Exercise 6

Aim: Study of mitosis.

Principle: Somatic growth of both plants and animals takes place by increase in the number of cells. The cells divide mitotically wherein number of chromosomes remains unchanged in the daughter cells from that in the maternal cells. Cells from the growing root-tips and apex of shoot buds are suitable for mitotically dividing cells. In animals mitotically dividing cells can be easily scored from the bone marrow of a vertebrate. The cell from the epithelium of gills in fishes and from the tail of growing tadpole larvae of frog are also good sources for scoring the mitotically dividing cells.

Requirement: Permanent slides available in the laboratory, compound microscope.

Procedure

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10X objective) to search the dividing cells. Observe the dividing cell under higher magnification (40X objective) of the microscope.

Observation

The stages of mitosis can be broadly put into two events: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell). Those cells, which are not in the phases of cell division are considered to be in interphase.

You will observe that most of the cells in a particular microscopic field are in interphase.

Interphase

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (coloured) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli *(Sing*: nucleolus) can also be observed inside the nucleus (Fig. 6.1a).

Stages of Mitosis

(a) Prophase

Intact nuclear outline is seen. The chromatin (seen as a homogeneous material in the nucleus at interphase) appears as a network of fine threads (chromosomes). Nucleoli may or may not be visible (Fig. 6.1b).

If the cell under observation is in the early stage of prophase then the chromatin fibres (chromosomes) are very thin. However, in the cells at late prophase, comparatively thicker chromatin fibres would be visible. Besides this, in the late prophase the nuclear membrane may not be noticed.

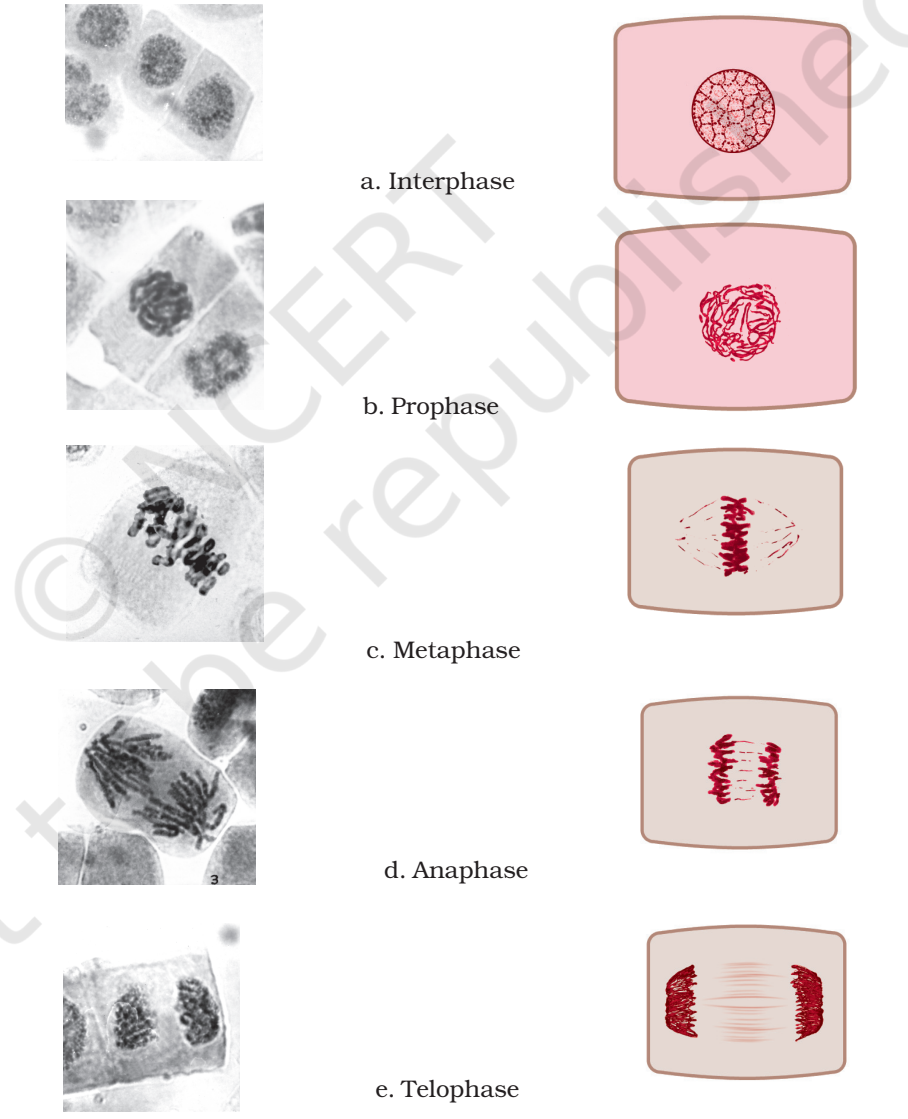


Fig. 6.1 Interphase (a) and stages of mitosis (b - e)

(b) Metaphase

The nuclear membrane disappears. Chromosomes are thick and are seen arranged at the equatorial plane of the cell (Fig. 6.1c). Each chromosome at this stage has two chromatids joined together at the centromere, which can be seen by changing the resolution of the microscope. Nucleolus is not observed during metaphase.

(c) Anaphase

This stage shows the separation of the chromatids of each chromosome. The chromatids separate due to the splitting of the centromere. Each chromatid now represents a separate chromosome as it has its own centromere. The chromosomes are found as if they have moved towards the two poles of the cell. The chromosomes at this stage may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them. Different anaphase cells show different stages of movement of chromosomes to opposite poles, and they are designated to represent early, mid and late anaphase (Fig. 6.1d).

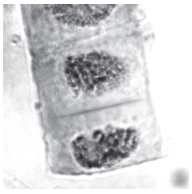
(d) Telophase

Chromosomes reach the opposite poles, lose their individuality, and look like a mass of chromatin (Fig. 6.1e). Nuclear membrane appears to form the nuclei of the two future daughter cells.

CYTOKINESIS

In plants, a cell plate is formed in the middle after telophase. The plate can be seen to extend outwards to ultimately reach the margin of the cell and divide the cell into two. Such cell plates are characteristic of plant cells (Fig. 6.2). However, in an animal cell, the two sides of the cell show constrictions formed from the peripheral region in the middle of the cell, which grow inward and meet to divide the cell into two daughter cells.

Draw labelled diagrams of all the phases of mitosis.



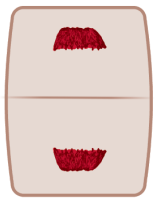


Fig.6.2 Cytokinesis

Questions

1. What would happen if the chromosomes fail to move during anaphase?
2. What would happen if cytokinesis does not take place after karyokinesis?
3. Which cellular elements participate in anaphase movement?
4. What would happen if mitosis
   (a) does not take place at all?
   (b) takes place at a rate faster than the normal rate?
5. Why is chromatin network seen during the prophase stage of mitosis ?

Exercise 7

Aim: To study modifications of root.

Principle: Roots of plants are generally meant for anchorage and absorption of water and nutrients from soil. However, in certain plants, roots perform additional functions, particularly as storage organs of photosynthates. Roots are also modified to provide additional support to weak stems or to trees which are massive. In some cases roots may help in gaseous exchange or for floating (aquatic plants). Consequently, the root morphology and structure undergoes certain modifications to perform these additional functions.

Requirement: Samples/charts of radish, carrot, beet, turnip, *Asparagus*, sweet potato, pneumatophores, stilt roots, climbing roots, leguminous plants showing root nodules.

Procedure

- Carefully observe the shape and external morphology of each specimen.
- Draw diagrams and observe the morphological differences between the samples.

Observation

Some modifications of roots are discussed below:

(i) **For storage of food**

Roots are modified in some plants for storing reserve food materials. These modified roots usually are swollen and assume different forms such as spindle shaped, e.g., radish; top shaped, e.g., beet, turnip; conelike, e.g., carrot; indefinite shape, e.g., sweet potatoes (Fig. 7.1). *Dahlia*, *Asparagus*, *Portulaca* are some other examples of plants with modified roots for food storage.

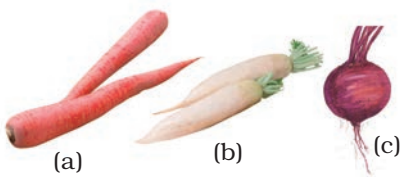


Fig. 7.1 Roots modified for storage of food (a) Carrot (b) Radish (c) Turnip

(ii) **Nodulated roots**

The roots of pea and other leguminous plants have numerous swollen nodules on fine branches of roots. These nodules are formed due to symbiotic association of *Rhizobium* (bacterium) that live inside the root cortical cells of the roots. They fix nitrogen. An active nodule is pink in colour (Fig. 7.2).

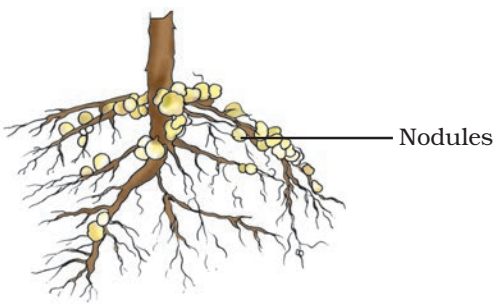


Fig. 7.2 Nodulated roots

(iii) **For mechanical support**

Roots are modified to provide mechanical support as seen in banyan tree which has roots growing vertically/obliquely downwards (prop roots); sugarcane/maize in which roots arise from the nodes in cluster at the base of the stem (stilt roots) and betel/black pepper in which nodes and internodes bear roots which help in climbing (Fig. 7.3).

Fig. 7.3 Prop root of banyan tree

(iv) **For gaseous exchange**

Pneumatophores or breathing roots are found in plants growing in mangroves or swamps with saline water for exchange of gases. They are erect peg like structures with numerous pores through which air circulates e.g., *Rhizophora mangle* (Fig. 7.4).

