour of unit time. Wind passes the exposure point and it is directed at the series of counter wheels, this is done for 5 minutes. The average wind velocity can be calculated.

The instrument is consists of 3 conical cups each of 5 inch diameter. They are supported by arms of rods on vertical spindle. Conical cup is suitable to keep the overestimation of stormy conditions. A suitable high raised point should be chosen to install the instrument.

Soil pH:

For field test – pH of soil is recorded with the help of universal indicator. For pH determination, small amount of each type of soil sample is taken in different test tubes and to each equal amount of barium sulphate and 15 ml of water are added. The solution is kept for some time till a clear supernatant fluid is obtained. After transferring the contents in a separate test tube, equal amount of universal indicator is added to it. The colour produced is matched with the colour chart for different values pasted on the indicator bottle and pH value of each is noted. Besides such a colorimetric determination of pH, an electronic pH meter is also used for this purpose.

4. pH meter: pH is a negative logarithm of hydrogen ion concentration. pH of any given substance (in solution form) is most reliably found by using a pH meter pH meter is consists of two electrodes, viz, a glass electrode and a calomel reference electrode. These two electrodes are connected to a potentiometer which measures the electromotive force (EMF) generated when both the electrodes are immersed in the solution to be tested. The EMF is read on the potentiometer.

1. **Glass electrode** – This electrode is made up of a small glass bulb (0.1 mm thick), which is attached at one end of glass tube of high resistance. The bulb contains a solution of 0.1 mol / l HCl connected to a platinum wire via a silver-silver chloride electrode which is reversible to hydrogen ions. A potential develops on the thin glass of the bulb when it is immersed in a given test solution. The glass electrode constitutes a half cell and the measuring circuit is completed by a reference electrode not sensitive to hydrogen ions.
2. **Calomel electrode** – Calomel electrode is the standard reference electrode in a pH meter. It consists of a glass tube filled with saturated KCl solution and with Potassium crystals at the bottom. Inside the tube, a platinum wire is suspended and its far end connected to Calomel paste. The calomel electrode is not resistant to H – ions and is the other half of the cell of the pH meter.

Working and precaution – Calibrate the pH meter before use, by means of a standard solution. To prepare the standard solution tablets of known pH, readily available (BDH), are used. Immerse both the electrodes in the standard solution and calibrate the meter according to the indicated pH of the tablet solution. Wash the electrodes in distilled water and rinse with test solution to remove any possible traces on the electrodes, left, which affects the pH. Dip the electrodes in the test solution and read the pH indicated. When not in use, the electrodes are to be kept in distilled water.

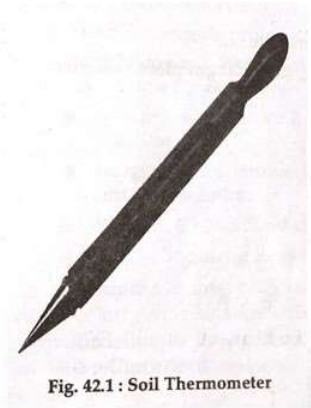


Fig. 42.1 : Soil Thermometer

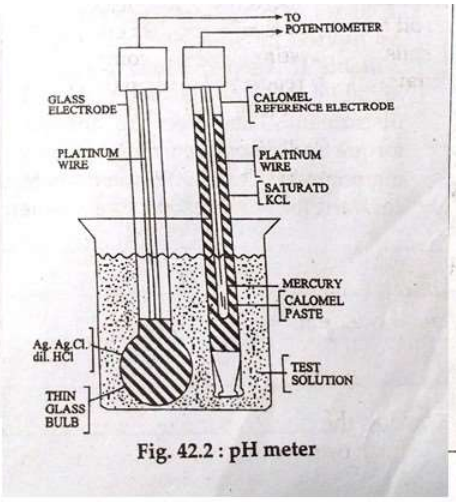


Fig. 42.2 : pH meter

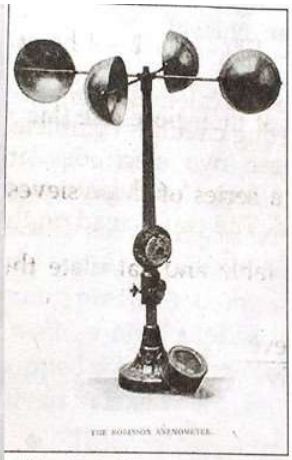


Fig. 42.3 : Hemispherical cup anemometer

MECHANICAL ANALYSIS OF SOIL BY THE SIEVE METHOD

Aim – To determine the mechanical composition of soil by sieve method

Requirements – Meshes of different pore size, weighing machine, filter papers, trays, spatula, and oven dried garden soil.

Procedure – Collect soil from garden and allow it to dry for a day or two (or make it dry by keeping it in pre-heated oven at 100^0 C for 2 – 4 hours). Take definite quantity of soil and pass through a series of meshes of different pore size in descending manner of the pore size. Collect the soil from different sieves and weigh. Find out the mechanical composition of soil from standard table.

Texture	**% Composition**
Sandy soil	60- 80 % Sand + 20- 40 % Silt and Clay
Loamy soil	50 % Sand + 50 % Silt and Clay
Clayey soil	60- 70 % Silt and Clay + 30- 40% Sand

Observation Table –

Amount of soil taken - _____ gm

Texture	**Amount of soil after segregation in gm**
Gravel	
Coarse sand	
Fine sand	
Silt and Clay	

Result: The given soil sample of _____ type.

Determination of pH of soil

Aim – To determine the pH of soil

Requirements: Different soil samples, spatula, and oven dried garden soil.

Procedure: For pH take 10 gm of different soil samples in beakers. Add 100 ml of distilled water to each beaker. Stir the solution and allow it to stand for minimum 1 hour. Filter and check the pH using a pH meter.

Soil having pH below 7 is acidic, while that having pH above 7 is alkaline. Agricultural soil has a pH of 6.5 -7.5.

Organic matter content of soil sample = % Carbon X Alison's factor

= % Carbon X 1.72

Result: The given Soil sample has pH_____.

QUANTITATIVE ESTIMATION OF ORGANIC MATTER OF THE SOIL

Aim – To estimate organic matter content in the given soil sample by Walkley and Black's method.

Requirements:

Glassware and apparatus: Burette, pipettes, conical flasks, absorbent sheet etc.

Chemicals: Conc. H_2SO_4, 1 N $K_2Cr_2O_7$, 1 N Ferrous ammonium sulphate, 85 % orthophosphoric acid, diphenylamine indicator, distilled water etc.

Material: Oven dried garden soil

Principle: $K_2Cr_2O_7$ acts as an oxidizing agent in the presence of strong acid like Conc. H_2SO_4. It oxidizes the organic carbon. Excess amount of $K_2Cr_2O_7$ solution (unused quantity) is then estimated by titrating against ferrous ammonium sulphate using diphenylamine indicator. Orthophosphoric acid prevents the interference of oxides of nitrogen and other minerals. Alison's factor (1.72) is used for calculation. The other constant used is 1 ml of = 0.003 gm of Carbon

Procedure:

1. Take 0.5 gm of dried and finely powdered soil in conical flask of 250 ml capacity.
2. Add 5 ml of 1 N $K_2Cr_2O_7$ solution and 10 ml of Conc. H_2SO_4.
3. Plug the conical flask. Shake the contents and allow it to stand for 30 minutes on an asbestos sheet.
4. Add 50 ml of distilled water and 5 ml of orthophosphoric acid. Add 6 – 8 drops of diphenylamine indicator and titrate it against 1 N ferrous ammonium sulphate.
5. For blank reading repeat the same procedure without soil sample.
6. End point is purple or blue colour initially, on titration very slowly and drop wise it changes to parrot green for blank and dirty green for soil sample.

Observations:

Solution in burette: 1 N Ferrous ammonium sulphate.

Solution in conical flask – For experimental - 0.5 gm soil sample + 5 ml of 1 N $K_2Cr_2O_7$ + 10 ml of Conc. H_2SO_4 + 50 ml of distilled water + 5 ml of orthophosphoric acid

For blank - 5 ml of 1 N $K_2Cr_2O_7$ + 10 ml of Conc. H_2SO_4 + 50 ml of distilled water + 5 ml of orthophosphoric acid

Indicator – diphenylamine

End point – For experimental – purple or blue colour changes to dirty green

For blank – purple or blue colour changes to parrot green.

Burette reading for blank (V_1)- _____ ml

Burette reading for experimental (V_2)- _____ ml

Calculations –

$$\% \text{ organic carbon in soil sample} = \frac{V_1 - V_2}{W} \times 0.003 \times 100$$

Where –

V_1 = Volume of 1 N Ferrous ammonium sulphate for Blank

V_2 = Volume of 1 N Ferrous ammonium sulphate for experimental

W = Weight of soil taken

Result - The organic matter in the given soil sample is _____ %.

STUDY OF VEGETATION BY THE LIST QUADRAT METHOD.

Aim: To study the vegetation of an area by list quadrat method.

Requirements – Nails, string, hammer, one meter scale, graph paper etc.

Procedure – It is not possible to count each and every plant in a large area hence sampling method which gives valid results is to be used. One of such methods is quadrate and it is of two types viz list quadrat and chart quadrat.
List quadrat – the entire experiment is carried out in two phases. –

I. To find out the minimum size of the quadrat – Select a point randomly in study area and fix a nail at that point. Put another nail at 10 cm away from the first nail and tie the thread or string between these two nails. With the help of other two nails and string, make a quadrat of 10 square cm. area. Count the number of species occurring in it. Make a list of these species. Likewise, increase the area to 20 square cm, 30 square cm, 40 square cm,……. so on. Find out the area of quadrat beyond which there is no increase in number of species.
Plot a graph on which size of quadrat on X – axis and number of species on Y – axis. The curve obtained will flatten out at certain point. The perpendicular line at X – axis from that point gives the minimum size of quadrat necessary to sample the area. This curve is known as species area curve.

II. Study of area – Number of quadrats of minimum size are necessary for sampling the area where the vegetation appears usually different. Put one quadrat of minimum size in this area and in the same manner lay out numbers of such quadrat (5 – 10). Record the number of species obtained in all the quadrats with positive or negative sign or with thick mark. The above data can also be used to find out the percentage frequency of each species.

$$\% \text{ frequency} = \frac{\text{Total number of quadrats having a single species}}{\text{Total number of quadrats studied}} X\,100$$

Frequency class table –

Sr. No	Frequency	Class
1.	Up to 20 %	A
2.	21 – 40 %	B
3.	41 – 60 %	C
4.	61 – 80 %	D
5.	81 – 100 %	E

Observation Table:

Minimum size of quadrat = _____ cm2

Sr. No	**Name of the plant**	**I**	**II**	**III**	**IV**	**V**	**Total**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Sr. No	**Name of the plant**	**Frequency**	**Class**
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

PRACTICAL: III

STUDY OF PLANTS FOR EACH OF THE GARDEN LOCATIONS

Avenues -

1. *Delonix regia*: (Gulmohar) Fam Leguminoceae. S.Family: Caesalpinae
2. *Peltophorum pterocarpum* (Copper pod): S.Family: Caesalpinae
3. *Thespesia populnea*: Family Malvaceae.
4. *Polyalthia longifolia* Family: Annonaceae.
5. *Roystonia regia* (Royal Palm) Family: Palmae.

Hedges -

1. *Duranta plumieri* Family: Verbenaceae
2. *Clerodendron inerme* Family: Verbenaceae.
3. *Lantana camara* Family: Verbenaceae.
4. *Hamelia patens* Family: Rubiaceae.
5. *Lawsonia alba* Family: Lythraceae

Edges -

1. *Catharanthus roseus* Family: Apocynaceae (Common name: Periwinkle)
2. *Coleus blumeii* Family: Labiateae
3. *Ixora coccinea* Family: Rubiaceae.
4. *Portulaca sps.* Family: Portulacaceae

Flower beds -

1. *Salvia coccinea* Family: Labiatae
2. *Tagetus erecta* Family: Compositae
3. *Rosa indica* Family: Rosaceae.
4. *Dianthus caryophyllus* Family: Caryophyllaceae

Lawns -

1. *Cynodon dactylon* (Doob-grass) Family: Gramineae
2. *Poa pratensis* (Kentucky blue-grass) Family: Gramineae
3. *Agrostis tenuis* Family: Gramineae

GARDEN PLANS – FORMAL AND INFORMAL GARDENS

FORMAL GARDENS –

A formal garden is a neatly trimmed, geometric and often symmetrical garden. It relies on handsome garden accessories of a classic nature, such as a pair of large urns on either side of the door, planted identically. If there is a path, it is likely to lead to a finely crafted bench. Massed ground covers, lines of trees or shrubs equidistantly planted along a long drive and tidy lawns fit the scene. Well-kept evergreens such as boxwood are in keeping with formal style. Pavements for pathways and terraces may be of brick, stone or concrete. Outdoor furniture is classic and looks more civilized than rustic. However, well made modern furnishings and accessories can also be used in a formal manner.

INFORMAL GARDENS –

An informal garden is naturalistic and usually includes asymmetrically placed design elements, naturally shaped plants and beds, curved spaces, rustic – looking furnishings and more casual paving, like decking and even wood chips. It can be flowery and furnishing can be of either traditional or modern design, but they are comfortable looking. This style sometimes employs unusual accents, such as back-door frame painted to match nearby plantings or a sweep of tall ornamental grasses in a broad, cloudlike band.