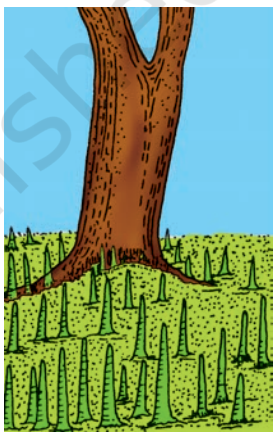


Fig. 7.4 Pneumatophores of *Rhizophora*

Questions

1. Why are healthy root nodules pink in colour?
2. Mention characteristics by which we can identify the modified roots as roots?
3. Prop and stilt roots are aerial in origin yet they are called roots. Why?

Exercise 8

Aim: To study modifications of stem.

Principle: The stem is the central axis that provides support to all the aerial parts of the plant. Besides, in some plants these also help in perennation, vegetative propagation, food storage, photosynthesis etc. through various modifications.

Requirement: Specimens of ginger, potato, onion, arbi *(Arum)*, yam, whole plant of *Oxalis*, mint, water lettuce/*Eichornia*, *Chrysanthemum*, tendrils of *Vitis*/ passion flower, thorns of Pomegranate/Bougainvillea/Acacia, *Opuntia, Ruscus, Asparagus*, or locally available specimens.

Procedure

- Observe the external morphology of each specimen.
- Draw diagrams and bring out the differences in each type of the stem modifications.

Observation

(i) **For storage of food**

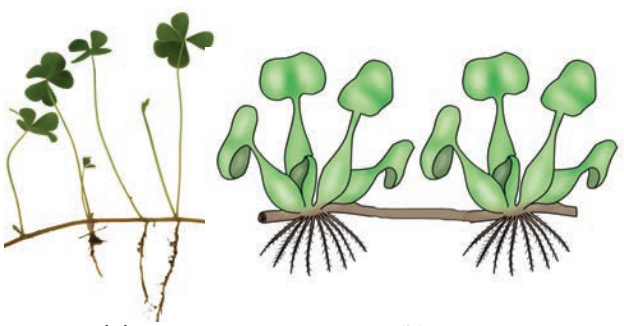
Stems get modified into underground structures for storage of food as seen in potato (tuber) (Fig. 8.1a), ginger (rhizome) (Fig. 8.1b), garlic (bulb), yam (corm). Presence of an eye (node) in potato, distinct nodes with internodes and scaly leaves in ginger/yam, a cluster of roots at the base of the reduced stem in garlic/ onion, all indicate that these underground plant parts are modified stem.

(a) (b)

Fig. 8.1 Stems modified for storage
(a) Potato
(b) Ginger

(ii) **For vegetative propagation**

Plants besides reproducing sexually also propagate through vegetative parts. For this purpose, stems may be modified into **runner** (*Cyanodon dactylon, Oxalis*) (Fig. 8.2a). Runners are a slender prostrate branches arising from axillary buds; **stolon** (e.g., mint, strawberry) which is a slender lateral branch



(a) (b)

Fig. 8.2 (a) Runner of *Oxalis*
(b) Offset of *Eichornia*

arising from the base of stem grows upward and then down to develop new daughter plants; **offset** having a single long horizontal internode growing upto some distance and producing a tuft of leaves above and cluster of roots below at the apex (*Eichornia, Pistia*) (Fig. 8.2b) and **sucker**, which arises from underground part of stem, grows obliquely and gives rise to a new shoot. (*Chrysanthemum*, Banana, Pineapple).

(a)

(b)

Fig. 8.3 Thorns of (a) *Acacia* (b) *Bougainvillea*

(iii) **For protection**

Some modified stem provides protection as thorns which are hard, pointed structures each representing a branch that arises from the axil of leaf. Thorns are found in plants like *Duranta*, Pomegranate, *Acacia*, Ber, *Prosopis*, *Bougainvillea, Citrus*, etc (Fig. 8.3).

Fig. 8.4 Tendrils of Passion flower

(iv) **For support**

Tendrils are modifications of stem to provide support to plants, e.g., *Vitis*, passion flower, *Bignonia* etc (Fig. 8.4).

(v) **For photosynthesis**

Stems are also modified into **Phylloclade**, to facilitate photosynthesis. Phylloclades are flattened/cylindrical stem or branches of unlimited growth (*Cactus*) (Fig. 8.5).

Fig. 8.5 Green stem of *Cactus*

Discussion

In all the examples cited above, the stems are modified to perform the additional function of storage, perennation, vegetative propagation, photosynthesis, etc. Accordingly, their morphology and structure have been modified to suit the function they perform.

Questions

1. Mention any one stem character by which ginger rhizome and onion bulb are recognized as stem.
2. Though potato tuber is non-green and underground, it has plenty of starch. Where does this starch come from?
3. Comment on the feature of photosynthetic stem of *Opuntia*.

23-04-2018

Exercise 9

Aim: To study the modifications of leaf.

Principle: Leaf is the most important vegetative organ of the plant. It is a lateral appendage borne at nodes of stem and is associated with photosynthesis, gaseous exchange and transpiration. Despite it's wide variety in shapes, sizes and form, leaves of many plant species are also modified to perform some other special functions. These modifications may be with respect to mechanical support, protection, reducing transpirational rate or to trap insects.

Requirement: Specimens of pea/lentil plant with tendrils, Cactus/*Argemone*, Pitcher Plant/*Utricularia*, bulbs of onion/garlic/Crocus.

Procedure

- Observe the external morphology of each specimen.
- Draw diagrams to bring out the differences in each modification.

Observation

(i) **For protection**

Leaves are modified into sharp, pointed structures or spines to act as defensive mechanism against excessive grazing and to check transpiration, e.g., *Cactus, Argemone* (Fig. 9.1).

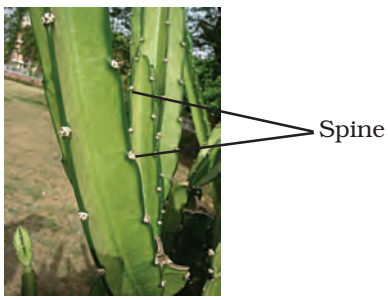


Fig. 9.1 Spines of a cactus

Fig. 9.2 Tendrils of sweet pea

(ii) **For mechanical support**

To provide support and help in climbing, leaves are modified into long, slender, thread like spirally coiled structures called tendrils in plants like sweet pea, lentil etc (Fig. 9.2).

(iii) **For storage of food**

Like stems and roots, leaves are also modified to store reserve food in plants as in the fleshy leaves of garlic, onion (Fig. 9.3).

Fig. 9.3 Onion bulb with scaly leaves

(iv) **For trapping insects**

Leaves are modified as pitcher to capture insects and digest them in **pitcher** plant (*Nepenthes*) and as **bladder** in bladderwort (*Utricularia*) (Fig. 9.4).

Fig. 9.4 Pitcher of *Nepenthes*

Discussion

In all the specimens studied it will be observed that the leaves show modifications in structure and morphology to perform some special functions.

Questions

1. How does a leaf spine differ from stem spine?
2. How does a leaf tendril differ from stem tendril?
3. Is there any significance attributed to insectivorous habit?

Exercise 10

Aim: To study and identify different types of inflorescences.

Principle: In angiosperms the flowers are borne either singly or in clusters. Flowers borne singly are solitary, and those borne in clusters together on a common stalk or peduncle form an **inflorescence**. It is the reproductive shoot composed of a number of shoots of limited growth (dwarf shoots) termed flowers. Pedicel is the stalk of a flower.

Requirement: Inflorescences of locally available plants, hand lens, beaker, water.

Procedure

- Collect inflorescences of locally available plants, keep them in a beaker with water.
- Make yourself familiar with the flow chart of types and characters of inflorescences, given in the Annexure 1 at the end of the exercise.
- Sort out the inflorescences into racemose/cymose and list the plant species in a tabular form as in Table 10.1.
- Identify the type of **raceme/cyme** giving reasons.
- Note the position of the inflorescence in the plant (axillary/terminal).
- Draw labeled diagram of inflorescence (of each plant species collected by you) showing the arrangement of the oldest and youngest flowers on the peduncle.
- Draw a diagram of a flower of each inflorescence, identify and label its parts. Note the position of ovary with respect to arrangement of other floral parts **(epigynous, perigynous, hypogynous)**.

Discussion

- Inflorescences may be of definite or indefinite types classified on the basis of position on the mother axis (axillary/terminal), number of flowers, maturity of flowers, etc.
- Inflorescence and flower characters help us in taxonomic classification and identification of plants.
- Note the type of inflorescence which is more common among the locally available plants.

Table 10.1

Name of plant	Inflorescence		Position of ovary in flower
	Type	Position	
1.			
2.			
3.			

Questions

1. How is a floret different from a flower?
2. Observe a pomegranate fruit and note whether it has developed from a solitary flower or an inflorescence.
3. Cite an importance of studying inflorescences of different plants.

Annexure 1

Types of Infloresence

The inflorescences are classified on the basis of position in the shoot system into the following four types:

1. *Terminal inflorescence:* A strictly terminal inflorescence, terminates the growth of the branch.
2. *Axillary inflorescence:* It is terminal on a short axillary branch, which is reduced to an inflorescence.
3. *Intercalary inflorescence:* These are terminal clusters that have been left behind by continuing apical growth of the main axis, which form alternately fertile and sterile sections.
   Example: Callistemon (Bottle brush)
4. *Cauliflory:* The development of inflorescences on older branches.
   Example: Theobroma cacao (Cocoa plant), *Arctocarpus* (Jackfruit), Cannon ball tree.

On the basis of sequence of development of flowers on the peduncle the inflorescences are of the following kinds:

1. **Racemose** (Indefinite or indeterminate)
2. **Cymose** (definite or determinate)

INFLORESCENCE

Racemose (Indefinite) The main axis grows indefinitely bearing flowers either directly on itself or on its branches. The flowers borne at the lowest are the oldest and the first to mature or open.

Cymose (Definite) The growth of main axis is arrested as it terminates in a flower. Other flowers are produced lower down the main axis or on its branches which also terminate in a flower. The oldest flower is at the top.