TERRARIUM

Terrarium – A unit with a high capacity to retain moisture that is used for displaying plants.

Types of terrarium – In terms of design, there are two basic types. In one design, the container for retaining moisture and maintaining high humidity does not come into direct contact with the growing medium. Instead, a potted plant is placed inside of a large container with a lid. In the second design, the potting medium is placed directly in the container. A terrarium may be sealed or openable.

Selection of container – The container should be of clear transparent (preferred) or translucent material (glass or plastic). Wine glasses, bell jars, glass bowls, glass bottles and aquarium tanks are a sample of the containers that can be used

Selection of Plants – Cacti and succulents are not recommended as in terrarium, maintenances of high humidity are observed. Plants that are adapted to low or medium light intensities and are slow growing are desirable.

BOTTLE GARDEN

Bottle garden is a closed type of terrarium in which the container is a bottle.
In an enclosed environment the plant prepares it's food through photosynthesis during daytime in presence of water and carbon dioxide present in empty space of the bottle. During this process oxygen and water vapour is released. This oxygen is used in respiration and carbon dioxide is liberated while the water vapor forms water by condensation and is absorbed by the soil. Thus, recycling of carbon dioxide, oxygen and water takes place in the bottle.
The plants commonly used in bottle garden are: Croton, *Pilea*, Ferns, *Pepromia*, *Selaginella*

DISH GARDEN

Dish garden is an open type of terrarium in which the container is a shallow.

The plants in the dish garden are grown in an aesthetically pleasing arrangement based on how the dish garden will be viewed. If the garden is to be viewed from all sides, the tallest plant should be set in the centre. If the garden is to be viewed from one side then the tallest plants are grown at the back, the intermediate plants in the middle and finally the smallest plants at the front. The plants which may be commonly cultivated in the dish garden are Cacti, *Asparagus*, *Pilea*, etc.

BIOTECHNOLOGY

STERILIZATION TECHNIQUES

Aim: To study various sterilization techniques

Sterilization: Sterilization is a technique used to destroy or remove all living Microorganisms present on glass wares and containers of the media. Before actual sterilization of glass wares they should be cleaned and Sterilization could be mainly of dry or wet type.

Procedure: Depending upon the material to be sterilized different sterilization techniques are adopted. They can be broadly divided into following categories.

DRY HEAT STERLIZATION

Dry sterilization is done with use of **hot air oven**. It is used to sterilize the glass wares, syringes, needles and inoculation loops. Hot air oven is also used to heat the chemicals to prepare standard solutions/media. In oven when electricity is passed through the heating coil, the electrical energy is converted to heat energy. The temperature is controlled by a thermostat. The clean articles are kept on the shelves in oven. The doors are closed firmly. Main switch is put on and temperature is controlled (for 30 minutes at 220^0 c) by adjusting the control knob. Oven is kept on for the required time and then main switch is put off. The articles are removed after 30 mins.

WET STERILIZATION

Wet sterilization is done with **autoclave.** Culture media, glass wares, aprons, rubber tubing etc. is sterilized in autoclave using steam under pressure. Autoclave works on a principle '**Boiling point of water is directly proportional to the pressure when volume is constant**'. When the pressure

is increased in closed vessel, the temperature increases proportionately. e.g. at 15 lbs/sq. inch pressure, the temperature inside autoclave is 121.6 °C. This pressure and temperature is kept constant for 20 mins and is sufficient to kill all the microorganisms and vegetative forms and spores of the organisms. The autoclave has a heating element, a perforated basket for placing the material which is to be sterilized, an outer jacket for water, an arm lid which is made air tight with the help of knobs and a gasket. In case of liquid media, a temperature of 121^0 C and a pressure of 15 lbs/sq inch for 20 minutes is adequate.

CHEMICAL STERILIZATION

Sterilization of living plant materials is carried out by using chemical sterilization agents like $CaOCl_2$ and in field grown materials with 70 % ethanol for 3 minute followed by 0.1% $HgCl_2$ for 15 minutes.

ULTRAFILTERATION

Thermo labile substances like vitamins, amino acids , etc. are sterilized by ultra filtration. This is done by using membrane filters of pores size 0.2-0.4µm. In case of aqueous solvents, cellulose filters are used, but if the solution is an organic solvent then nylon filters are used. The ultra filtration unit may be a system with a vacuum pump attached to it or it can be in the form of disposable cassettes which can be used along with sterile hypodermic syringes for small volume liquids.

PREPERATION OF STOCK SOLUTION AND MS MEDIA

Aim: Preparation of stock solution and MS Media

Theory: Murashige and Skoog's Medium (1962) is the most used medium for all types of tissue culture work. The cells of most plants species can be grown on completely defined media. All media consist of mineral salts, a carbon source (generally sucrose), vitamins and growth regulators. The MS medium designed for tobacco.

PROCEDURE: PREPERATION OF STOCK SOLUTION

The nutrient medium for most plant tissue cultures comprises of 5 groups of ingredients inorganic nutrients, carbon source, vitamins and growth regulators. The various stock solutions prepared for M.S. media are macronutrients, micronutrients, iron stock and vitamin stock. Mix the different concentration of chemical listed, serially by dissolving each chemical completely before adding the subsequent one.

PREPARATION OF MS MEDIA

Medium is prepared by diluting the appropriate amount of stock solution for desired volume. All the ingredients are mixed. Sugar is added and the pH is adjusted to 5.8 -6.0. Medium is poured in flasks, covered with aluminum foil and autoclaved at 121^0 C for 15 minutes. Prepared media can be stored for few weeks before inoculation. Liquid medium for a given material is same as static medium used for callus culture except gelling agent, agar. All the ingredients must be thoroughly mixed before dispensing them in the vessels. Autoclaved media should be stored in cabinets placed in the inoculation room immediately after sterilization. Glass, distilled water and chemicals of the higher purity should be use.

SEED STERILIZATION

Aim: To sterilize seeds of a suitable plant in order to tissue culture the entire plant.

Requirement: Brassica oleraceae or Broccoli seeds, Calcium hypochlorite ($CaOCl_2$) solution, Phosphate buffer, Tween 80, Bacillocide solutions.

Procedure:

1. Ten viable seeds are washed in running tap water and immersed in a beaker containing a liquid detergent (Tween 80) for 10 -12 min. (Depending on the seed, testa is removed.)
2. Any traces of detergent are removed by washing explants properly with tap water.
3. All the subsequent steps must be carried out under aseptic condition.
4. The seeds are dipped in 70 % alcohol for 1 minute and washed 2-3 times in sterile D/W.
5. The seeds are then transferred in a beaker / bottle containing 0.1% hypochlorite / Mercuric chloride solution and kept for 10-12 minutes on shaker. They are raised 2-3 times with sterile DW.
6. Using sterile forceps, they are placed in sterile Whatman filter paper and transferred them to culture tubes containing MS medium. The culture tubes are sealed with parafilm.
7. Alternatively, the seeds are kept for germination on moist filter paper in petriplate and then germinated seedlings are transferred to M S medium and sealed with parafilm.
8. They are then kept for incubation at 25^0 C with 3000 lux illumination and 18 hours of photoperiod light and 6 hours of darkness.
9. The seed will germinate within 2-4 days.