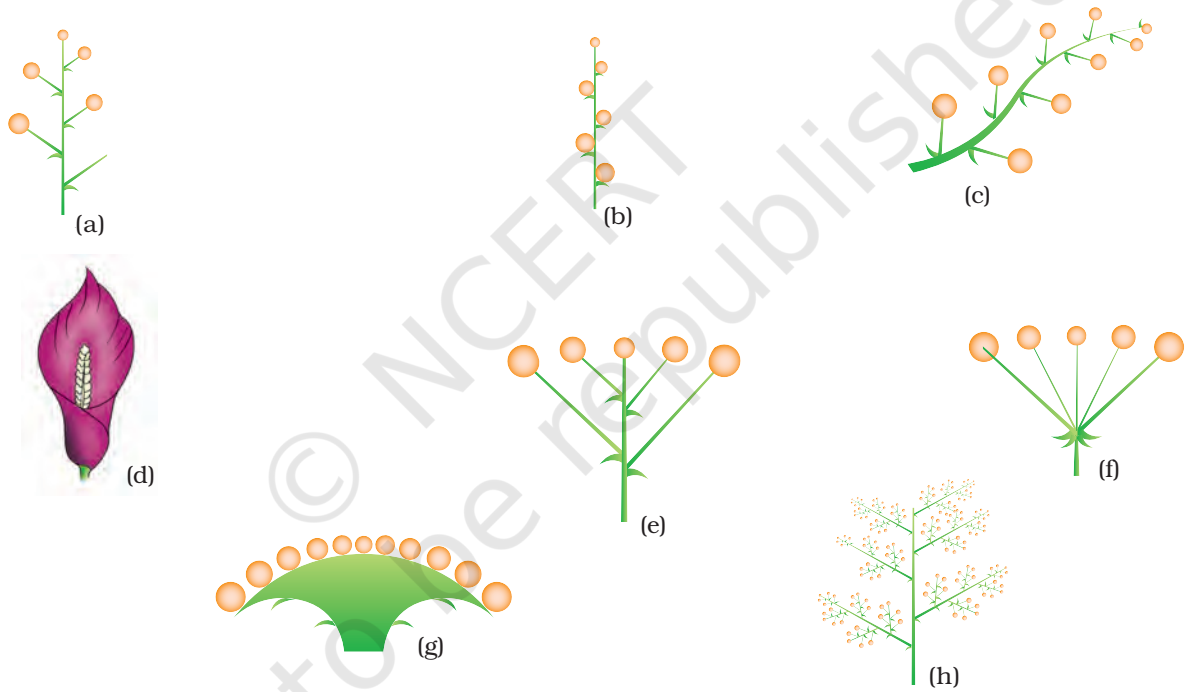


Fig. 10.1 Different types of racemose inflorescence
(a) simple raceme (b) spike (c) catkin (d) spadix
(e) corymb (f) umbel (g) capitulum (h) panicle

Racemose Inflorescence : The unbranched main axis bearing stalked (pedicellate) flowers as in mustard, radish, *Crotalaria* is **simple raceme** (Fig. 10.1 a). Sessile flowers borne on elongated axis as seen in amaranth is referred to as **spike** (Fig. 10.1 b). If the main axis is pendulous and bears stalkless (sessile) unisexual flowers, the inflorescence is **catkin,** e.g., mulberry (Fig. 10.1 c). Fleshy peduncle covered by long showy bract with spike inflorescnce as in banana and *Colocasia* is called **spadix** (Fig 10.1 d). In **corymb** inflorescence, which is a relatively shorter and broder raceme, the pedicel of lower flowers are longer than the upper ones and all the flowers reach the same level (Fig 10.1 e). e.g., *Cassia auriculata, Gynanadropsis,* candy tuft.

An inflorescence with extremely reduced main axis bearing a cluster of pedicellate flowers with more or less equal stalk is referred to as **umbel** (Fig 10.1), e.g., coriander, *Allium cepa* (onion). In **Head or Capitulum** type, sessile flowers are borne in a dense cluster in a common receptacle, which is the flattened main axis (Fig 10.1 g) e.g., sunflower.

If the main axis is branched then the inflorescence is termed as compound. A **panicle** as seen in mango and drumstick is a compound raceme(Fig 10.1 h). Likewise, there can be compound spadix, e.g., palm, compound umbel, e.g., coriander, compound corymb, e.g., candy tuft.

Cymose Inflorescence: There are mainly three types of cymose inflorescence viz. monochasial cyme, dichasial cyme, polychasial cyme.

In **monochasial cyme** a single flower arises in the axil of a leaf of an ordinary shoot or the peduncle ends in a single flower (Fig 10.2 a), e.g. *Hibiscus rosasinensis* (shoe flower).

Dichasial cyme consists of only three flowers, out of which the central one is the oldest and the two lateral ones arising in the axils of bracts below the older flower are youngest (Fig 10.2 b, c), e.g., *Jasminum.*

In **polychasial cyme** the main axis ends in a flower with more than two branches arising from the peduncle below the terminal flower (Fig 10.2 d)., e.g., *Calotropis.*

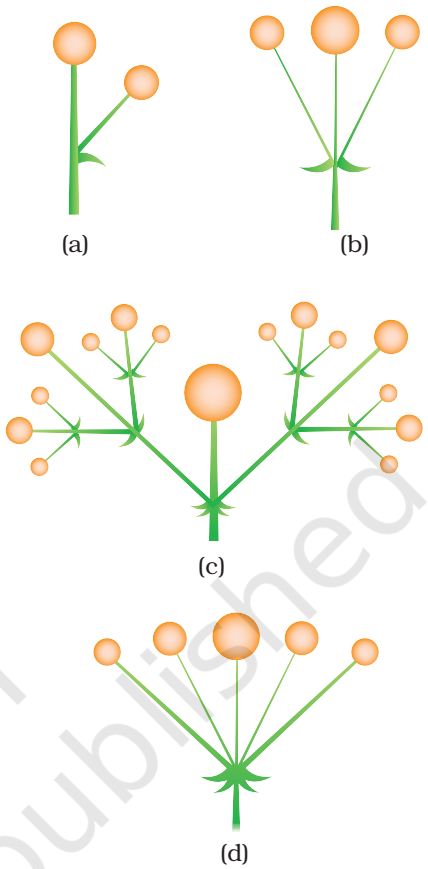


Fig.10.2 Different types of cymose inflorescence (a) monochasial cyme (b) simple dichasial (c) compound dichasial (d) polychasial

Exercise 11

Aim: Study and describe flowering plants of families Solanaceae, Fabaceae and Liliaceae.

Principle: Taxonomy deals with identification, nomenclature and classification of organisms. Bentham and Hooker's system of classification is universally used for classification of plants. Field identification of plants is based primarily on morphological features particularly the floral characters.

Requirement: Locally available plant specimens of Solanaceae, Fabaceae and Liliaceae. (minimum 3 species for each family other than the ones described for reference in the manual); each specimen should have at least a small branch with a few inter nodes, leaves, flowers and fruits; glass slides, cover glass, water, 100 ml beakers, petridish, razor, blade, needles, brush, hand lens, dissecting microscope and compound microscope.

Procedure

Keep the twigs in beakers containing water. Make yourself familiar with the terms given to describe the habit of plant, its root system, stem and leaf, inflorescence and flowers. Describe the vegetative and floral features of the plant in the same sequence using terms described therein. Observe the flower bud under dissection microscope or a hand lens and note the aestivation patterns of calyx and corolla, number of sepals and petals (tri, tetra, penta-merous), number of stamens. Cut LS of the flower, place it on a slide and observe under the dissecting microscope to study:

- Position (attachment) of stamens – opposite/alternate to petals; free or epipetalous; extrorse/ introrse anthers (anther lobes in the bud face away from axis – extrorse; anther lobes in the bud face towards the main axis – introrse).
- Number of carpels (mono, bi, tri- carpellary); Position of the ovary (epigynous, perigynous, hypogynous).

Mount a stamen on a slide and study the attachment of filament to anther (basifixed, dorsifixed, versatile, adnate), dehiscence pattern of anther (porous, longitudinal), number of anther lobes (monothecous, dithecous). Mount the pistil and study the ovary, style and stigma. Also cut a TS of the ovary to study the number of locules and placentation. Write the floral formula and

draw the floral diagram of each specimen based on the description. Identify features of the different parts of flower on the basis of descriptions given in Table 11.1.

Observations

Compare the characters with those given in the table and identify the family to which the plant belongs to.

Note: For ready reference some plants are described for each family. The students are required to study the plants other than one described here-under.

Questions

1. Draw the floral diagram and write the floral formula from the below given description of a flower-

   Bisexual, actinomorphic, hypogynous, sepals 5, gamosepalous, petals 5, free, imbricate aestivation, stamens 6, arranged in 2 whorls, ovary superior, trilocular, axile placentation.
2. In which type of placentation would the ovary be always unilocular?
3. If a flower is epigynous what is the position of floral parts?
4. What in the fruit is equivalent to the ovule of the ovary?