CALLUS INDUCTION

Aim: To induce the formation of callus and regenerate plant from it.

Requirement : Sterile beakers, sterile petridishes, sterile cork borer (No.2), Sterile forceps, sterile DW, 0.5 % hypochlorite solution, explant, Murashige and Skoog's solid medium slants.

Theory - Callus is defined as unorganized mass of cells derived from a variety of plant organs (e.g. Roots, Shoots , Leaves) or special cell types (e.g. Endosperm , Pollen). The term explants refers to initial piece of plant induced in-vitro. For callus initiation, the explants are aseptically transferred to the required semi- solid medium and are gently pressed onto agar so that good contact is made.

Plant regeneration can be done using any one of the 3 method-

1. Embryo culture – aseptic transfer of zygote embryo
2. Somatic or asexual embryogenesis—production of embryo-like structures from somatic cells.
3. Organogenesis—formation and outgrowths of shoots from callus or initiation and outgrowth of axillary buds generated from cultured tips and their subsequent adventitious rooting.

For this, different plant growth regulators are used. The choice depends on the plant of interest.

PROCEDURE:

A) Surface sterilization (eg. Broccoli): same as before.

B) Induction of callus

1. The germinated seedling may serve as explants for callus induction in this case.

2. The explants are transferred to culture tubes containing MS medium with

1 mg/L 2,4- D.

3. They are then kept for incubation 25^0 C with 3000 lux illumination for 16 hours photoperiod.

4. After few days the explants become slightly rough in texture. This is the beginning of callus formation.

5. The fragile callus is sub-divided and transferred into fresh medium.

C) Regeneration: Differentiation of Sugarcane callus into shoots and roots

1. A variety of phytochromes are used like auxins (IAA, IBB, NAA); cytokinins (kinetin, BAP) etc. for inducing shoots and roots in callus.

2. MS medium slants containing 1mg/L BAP and 0.5 mg/L NAA is used for shoot Induction.

3. They are kept for incubation at 25^0 C with white cool florescent tubes for 16 hours photoperiod.

4. Within 3-4 weeks, multiple shoots are developed. They are then subcultured.

5. For the induction of roots, the multiple shoots are removed from culture tubes and placed in sterile petriplates.

6. The adhering callus tissue, if any, is removed and kept in rooting medium (MS medium with 1mg/L IBA).

7. They are incubated at 25^0 C under 12 hrs. light. Roots are developed within 1-2 weeks.

8. Thus, whole plant is regenerated in-vitro. They are then acclimatized, hardened and transferred into field.

IDENTIFICATION OF CLONING VECTORS

A **plasmid** is a **circular dsDNA molecule** a few hundred or thousand base pairs in circumference. Naturally-occurring plasmids are extrachromosomal material of bacteria.

pBR 322

This is one of the first artificial cloning vectors to be constructed. It was engineered in 1977 by Bolivar and Rodriguez. It is a 4.36 kb long, circular, double stranded cloning vector with a molecular weight of 2.83×10^6 Da. This plasmid vector has been put together from fragments originating from three different naturally occurring plasmids. It is having recognition sites for 20 different types of restriction endonuclease, ampicillin and tetracycline resistant genes and an ori- site for self-replication in E.coli cell. The bacterial cell with this plasmid can grow in a medium containing ampicillin and tetracycline while natural bacteria die. It can carry desired gene inside the bacterial cell in the form of recombinant DNA. The incorporated gene is cloned inside the cell and gives its product i.e. protein.

pUC 18

The artificial plasmid **pUC18** has been genetically engineered to include
(**1**) a gene for **antibiotic resistance** to **Ampicillin** (**amp^R**), and
(**2**) a gene (and its **promoter**) for the enzyme **beta-galactosidase** (**lacZ**). The **lacZ** gene contains a (**3**) **polylinker** region, with a series of unique restriction sites found nowhere else in the plasmid. Digestion with any one of these endonucleases will make a single cut that linearizes the circular plasmid **DNA**, and allow it to recombine with foreign **DNA** that has been cut with the same endonuclease.

T_i - PLASMID

Agrobacterium tumifaciens is a Gram –ve soil bacterium. It is a natural genetic engineer infecting mainly dicotyledonous plant species, resulting in the formation of 'Crown Gall' disease.

The virulence of the bacterium is due to the plasmid called T_i plasmid (Tumour inducing plasmid), which during the infection process, transfers a portion of DNA called T-DNA (Transferable DNA) to the host plant. The genes in the T-DNA portion consist of auxins, cytokinins and opines.

Opines are used by bacterium for its multiplication. Auxins and cytokinins together result in rapid proliferation of the cells at the site of infection resulting in the formation of tumourous gall. T-DNA portion is flanked by T_L and T_R borders. 'Ori' refers to the point of origin. 'Vir' region on plasmid participates in the virulence of the plasmid. When T_i plasmid is used as vector for delivery of foreign genes, the genes are replaced with auxin cytokinin genes.

BIOSTATISTICS

CHI SQUARE TEST

Aim – Using the given data to analyze the 'Chi-square' value and test for 'Goodness of fit'.

Definition – When a statistical test is used to compare and observe ratio with an expected or a theoretical ratio and to determine how closely the former fits the later, it is known as testing the goodness of fit.

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Requirements – Yellow and Green pea seeds/ Tabulated data.

Procedure – The number of yellow and green seeds are counted and the results tabulated as shown. The chi – square value is calculated using the formula. Its distribution is checked in Table showing X^2 values for different probabilities under different degrees of freedom (n – 1)

The calculated value of X^2 should always be lower than the tabulated value at 0.05 probability in which case we describe it as being a good fit.

For any biological experiment, probability of 0.05 is taken as significant which means that the event (in this case deviation) is not likely to occur more than 5 times out of 100 tests. Thus, the hypothesis of Goodness of fit can be accepted.

Conclusion - X^2 = _____ which is less than the value of probability at 0.05 at _____^{0} freedom, hence it is a good fit.

Coefficient of Correlation

Aim: to determine the coefficient of correlation between the length and breadth of given leaf.

Requirements: *Polyalthia* leaves – 10, thread, scale, calculator etc.

Principle: The coefficient of correlation is a statistical tool. It is used to study correlation between one variable factor (x) on the other variable factor (y). If a linear relationship exists between the two variables x and y, then they are said to be correlated. When only two variables are involved it is called simple correlation. Multiple correlations involve more than two variables. When the coefficient is above 0.25 but below 0.5 it is considered significant. When the coefficient is above 0.5 but below or equal to +1 then it is considered significant. When the coefficient is above 0.5 but below or equal to +1 then it is considered to be perfect positive correlation. When the value of coefficient is -1 it is perfect negative correlation. The coefficient of correlation can range between -1 to +1.

If the coefficient value is closer to +1 or -1 then the correlation is strong. If it is closer to +1 or -1 then the correlation is strong. If it is closer to 0 the correlation is weak. If the coefficient value is 0, it means that there is no correlation between the 2 variables. The coefficient of correlation is denoted by "r".

Procedure:

1) Measure the length and breadth of each leaf.
2) Tabulate the data as shown in observation table.
3) Calculate the coefficient of correlation by using the formula.

$$r = \frac{\sum(dx.dy)}{\sqrt{\sum d^2x.\sum d^2y}}$$

Result: The coefficient of correlation is ----------

Conclusion: The correlation between length and breadth of *Polyalthia* leaves is

BIOINFORMATICS

Web Search- Google

Aim: To search the Internet via Google search engine and retrieve a data.

Requirements: Computer, internet connection.

Theory: The information in the Internet is achieved via a system of interconnected computer networks that share data by using the standardized Internet Protocol Suite (TCP/IP). A majority of widely accessible information on the Internet consists of inter-linked hypertext documents and other resources of the World Wide Web (WWW). Computer users typically manage sent and received information with web browsers; other software for users' interface with computer networks includes specialized programs for e-mail, online chat, file transfer and file sharing.

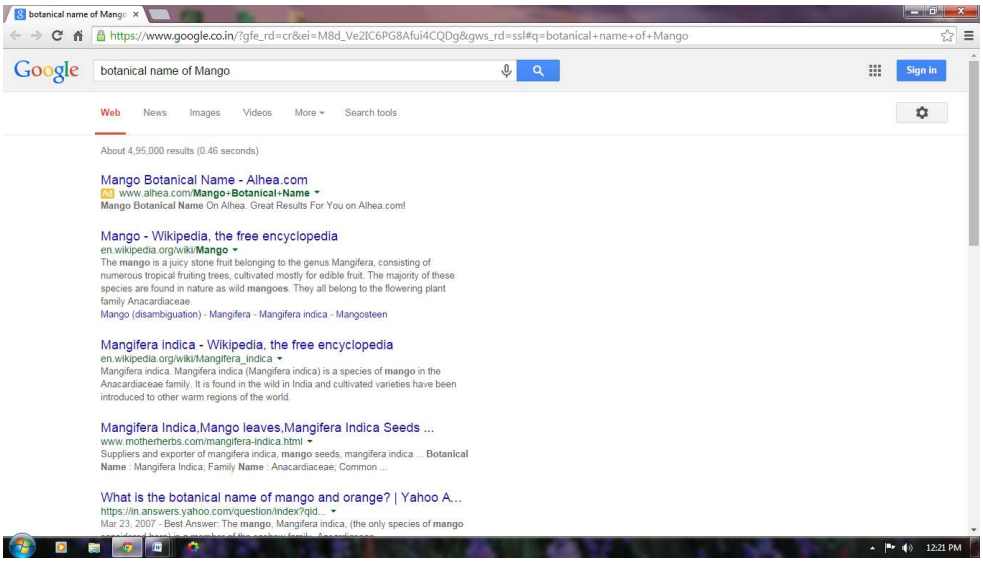
Procedure:

1. Start up your computer, and make sure that your modem is on.
2. Initiate the connection. Many access software packages will display the status of the connection process, which generally takes between 15 and 30 seconds.
3. Once you have successfully connected, access and launch your Web browser. Example: Internet explorer,; Mozilla, fire fox,; etc.
4. Once you have access to your Web Browser enter the search engine or navigator url to search against your query.
5. Type for the search engine www.google.com
6. Enter your query in to the query text box. Eg: Botanical name of Mango

Result:

1) By Google search: Results **1** - **10** of about **495,000** for the query (**0.46** seconds)

Mangifera indica



Conclusion: Thus, we successfully searched the internet regarding our query using google.

Entrez

Aim: To use Entrez as search engine so as to retrieve and study a nucleotide and protein sequence from NCBI database. (Biological Data-base).

Requirement: Computer, internet: url: http://www.ncbi.nlm.nih.gov/

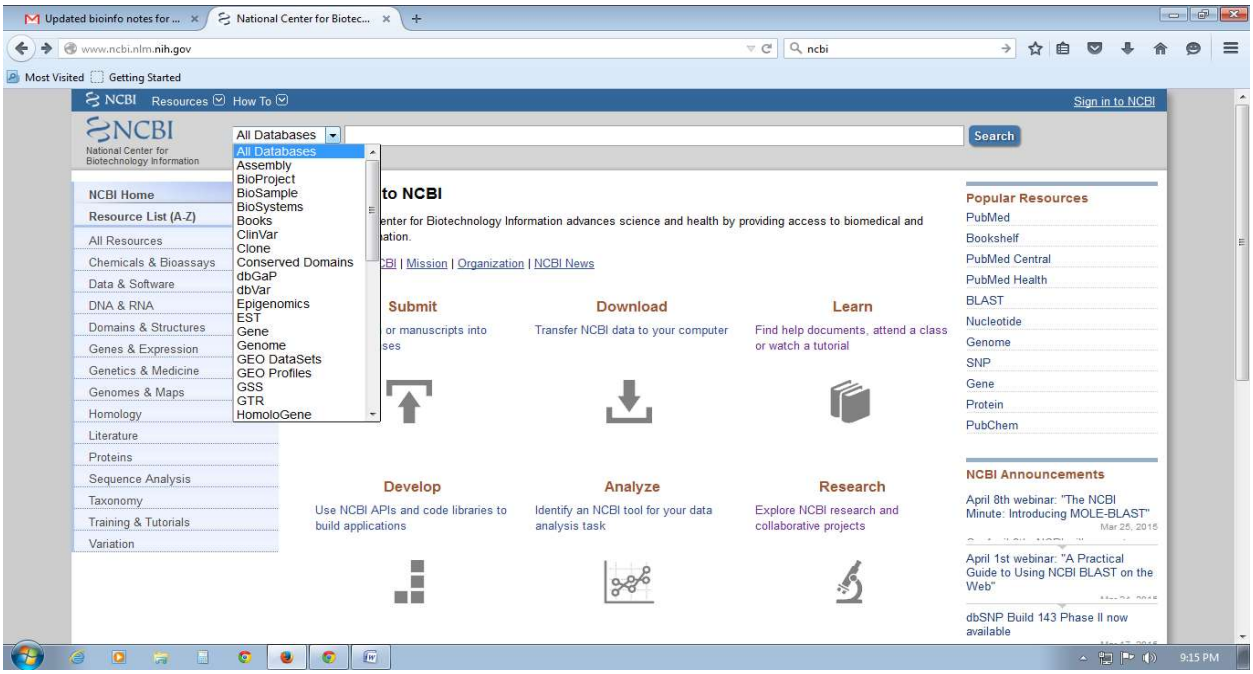
Theory:

- The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health.
- The NCBI is located in Bethesda, Maryland and was founded in 1988.
- All these databases are available online through the Entrez search engine.