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Table 11.1 Description of parts of flowers: Calyx/Corolla

Aestivation	**Arrangement of sepals and petals with respect to one another**
Aestivation (Fig 11.1 a–e)	**(i) Valvate:** The sepals/petals close to each other without overlapping or may be in contact with each other. **(ii) Twisted:** Overlapping is regular, i.e., one margin of the sepal/petal overlap the next member and the other margin is overlapped by the previous. **(iii) Imbricate:** Out of five sepals/petals one is completely internal being overlapped on both margins and one is completely external with the rest of the members arranged as in twisted aestivation. **(iv) Quincuncial:** Out of five sepals/petals two are completely internal, two external and one has one margin external and the other margin internal. **(v) Vexillary:** Out of five sepals/petals the posterior one is the largest and external almost completely covering two lateral members which in turn overlap the two small anterior sepals/petals
Number of stamens	**The number of stamens may vary from a few to many in different flowers**
Cohesion (Fig. 11.2 a-e)	Stamens may be free or united. If united they can be of the following type: **(i)** ***Syngenesious:*** Filaments free and anthers united, e.g., Sunflower. **(ii)** ***Synandrous:*** Stamens fused all through their length. e.g., *Cucurbita.* **(iii)** ***Adelphous:*** Anthers remain free and filaments are united. Adelphous condition can be:- (a) Monoadelphous - United to form 1 bundle. e.g., China rose. (b) Diadelphous - United to form 2 bundles. e.g., Pea. (c) Polyadelphous- United into more than two bundles. e.g., Lemon.
Adhesion (Fig. 11.3)	Fusion of stamens with other parts of the flower. **(i)** ***Epipetalous:*** Stamens fused with petals e.g., Sunflower, Datura. **(ii)** ***Epiphyllous:*** Stamens fused with perianth e.g., Lily.
Attachment of filament to anther (Fig. 11.4 a-d)	**(i)** ***Basifixed:*** Filament attached to the base of anther. e.g., Mustard. **(ii)** ***Adnate:*** Filament attached along the whole length of anther. e.g., *Michelia, Magnolia.*

	(iii) ***Dorsifixed:*** Filament attached to the back of anther, e.g., Passion flower. **(iv)** ***Versatile:*** Anther lobes attached with filament in the middle portion with both ends free. e.g., Gramineae family.
Lobes of anther (Fig. 11.5 a,b)	**(i)** ***Monothecous:*** Anther single lobed. **(ii)** ***Dithecous:*** Anther bi-lobed.
Dehiscence pattern (Fig. 11.6 a,b)	**(i)** ***Porous:*** Pollens released through pores, e.g., brinjal, potato. **(ii)** ***Longitudinal:*** Pollens released through the longitudinal slit of anther lobes, e.g., China rose, cotton.

Gynoecium

Position of ovary (Fig. 11.7 a-d)	**(i)** ***Epigynous:*** Position of ovary inferior to other floral parts. e.g., mustard, China rose. **(ii)** ***Perigynous:*** Other floral parts (organs) are attached around the ovary. e.g., apple, guava. **(iii)** ***Hypogynous:*** Position of ovary superior to other floral parts e.g., sunflower.
Cohesion (Fig. 11.8 a-c)	If number of carpels is more than one, they may be **(i)** ***Apocarpous:*** Carpels are free. Each carpel has its own style and stigma. e.g., rose. **(ii)** ***Syncarpous:*** Carpels are united, e.g., lady finger, tomato.
Number of locules in ovary	Vary from one to many **(i)** ***Unilocular:*** One locule, e.g., rose, pea. **(ii)** ***Bilocular:*** Two locules. e.g., datura. **(iii)** ***Multilocular:*** Many locules, e.g., lady's finger, China rose.
Placentation (Fig. 11.9 a-e)	**(i)** ***Marginal:*** The placenta forms a ridge along the ventral suture of the ovary and the ovules are borne on this ridge e.g., pea. **(ii)** ***Axile:*** The ovary is partitioned into several chambers or locules and the placentae are borne along the septa of the ovary. e.g., tomato, China rose. **(iii)** ***Parietal:*** The ovules develop on the inner wall of the ovary or on peripheral part. Ovary unilocular but in some cases becomes two chambered due to formation of a false septum. e.g., mustard. **(iv)** ***Free central:*** Ovules are borne on the central axis and septa are absent. e.g., carnation, chilly. **(v)** ***Basal:*** Placenta develops at the base of the ovary. e.g., sunflower.

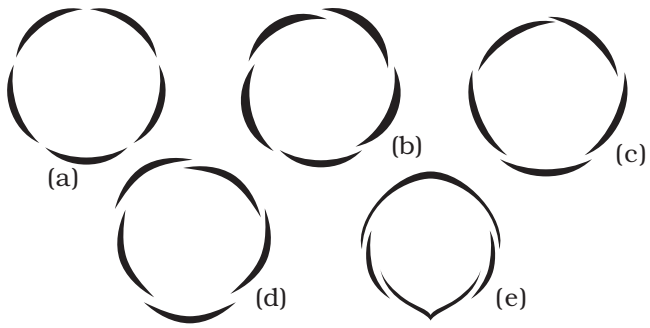


Fig. 11.1 Aestivation (a) Valvate (b) Twisted (c) Imbricate (d) Quincuncial (e) Vexillary

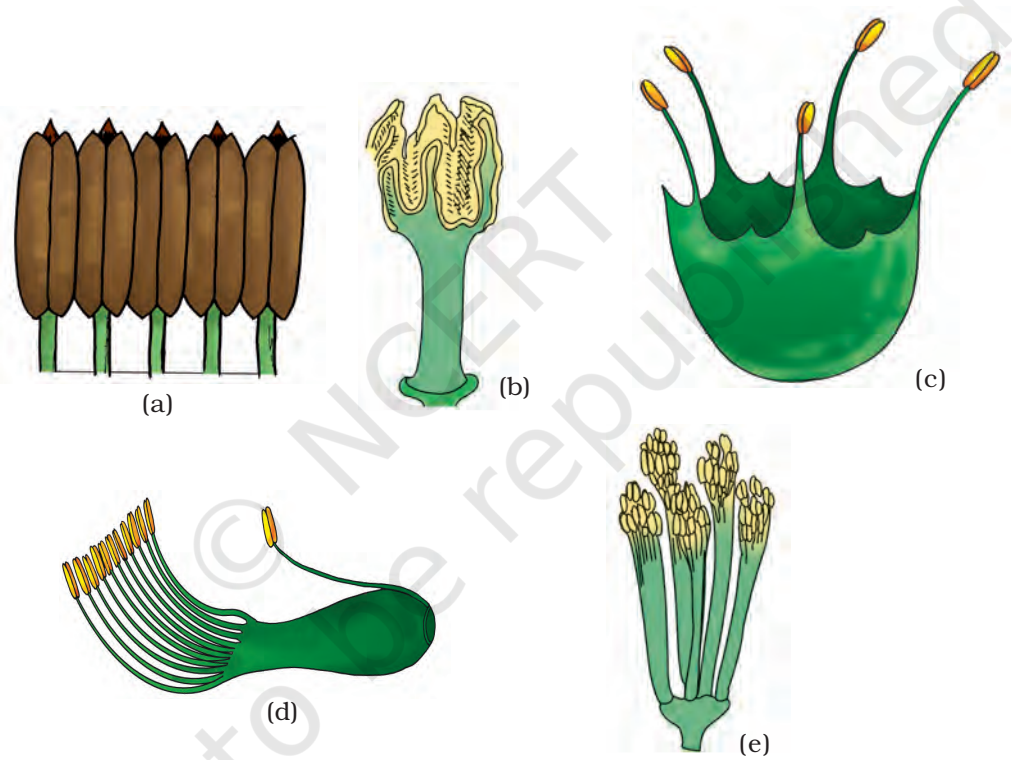


Fig. 11.2 Cohesion of stamens (a) Syngenesious (b) Synandrous (c) Monoadelphous (d) diadelphous (e) Polyadelphous

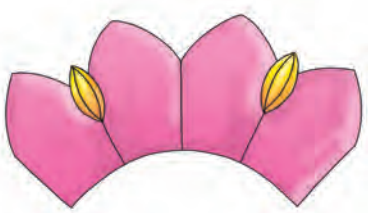


Fig. 11.3 Adhesion of Stamens-Epipetalous/Epiphyllous

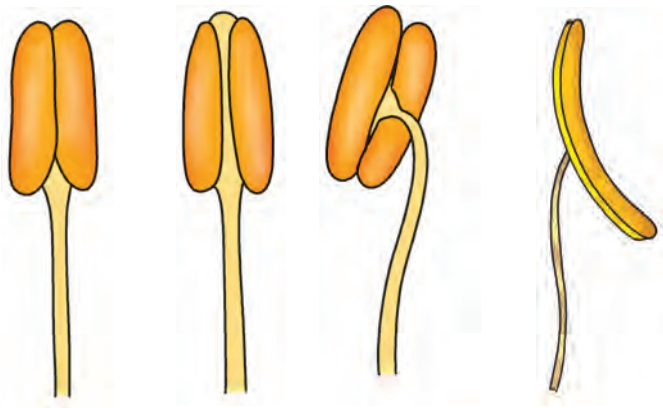


Fig. 11.4 Attachment of filament to anther (a) Basifixed (b) Adnate (c) Dorsifixed (d) Versatile