- Entrez is a WWW-based data retrieval tool developed by the NCBI.
- It can be used to search for information in many integrated NCBI databases, including GenBank and its subsidiaries like OMIM and the literature database MEDLINE, PubMED.
- Entrez is accessed via the NCBI homepage and text search terms and sequences can be used as queries with utilities such as BLAST.
- Entrez can efficiently retrieve related sequences, structures, and references. It can provide views of gene and protein sequences and chromosome maps. Some textbooks are also available online through the Entrez system.

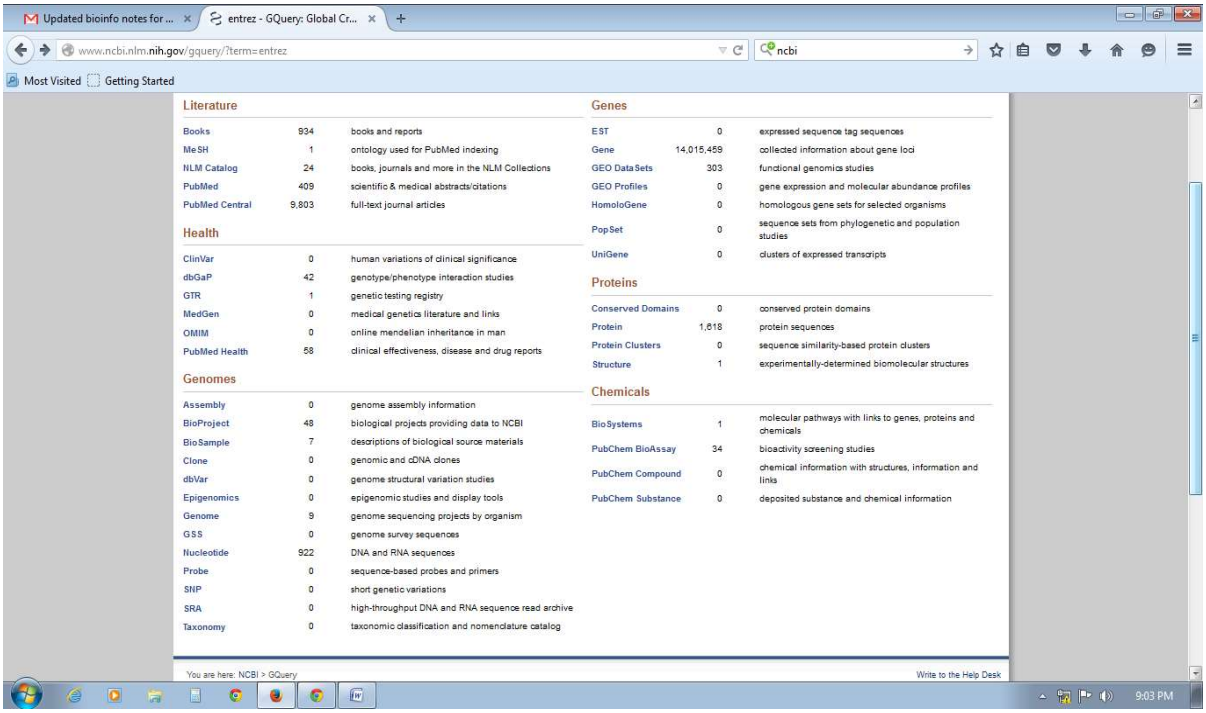
Procedure:

1) Type a url into the address bar of **Global query page: click search on ncbi home page-http://www.ncbi.nlm.nih.gov/gquery**

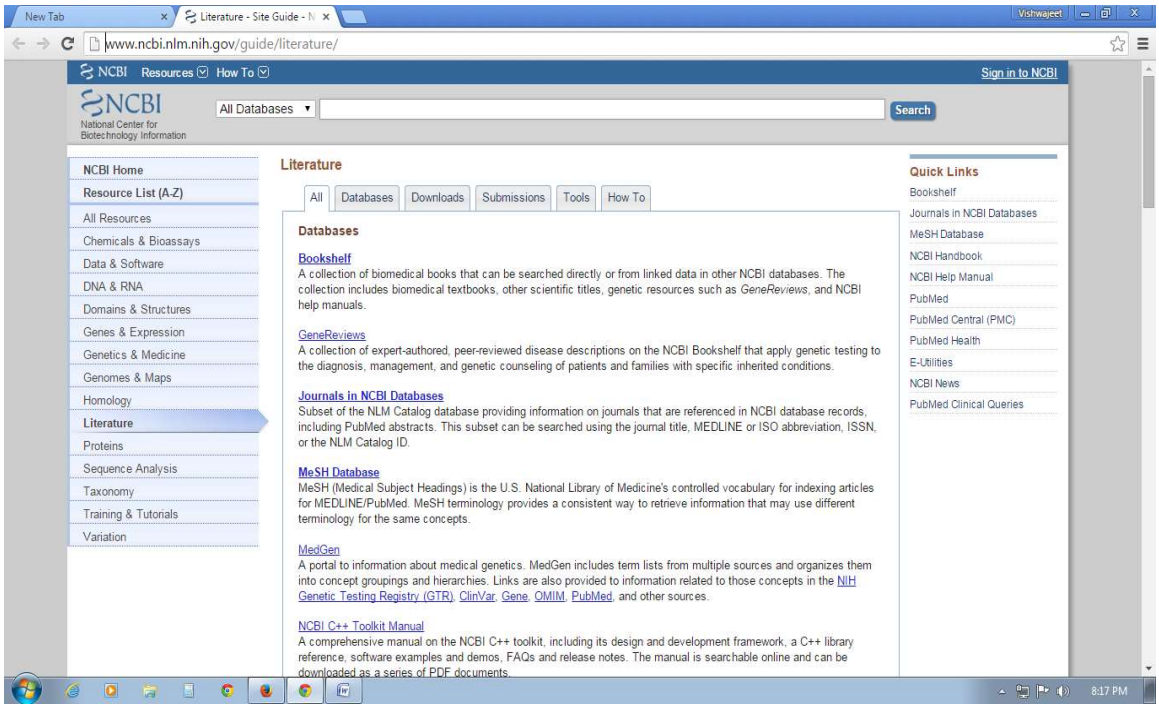


2) Click on search. Entrez options are displayed.