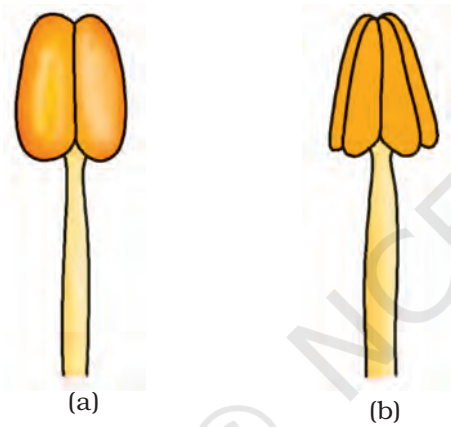


Fig. 11.5 Anther lobes (a) Dithecous (b) Monothecous

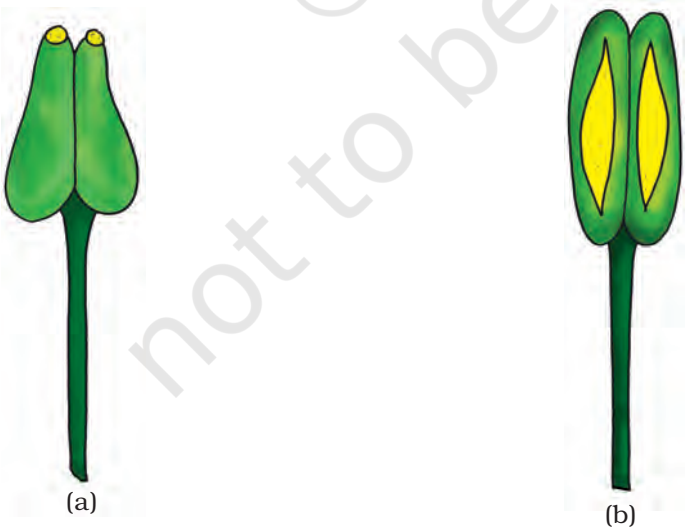
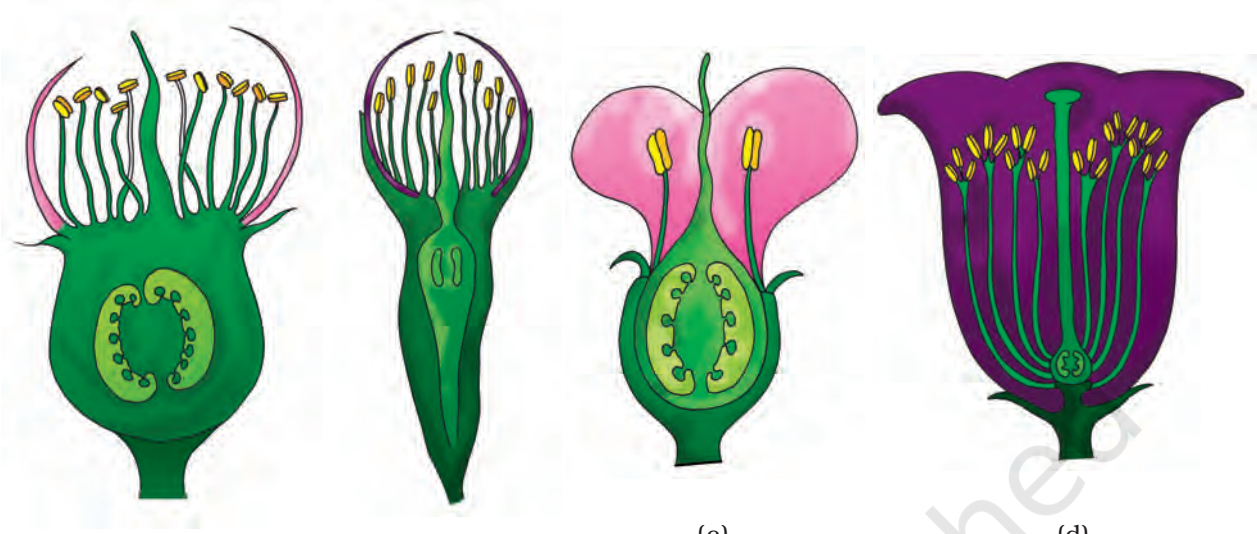


Fig. 11.6 Dehiscence pattern of anther (a) Porous (b) Longitudinal



(a) (b) (c) (d)

Fig. 11.7 Position of ovary (a) Epigynous (b-c) Perigynous (d) Hypogynous

(a) (b) (c)

Fig. 11.8 Cohesion of carpels (a) Apocarpous (b-c) Syncarpous

Fig. 11.9 Placentation (a) Marginal (b) Axile (c) Parietal (d) Free central (e) Basal

Annexure 1

Characteristics	*Solanum nigrum*(Makoi, Black night shade)	*Petunia alba*	*Lathyrus* sp.	*Pisum sativum*	*Asphodelus tenuifolius*
Habit	Herbaceous annual	Herbaceous annual	Herbaceous annual, climber	Herbaceous annual, climber	Herbaceous annual
Root	Tap root	Tap root	Taproot, the lateral roots may have nodules which contain nitrogen fixing *Rhizobium* bacteria	Taproot, the lateral roots may have nodules which contain nitrogen fixing *Rhizobium* bacteria	Fibrous root
Stem	Erect, herbaceous, branched, solid, cylindrical, green	Erect, herbaceous, branched, solid, cylindrical, green	Weak, cylindrical, branched, herbaceous, aerial, climbing with help of leaf tendrils, green	Weak, cylindrical, branched, herbaceous, aerial climbing with help of leaf tendrils, green	Very small but scape formed in reproductive phase
Leaf	Ex-stipulate, petiolate or sessile, simple, alternate, reticulate venation	Ex-stipulate, sessile, simple, alternate in the basal parts and opposite decussate in upper parts, reticulate venation	Stipulate (stipules foliaceous and in pairs), modified into a tendril, simple, alternate, reticulate venation	Stipulate (stipules large, ovate, foliaceous), petiolate, imparipinnately compound, (leaf lets 4 or 6) the common rachis ends in a branched tendril, terminal leaflet is always a tendril; alternate leaflets with reticulate venation	Fistular, slender
Inflorescence	Cymose	Solitary	Racemose	Racemose	Racemose

Characteristics	*Solanum nigrum*(Makoi, Black night shade)	*Petunia alba*	*Lathyrus* sp.	*Pisum sativum*	*Asphodelus tenuifolius*
Flower	Ebracteate, ebracteolate, pedicellate, complete, actinomorphic, bisexual pentamerous, hypogynous	Bracteate, ebracteolate, pedicellate, complete, actinomorphic, bisexual, pentamerous, hypogynous	Bracteate, bracteolate, pedicellate, complete, zygomorphic, bisexual pentamerous, hypo-or perigynous, papilionaceous	Bracteate, bracteolate, pedicellate, complete, zygomorphic, bisexual pentamerous, hypo-or perigynous, papilionaceous	Bracteate, ebracteolate, pedicellate, actinomophic, bisexual, trimerous, hypogynous
Calyx	Sepals 5, persistant, gamosepalous, green, valvate aestivation	Sepals 5, persistant, gamosepalous, green, valvate aestivation	Sepals 5, gamosepalous, ascending imbricate aestivation, odd sepal anterior, green	Sepals 5, gamosepalous, ascending imbricate aestivation, valvate aestivation, odd sepal anterior, green	
Corolla	Petals 5, gamopetalous, white, valvate aestivation	Petals 5, gamopetalous, white/purple, valvate aestivation	Petals 5, polypetatous papilionaceous (The 5 petals are unequal and have a bilateral symmetry. The posterior or outer most largest petal is called **standard,** the lateral pair of petals which are clawed are called the **wings** and the two anterior petals are united to form the **keel** i.e., 1+2+2 arrangement), which encloses the stamens and the carpel descending imbricate (vexillary) aestivation	Petals 5, polypetatous papilionaceous (The 5 petals are unequal and have a bilateral symmetry. The **posterior** or outer most largest petal is called **standard**, the lateral pair of petals which are clawed are called the **wings** and the two anterior petals are united to form the **keel** which encloses the stamens and the carpel, i.e., 1+2+2 arrangement), descending imbricate (vexillary) aestivation	Perianth tepaloid, tepals 6 in two whorls of 3 each (3+3), free, valvate aestivation

Characteristics	*Solanum nigrum*(Makoi, Black night shade)	*Petunia alba*	*Lathyrus* Sp.	*Pisum sativum*	*Asphodelus tenuifolius*
Androecium	Stamens 5, epipetalous, alternate with corolla lobes, polyandrous, anthers dithecous, introrse, dehiscence by apical pores	Stamens 5, epipetalous, alternate with corolla lobes, filaments unequal, polyandrous, anthers basifixed, dithecous, introrse, dehiscence by apical pore	Stamens 10 arranged in a single whorl, diadelphous, (9+1 arrangement, 9 unite at the base and form a tube around the ovary and the 10th posterior stamen is free) anthers basifixed, dithecous, introrse, longitudinal dehiscence	Stamens 10 arranged in a single whorl, diadelphous, (9+1 arrangement, 9 unite at the base and form a tube around the ovary and the 10th posterior stamen is free) anthers basifixed, dithecous, introrse, longitudinal dehiscence	Stamens 6 in 2 alternate whorls of 3 each, epiphyllous opposite to tepals, basifixed, dithecous. introrse, dehiscence by longitudinal slits
Gynoecium	Bicarpellary syncarpous, ovary superior, bilocular, ovary obliquely placed in the flower, ovules many per locule, axile placentation, placenta swollen,	Bicarpellary syncarpous, ovary superior, bilocular, ovary obliquely placed in the flower, ovules many per locule, obliquely transverse septum, axile placentation, placenta swollen,	Monocarpellary, ovary superior, unilocular, ovules many, placentation marginal,	Monocarpellary, ovary superior, unilocular, ovules many, placentation marginal	Tricarpellary syncarpous, ovary superior trilocular, two ovules in each locule, axile placentation,
Fruit	Berry	Capsule	Legume	Legume	Berry
Floral formula	Ebr, Ebrl, ♂,Å, $K_{(5)}\ \overset{\frown}{C_5\ A_5}\ G_{\underline{(2)}}$	Ebr, Ebrl, ♂,Å , $K_{(5)}\ \overset{\frown}{C_5\ A_5}\ G_{\underline{(2)}}$	Br, brl, ♂, %, $K_5\ C_{1+2+2}\ A_{(9)+1}\ G_{\underline{1}}$	Br, brl, ♂, %, $K_5\ C_{1+2+2}\ A_{(9)+1}\ G_{\underline{1}}$	Br, Ebrl, ♂,Å, $\overset{\frown}{P_{(3+3)}\ A_{3+3}}\ G_{\underline{(3)}}$

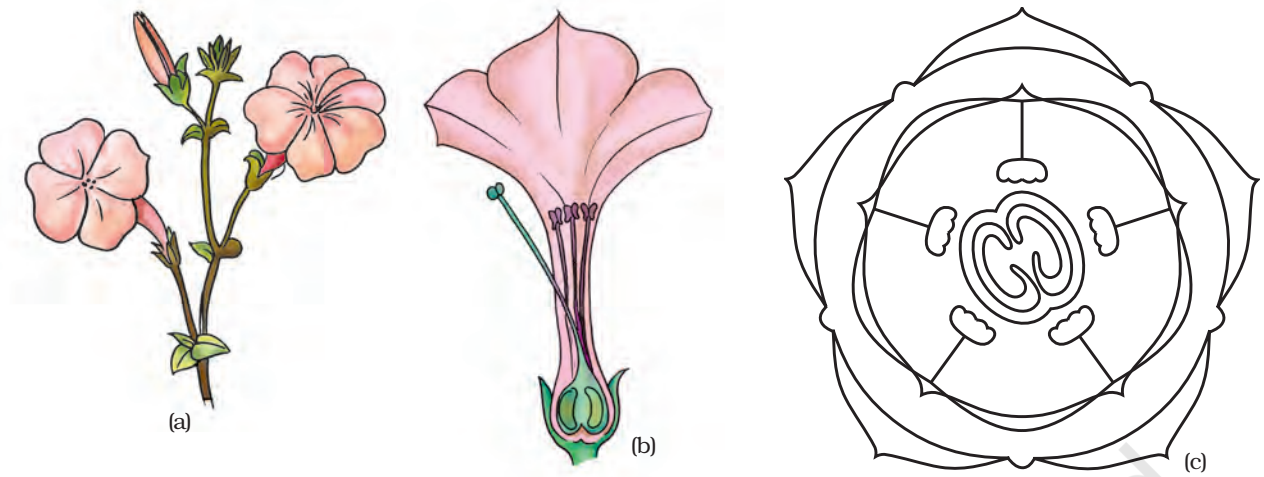


Fig. 11.10 *Petunia* (a) A twig (b) LS of flower (c) Floral diagram

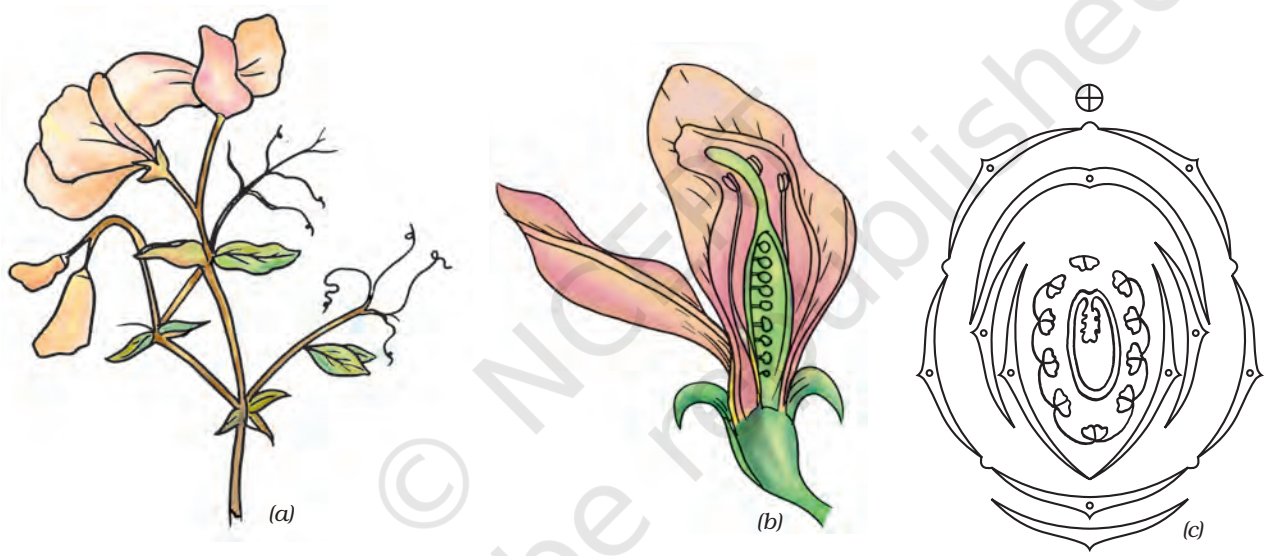


Fig. 11.11 *Lathyrus* (a) A twig (b) LS of flower (c) Floral diagram

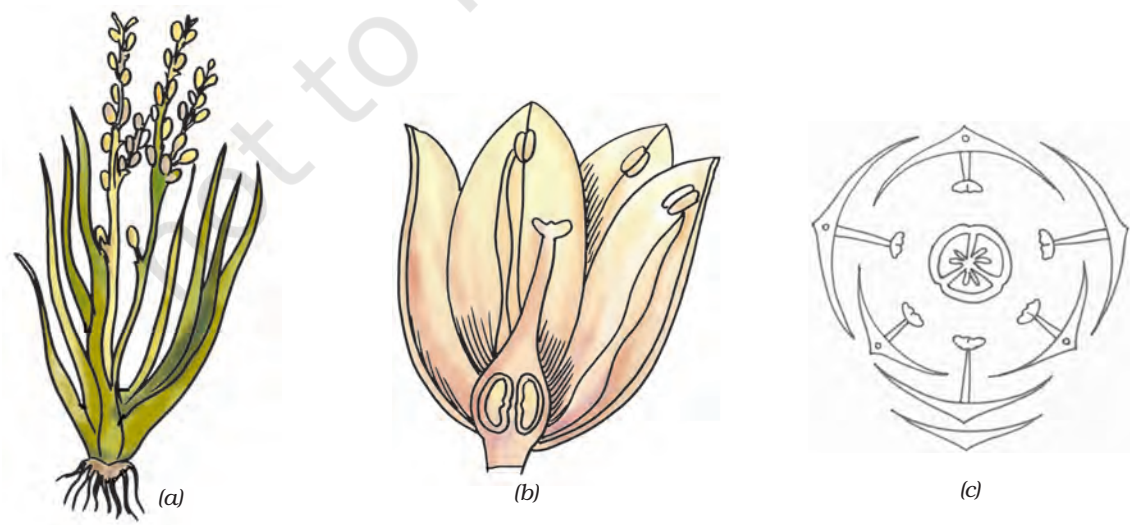


Fig. 11.12 *Asphodelus* (a) A twig (b) LS of flower (c) Floral diagram

Annexure 2

Other Examples

Family : Solanaceae	Family : Fabaceae	Family : Liliaceae
Physalis *Solanum xanthocarpum* *Solanum melongena* *Solanum tuberosum* *Nicotiana tabacum* *Hyocyamus* *Atropa belladonna* *Withania somnifera* *Cestrum nocturnum* *Datura*	*Phaseolus moong* (Urad) *P. vulgaris* (Kidney bean, French bean) *P. aureus* (Moong) *Trigonella* (Fenugreek) *Cajanus cajan* (Arhar, pigeon pea) *Dolichos lablab* (Sem, Hyacinth bean) *Cicer arietinum* (chana, gram, chickpea *Indigofera* (Indigo) *Abrus* (Ratti) *Arachis hypogea* (groundnut) *Medicago sativa* (Alfalfa)	*Allium cepa* (onion) *Gloriosa superba* *Aloe barbendesis* *Heterosmilax* *Asparagus officinale* *Yucca gloriosa* *Lilium candidum* *Smilax spp*

Identification and systematic Position- Family : Solanaceae

1.	Leaves reticulate venation, flowers tetra or pentamerous, tap root system.	**Dicotyledons**
2.	Petals fused,	**Gamopetalae**
3.	Ovary superior, carpels usually two, stamens alternate with the corolla lobes, number of stamens equal or fewer to the number of corolla lobes.	**Bicarpellatae**
4.	Herbs or twiners, leaves alternate, flowers actinomorphic, stamens epipetalous, ovary superior two carpels, bilocular, axile placentation, ovules few or many in each carpel.	**Polemoniales**
5.	Herbs and shrubs, leaves simple, alternate, gamosepalous, stamens 5, epipetalous, ovary superior, bicarpellary syncarpous, bilocular, sometimes four locules due to false septum, many ovules in each locule, swollen placenta, ovary obliquely placed in the flower, axile placentation, fruit a berry or a capsule.	**Solanaceae**

Identification and systematic Position - Family : Fabaceae

1.	Leaves with reticulate venation, flowers tetra or pentamerous, tap root system.	**Dicotyledons**
2.	Petals free or not united.	**Polypetalae**
3.	Flowers hypo or perigynous; regular or irregular (vexillary).	**Calyciflorae**
4.	Flowers zygomorphic and *papilionaceous*, descending imbricate aestivation of corolla, 1 standard, 2 wings and 2 keels; stamens10, mono or diadelphous (9+1) ovary superior, marginal placentation, ovules many.	**Fabaceae**

Identification and systematic Position - Family : Liliaceae

1.	Leaves usually with parallel venation, flowers trimerous, fibrous root system, embryo with one cotyledon	**Monocotyledonous**
2.	Ovary superior, trilocular, 6 tepals in 2 whorls of 3+3, petaloid	**Coronariae**
3.	Perianth petaloid, 6 tepals free or connate below. stamens 6 in two whorls of 3+3, opposite to tepals, epiphyllous, ovary tricarpellary, syncarpous, trilocular, 2 or more ovules per locule fruit 3 celled berry or capsule.	**Liliaceae**

Exercise 12

Aim: To study anatomy of stem and root of monocots and dicots.

Principle: The study of internal morphology, i.e., cells of various tissues in an organ of a living body is called Anatomy. Tissue, which is a group of cells performing a common function, may be simple (parenchyma, collenchyma and sclerenchyma) or complex containing more than one type of cells (xylem and phloem). The tissues may be temporary (meristematic) or permanent (sclerenchyma, parenchyma, collenchyma).

The internal organisation of these tissues differ in root, stem and leaves. These differences are given in tabular form for easy identification. Various tissues which constitutes roots and stems are described briefly.

Requirement: Samples of stem and root of sunflower, *Cucurbita*, maize, *Canna*, etc., or any other locally available plant, safranin stain, dilute acid water, glycerine, watch glass, slide, cover slip, brush, razor/scalpel blade, blotting paper, microscope.

Procedure

- Collect a few thin green branches of recent growth (i.e., non-woody/ herbaceous without any secondary growth) from the examples given above, preferably of the thickness of a tooth-pick.
- Use pith of potato piece/*Calotropis* stem/raw papaya fruits for embedding the material to be sectioned. It is advisable to first stain roots before sectioning. If material is thick like that of maize, it can be directly sectioned without embedding them in pith.
- Hold the material between the thumb and index finger in such a way that the tips of the finger and smooth cut surface of the material are in a line, while the tip of the thumb is just a few mm below the upper surface of the material.
- Wet the surfaces of razor blade/scalpel blade.
- Carefully move the blade horizontally over the surface of material in quick succession in a manner that a very thin and complete slice of the material is cut and obtained over the surface of razor blade.
- After cutting several sections in this manner, transfer all these into a watch glass containing water.