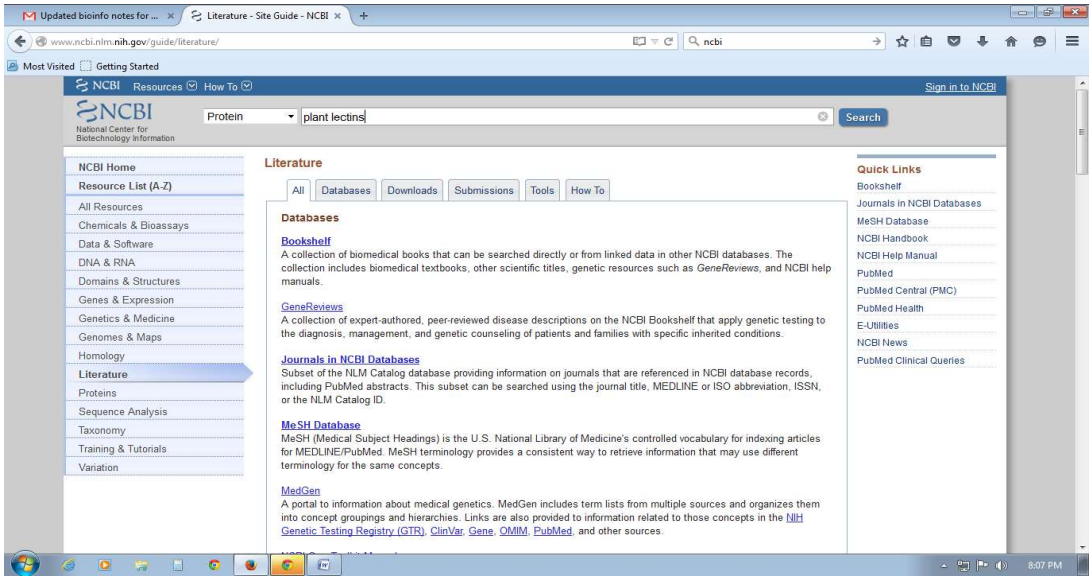
3)



3) Point to http://www.ncbi.nlm.nih.gov/guide/literature/



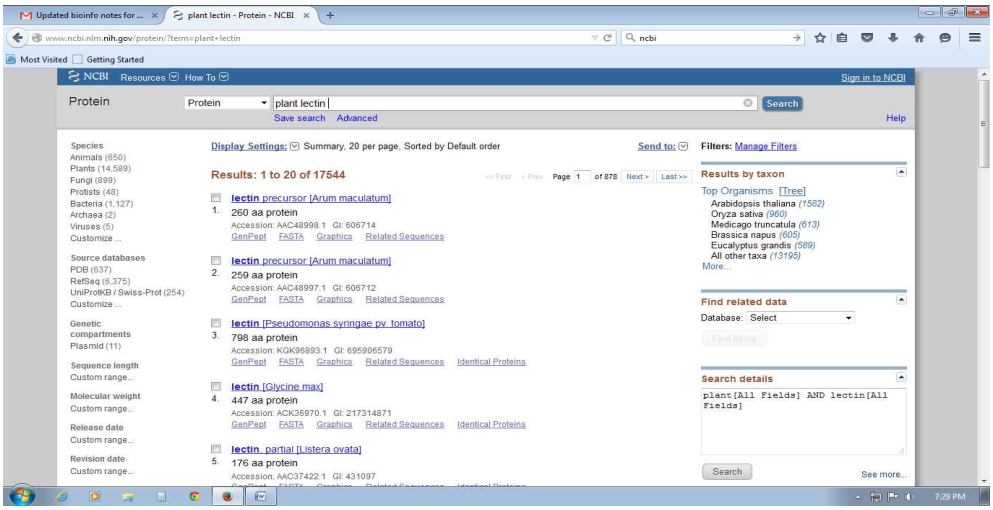
4) Select Protein in search window of database and type for Plant Lectin in the space provided.



Clicking on 'Search' initiates the search.

Results:

Result pages 1 to 20 shows 17544 entries.



1. Hits that are matching the selected querry-i.e. plant lectin and other matching records in other Entrez databases are also shown.
2. The Hits obtained from the above querry are arranged based on similarity by precomputed analysis of sequences/structures or the literature.
3. In addition to using the search engine forms to query the data in Entrez, NCBI provides the
4. Entrez Programming Utilities (eUtils) for more direct access to query results.
5. One can further study the desired hit obtained.

Conclusion: Thus we have searched a query using Entrez as search engine.

BLAST

Aim: Identify the homologous sequence with respect to our query sequence using BLAST.

Requirement: Computer, Internet: url: http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Theory: Sequence alignments provide a powerful way to compare novel sequences with previously characterized genes. Both functional and evolutionary information can be inferred from well designed queries and alignments. BLAST 2.0, (**B**asic **L**ocal **A**lignment **S**earch **T**ool), provides a method for rapid searching of nucleotide and protein databases. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

Types of BLAST:

Nucleotide-nucleotide BLAST (blastn)
Protein-protein BLAST (blastp)
Position-Specific Iterative BLAST (PSI-BLAST)
Nucleotide 6-frame translation-protein (blastx)
Nucleotide 6-frame translation-nucleotide 6-frame translation (tblastx)
Protein-nucleotide 6-frame translation (tblastn)

Methodology:
Setting up a querry
Identify a sequence (Query-sequence), to BLAST.

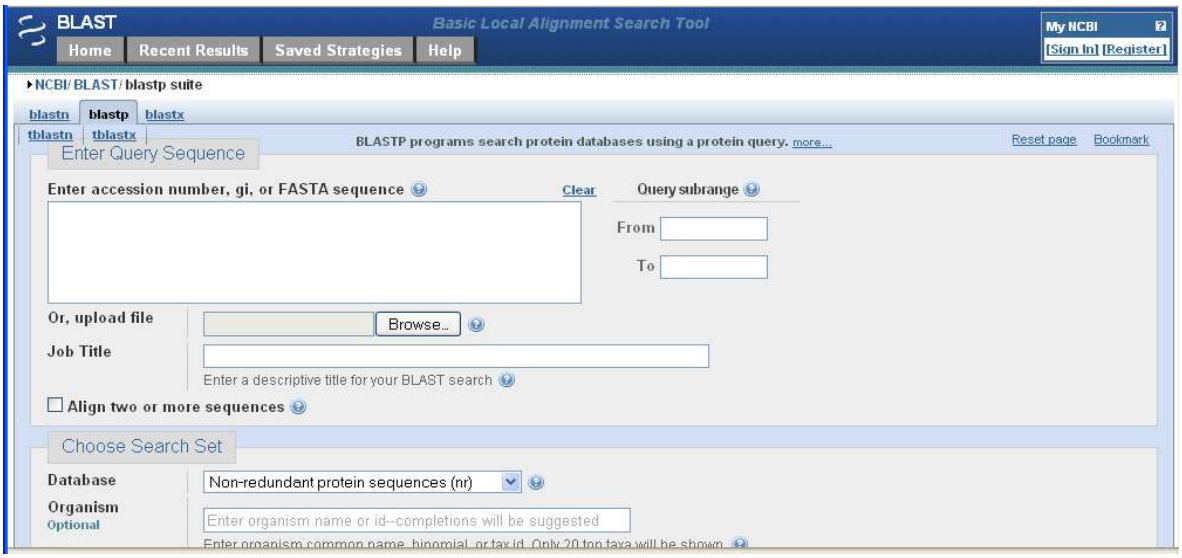
Query: = gi|2501594|sp|Q57997|Y577_METJA PROTEIN MJ0577 (162

letters)

Type a url in search text window -- http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Choose a BLAST program to run.