- Make a visual observation of the sections cut and pick the thinnest possible and complete sections from the lot and transfer it into a watch glass containing safranin and allow these to remain there for about 2 mins.
- With the help of a brush gently transfer the section into another watch glass containing water to remove excess of safranin stain. Keep the material for few minutes and transfer it into a watch glass containing a few drops of dilute acid in water to remove excess of safranin stain. Wash with water and transfer the section on to a clean slide containing 1 drop of glycerine. Place a cover slip over it avoiding air bubbles.

Observation

Note all tissues which are lignified (as in sclerenchyma, collenchyma) are stained red with safranin. Observe the outline of the cut sections. Make a note of the presence and composition of various tissues (epidermis, cortex, endodermis, pericycle, vascular bundle) and characteristics of vascular bundle. List the differences between root and stem of monocots and dicots. Use the information given in Annexure 3 for identification.

Anatomically root differs from stem by the following points:

S.No.	Root	Stem
1.	Cuticle absent	Cuticle present
2.	Epidermis does not have stomata	Epidermis contains stomata
3.	Unicellular root hairs present	Epidermal hairs are usually multicellular
4.	Collenchyma absent	Collenchyma present
5.	Green plastids absent (achlorophyllous)	Green plastids present (chloropyllous)
6.	Vascular bundles are radial in arrangement (xylem and phloem are on different radii)	Vascular bundles are conjoint and collateral in arrangement (xylem and phloem are on the same radius)
7.	Xylem development is centripetal and protoxylem is exarch, i.e., lies towards the periphery	Xylem development is centrifugal and protoxylem is endarch, i.e., lies towards the center

Annexure 3

Anatomy of the Root

The most distinguishing anatomical characters of the root are:

1. **Epidermis:** It is the outer most layer of thin walled parenchymatous cells with many unicellular root hairs. It does not have stomata and cuticle.
2. **Cortex:** It is multilayered and well developed. The cells are thin walled, parenchymatous and may contain leucoplasts. The intercellular spaces are well developed. Collenchyma is absent. The inner most layer of the cortex is called **endodermis**. The endodermis is a definite ring like layer consisting of barrel shaped cells compactly arranged without any intercellular spaces. **Casparian** thickenings in the form of strips are present on the radial and inner walls of the endodermal cells. Also, **passage cells** are present. The passage cells are thin walled and are usually located opposite the protoxylem.
3. **Pericycle:** The outer most layer of the **stele** (vascular tissue) is called pericycle. It is single layered and consists of compactly arranged thin walled parenchymatous cells with no intercellular spaces. The pericycle cells alternate with the endodermal cells suggesting that these two layers differ in their origin. The endodermis is derived from periblem initials of the apical meristem, whereas the pericycle is derived from the pleurome initials. Pericycle encloses the vascular system.
4. **Vascular system:** Bounded by the endodermal and pericycle layers, vascular system consists of xylem, phloem and the associated parenchyma tissue called conjunctive tissue.

   The vascular bundles are arranged in a ring. The bundles are radial and there are equal number of separate bundles of xylem and phloem. The number of xylem and phloem bundles varies from two to six (diarch, triarch, tetrarch, pentarch, and hexarch) in dicots and more than six, i.e., polyarch in monocots.

   The xylem consists of **protoxylem** which lies towards periphery and **metaxylem** which lies towards the centre or pith. This type of arrangement of xylem is called **exarch** (protoxylem is exarch in root and endarch in shoot). The protoxylem consists of annular and spiral vessels with narrow lumen (in cross section) and the metaxylem consists of reticulate and pitted vessels with broad lumen. (Recall the xylem maceration experiment)

   The phloem consists of sieve tubes, companion cells and phloem parenchyma.

   The parenchyma present in between the xylem and phloem bundles is known as **conjunctive** tissue.
5. **Pith:** It occupies the central area and may be large, small or even, absent. Generally in dicot roots the pith is small or absent. Total obliteration of pith occurs sometimes when metaxylem elements grow and meet in the centre. In monocot roots pith is large in size. Pith consists of parenchymatous cells with intercellular spaces.

Anatomy of the Shoot

The central ascending portion of the plant axis is called the shoot. It develops from the plumule of the embryo. The shoot bears lateral appendages called leaves.

The anatomical feature of stem are:

1. **Epidermis:** It is the outermost layer of cells, generally parenchymatous rectangular in shape. Multi-cellular **trichomes** or epidermal hairs, (no epidermal hairs in monocots) are generally present. The epidermis has an outer layer of cuticle made up of waxy material.
2. A multilayered **hypodermis** is present just below the epidermis. The hypodermis is generally collenchymatous in dicots and sclerenchymatous in monocots.
3. Cortex and pith are well defined in cases of dicots whereas in monocots only ground tissue is present. In dicots well defined endodermis and pericycle below the cortex are present. In monocots the endodermis is present around each vascular bundle. Distinction into cortex, pericycle, and pith is not seen. Vascular bundles are present in the ground tissue.
4. Each vascular bundle consists of xylem, phloem, cambium (absent in case of monocots) and associated parenchyma tissue. The vascular bundles are conjoint and collateral. They are open (i.e., cambium present between xylem and phloem) in dicot stems and thus show the secondary growth. Cambium is absent in monocot stems and therefore there is no secondary growth with a few exception.

   The vascular bundles are arranged in a ring in dicots whereas they are scattered in ground tissue in monocots. Each vascular bundle is surrounded by a sclerenchymatous bundle sheath.

   The vascular bundles are usually of equal size in dicots whereas in monocots they are of unequal size. In monocot stem the bundles near the periphery or closer to epidermis are smaller in size and the bundles nearer to the center are larger in size.
5. The protoxylem is endarch, i.e., towards the centre. The phloem consists of sieve tubes, companion cells and phloem parenchyma.

   In dicot stems, in between the xylem and phloem of the vascular bundle a procambium strip of 2-3 cells thickness (fascicular cambium) is present. The procambium between two adjacent vascular bundles is called interfascicular cambium. In young stems the cambial strips are confined only to the vascular bundles but as the stem becomes older, the interfascicular cambium develops and a continuous ring of cambium is formed. The secondary growth (formation of secondary phloem and secondary xylem) is due to the activity of cambium.
6. In dicot stem the central region of the stem is called pith (medulla). The pith consists of thin walled parenchymatous cell with intercellular spaces. The pith is well developed in dicot stem whereas in monocots it is absent.

From the anatomical point of view the monocot and dicot roots differ from each other in the following features (Fig. 12.1 and 12.2):

S. No.	Monocot Root	Dicot Root
1.	Polyarch condition	Diarch to hexarch (2-6 vascular bundles) condition
2.	Pith well developed	Pith is very small or absent
3.	Secondary growth absent	Secondary growth occurs due to the activity of vascular cambium

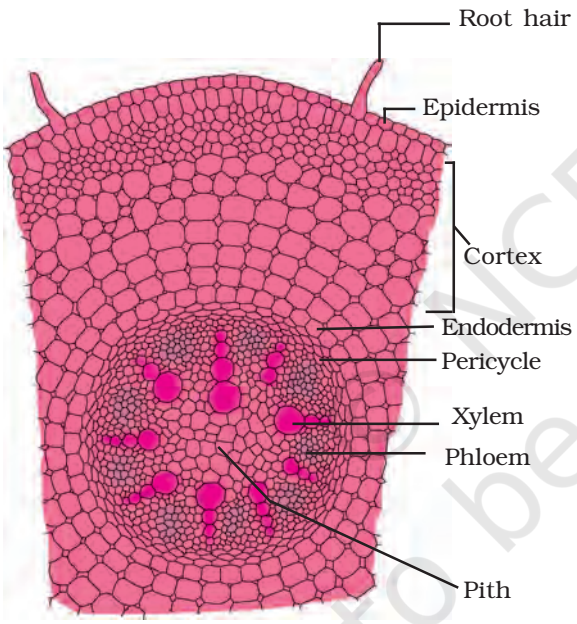


Fig. 12.1 TS of a monocot root

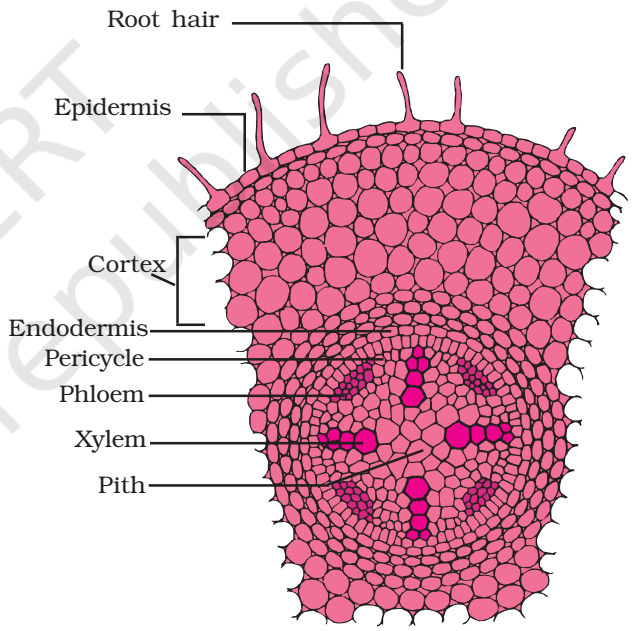


Fig. 12.2 TS of a Dicot root

A few examples of dicotyledonous and monocotyledonous roots which can be selected for anatomical study are given in the following table.