Types of blast will appear as: blastn; blastp; blastx; tblastn; tblastx; psi-blast; phi-blast.Select blastp because the query sequence is protein sequence.



Enter your Query Sequence by any of the following options:

Manual data entry. Type the data into the window. OR copy and paste using FASTA formatted sequence which consists of a greater-than symbol (>) followed first by a single-line description and then

(Starting a new line) by the sequence data.OR enter an NCBI Accession number or a GenBank Identification (gi) number.

Choose appropriate parameter or set as default parameter.

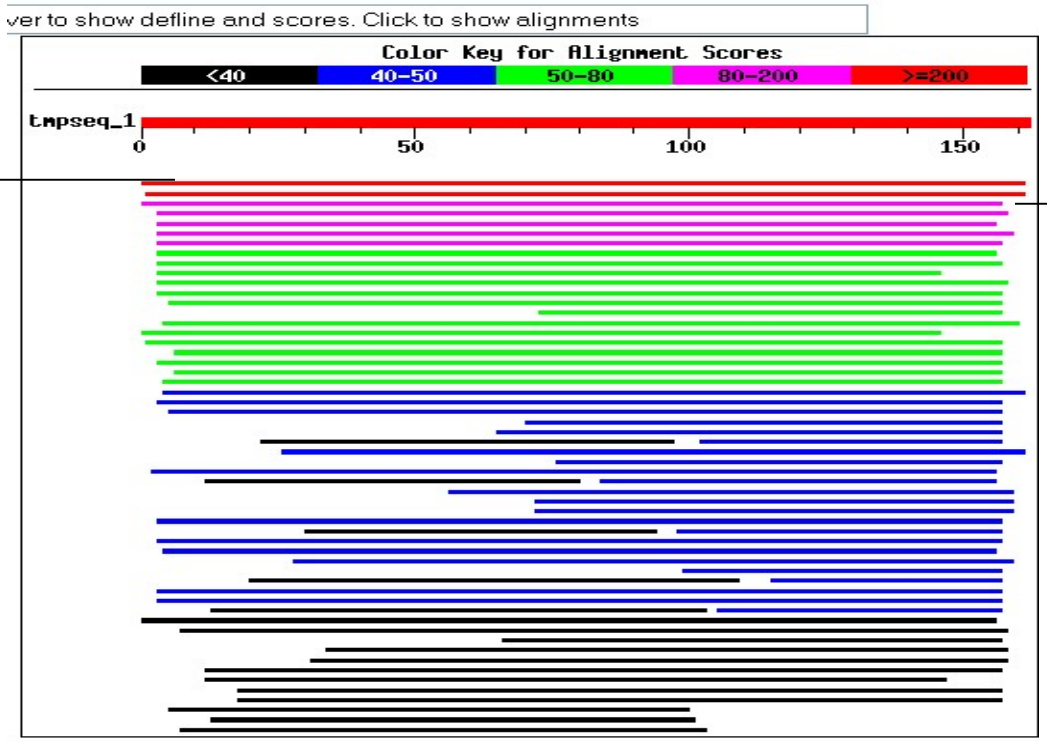
Submit the query.

Result: Output of BLAST result-

Details of Query

Query: = gi|2501594|sp|Q57997|Y577_METJA PROTEIN MJ0577 (162 letters)

Database: Non-redundant GenBank CDS translations+PDB+SwissProt+SPup+PIR 437,713 sequences; 134,605,311 total letters.



Sequences producing significant alignments: Score(bits) E Value

1. sp|Q57997|Y577_METJA PROTEIN MJ0577
>gi|2128018|pir||A64372...314 2e-85

2. pdb|1MJH| Structure-Based Assignment Of The Biochemical F...
272 1e-72

The description lines reveal that the sequence in the database with greatest similarity to MJ0577 is MJ0577 itself and appear as red bars. The second hit is to the database entry associated with the determination of the MJ0577 structure. The score for the structure entry is somewhat lower and E value somewhat higher. The missing residues will appear as dashes.

The set of entries corresponding to the pink bars in the overview are to orthologous sequences.

The set of entries corresponding to the green bars in the overview are to more distantly related Archaeal sequences. The scores and E values are respectable and most of the alignments extend the length of the query.

sp|Q57997|Y577_METJA MJ0577 - Methanococcus jannaschii >gi|5107801|pdb|1MJH|A
Chain A, Structure-Based Assignment Of The Biochemical Function Of Hypothetical Protein Mj0577: A Test Case Of Structural Genomics >gi|5107802|pdb|1MJH|B Chain B, Structure-Based Assignment Of The Biochemical Function Of Hypothetical Protein Mj0577: A Test Case Of Structural Genomics >gi|1591284 (U67506) conserved hypothetical protein [Methanococcus jannaschii]
Length = 162

Score = 314 bits (796), Expect = 2e-85

Identities = 162/162 (100%), Positives = 162/162 (100%)

Query: 1
MSVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIKKRDIFSLLLGV
A 60
MSVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIKKRDIFSLLLGV
A
Sbjct: 1
MSVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIKKRDIFSLLLGV
A 60

Query: 61
GLNKSVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAE
DEG 120

GLNKSVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAE
DEG
Sbjct: 61
GLNKSVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAE
DEG 120

Query: 121 VDIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS 162
VDIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS
Sbjct: 121 VDIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS 162

pdb|1MJH| Structure-Based Assignment Of The Biochemical Function Of Hypothetical Protein Mj0577: A Test Case Of Structural Genomics
Length = 287

Score = 272 bits (687), Expect = 1e-72

Identities = 145/161 (90%), Positives = 145/161 (90%), Gaps = 16/161 (9%)

Query: 2 SVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIKKRDIFSLLLGVA G 61

SVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIK

Sbjct: 143 SVMYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIK---------- 189

Query: 62 LNKSVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAED EGV 121

SVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAEDEGV

Sbjct: 190 --- SVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKDIIVVGIPHEEIVKIAEDEGV 246

Query: 122 DIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS 162

DIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS

Sbjct: 247 DIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVKRKNS 287

Conclusion: we have significantly identified the homologous sequence corresponding to the protein query sequence.