Dicotyledonous Roots	Monocotyledonous Roots
Phaseolus radiatus	*Canna*
Ranunculus	*Zea mays*
Cicer	*Smilax*
Ficus	*Allium cepa*

Anatomically, the dicot and monocot stems differ in the following features (Figs. 12.3 and 12.4):

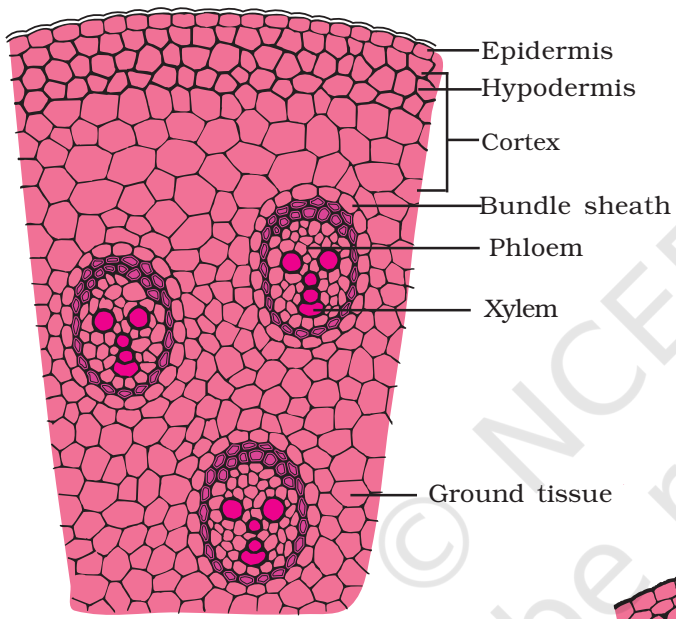


Fig. 12.3 TS of a monocot stem

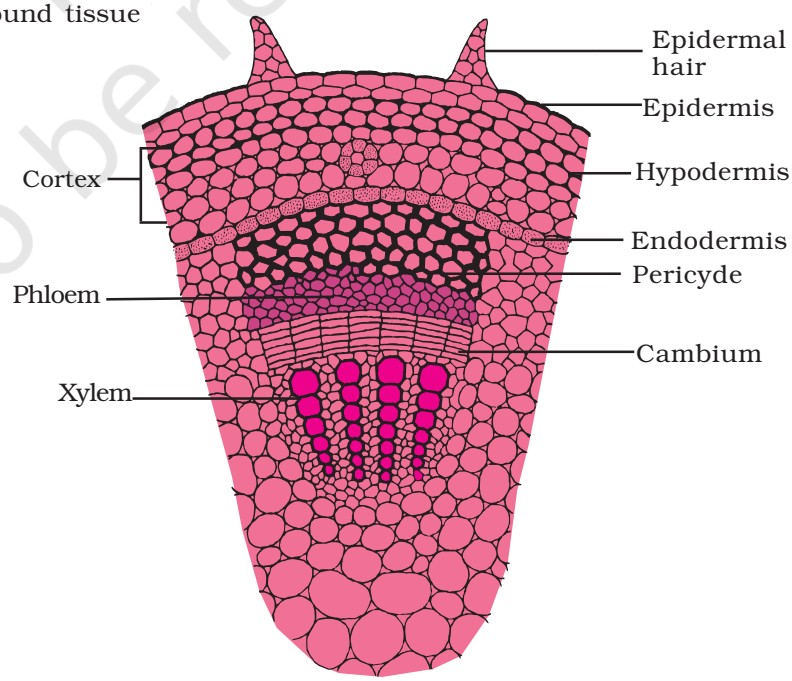


Fig. 12.4 TS of a dicot stem

S. No.	Monocot Stem	Dicot Stem
1.	Epidermis single layered and no epidermal hairs	Epidermis single layered and epidermal hairs are present
2.	Hypodermis sclerenchymatous	Hypodermis collenchymatous
3.	The vascular bundles are scattered in arrangement	The vascular bundles are arranged in a ring
4.	The vascular bundles at the periphery are smaller in size than those at the center	The vascular bundles are of the same size
5.	The vascular bundles are conjoint, collateral and closed; the sclerenchymatous bundle sheath is present; the vessels are arranged in V- or Y-shape; water cavity is present	The vascular bundles are conjoint, collateral and open; the bundle sheath is absent; the vessels are arranged in rows; water cavity is absent
6.	Only ground tissue is present	A well defined cortex, endodermis, pericycle and pith are present

A few typical dicotyledonous stems and monocotyledonous stems that can be selected for study of anatomical are given in the following table.

Dicotyledonous Stems	Monocotyledonous Stems
Helianthus (sunflower)	*Zea mays* (maize/corn)
Tinospora	*Canna*
Ricinus (castor)	*Asparagus*
Xanthium	*Cynodon dactylon* (*Doob* grass)

Questions

1. Arrange the following sequentially as you would see in a TS of a dicot stem-pericycle, epidermis, pith, cortex, xylem, phloem.
2. Where do you find radial, conjoint, collateral and open vascular bundles?
3. What type of xylem arrangement would be seen in TS root of lily plant?
4. Which part of dicot stem is meristematic?

23-04-2018

Exercise 13

Aim: Preparation of herbarium sheets of flowering plants.

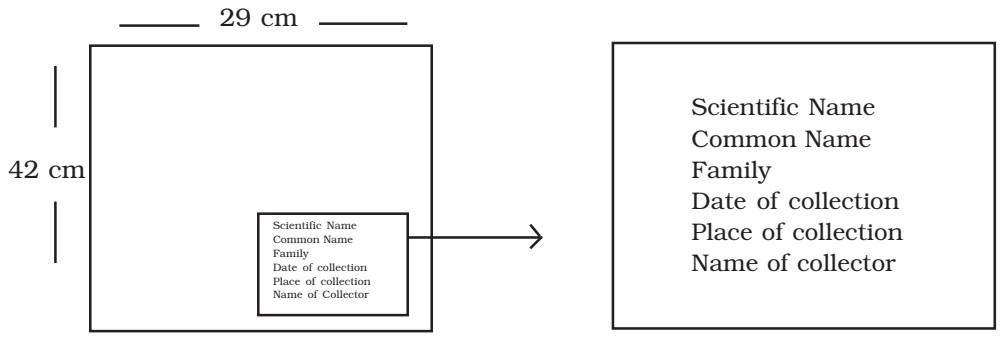
Principle: Taxonomists preserve plant specimen in dry state by mounting it on a thick sheet of paper 42 × 29cm size. Such a mounted sheet is called **herbarium sheet**. The place (Laboratory/Institute) where herbarium sheets are systematically stored is called **herbarium**. The primary function of a herbarium is to facilitate identification of plants and also aid in taxonomic research.

Requirement: A sharp knife, blotting sheets or old newspapers, 2% formalin solution, tray, forceps, thick white card sheets cut to 42 × 29cm size, gloves, field press, rope, gum/quick fix/needle and thread.

Procedure

- Collect twigs of a few wild plants (herbs, shrubs or trees) 20-25cm long with leaves and flowers. Record in a field diary its name, date and place of collection.
- Dip the specimens for 2-3 minutes in 2% formalin solution taken in a tray (wear gloves). Rinse the plant gently to remove excess formalin and blot to remove the fomalin traces.
- Keep the plant twig between folds of another blotting paper/ newspaper.
- Spread the plant on the sheet in a manner that at least ventral surfaces of 1-2 leaves are facing upwards and no overlapping of foliage or flowers occur. Likewise spread the attached flower(s) in a manner that all the floral parts of at least one flower are widely spread. If the plant is too long, fold it at one or two places so as to accommodate it on a single sheet.
- Repeat the process for the other plants you have collected. Keep the specimens in separate sheets.
- Place the sheets with plant specimens in a field press and secure it tightly with a rope. If a field press is not available, place the sheets under a heavy weight or box.
- Next day carefully transfer the plants to dry blotting sheets/old news papers and tie them up in the field press.
- Repeat this process of transferring the specimens to fresh sheets for a few days till specimens are dry.

- Mount the dried plant specimen on a herbarium sheet with gum or stitch it to the sheet at a few places with a needle and white sewing thread.
- Fill in the herbarium label with the details of the plant specimen and mount it on the right hand side bottom corner as shown in figure.



Prepare the following data of the plants you have collected:

Name of plant	Common name	Family	Time and place	Economic uses	Any threats

Note: There are many international, national, regional and local herbaria. The Royal Botanic Gardens, Kew, London, is one of the most prestigious international herbarium, where several thousand of herbarium sheets are preserved. In our country, the Botanical Survey of India (BSI) has regional herbaria located at Coimbatore, Pune, Dehradun, Kolkata and Lucknow. Many universities and colleges have small herbaria. It is worthwhile to visit a herbarium with your teacher.

23-04-2018

Exercise 14

Aim: Study of external morphology of animals through models.

Principle: The external morphology of any organism is normally studied using either their preserved (in formalin or ethanol) or stuffed specimens. Use of models for the purpose of its study becomes very important and relevant for such animals, which are rare, endangered or even extinct.

It is needless to mention that every fine detail of the external features of an organism is depicted in the model. Not only this, a magnified image of a few otherwise smaller or minute parts are also sometimes depicted in the model. In order to have an idea of the exact size of the animal, a scale is desired to be given on the platform on which the model is mounted.

Requirement: Model of the animal to be studied, note book, pencil and eraser

Procedure

For this purpose, take a model of the animal and observe its features and note down in your practical record notebook. It is always desirable to study the models of both male and female specimens that show sexual dimorphism. Also draw a labelled diagram of the animal.

Earthworm

The external features of earthworm (*Pheretima posthuma*) are as follows:

(i) Narrow, cylindrical and elongated body measuring approximately 150mm in length and 3 to 5 mm in diameter. The anterior end of the body is pointed whereas the posterior end is slightly depressed or blunt.

(ii) The dorsal surface of the body is darker than the ventral surface. Besides this, a median dark line due to underlying dorsal blood vessel is also visible on the dorsal surface all along the length of the body.

(iii) Entire body is divisible into more than 100 segments of almost equal size. These are called **metameres** (Fig. 14.1).

(iv) Mouth is situated anteriorly in the first metamere called the **peristomium**.

(v) Anus is situated at the tip of the last metamere.

(vi) In the adult earthworm, the skin or body wall around the segments 14th to 16th is comparatively thick, and the segmentation is not conspicuous. This thickened region is called **clitellum**.

(vii) The animal is hermaphrodite. Female and male genital apertures are present ventrally in the 14th and 18th segments respectively. The female genital aperture is situated mid ventrally, whereas the two male genital apertures are ventro-lateral in position.

(viii) A pair of genital papillae is also present ventrolaterally in the 17th and the 19th segment just above and below the male genital apertures.

(ix) On the ventral surface itself are four pairs of openings of the **spermatheca** situated ventrolaterally in the grooves between 5/6, 6/7, 7/8 and 8/9 segments.

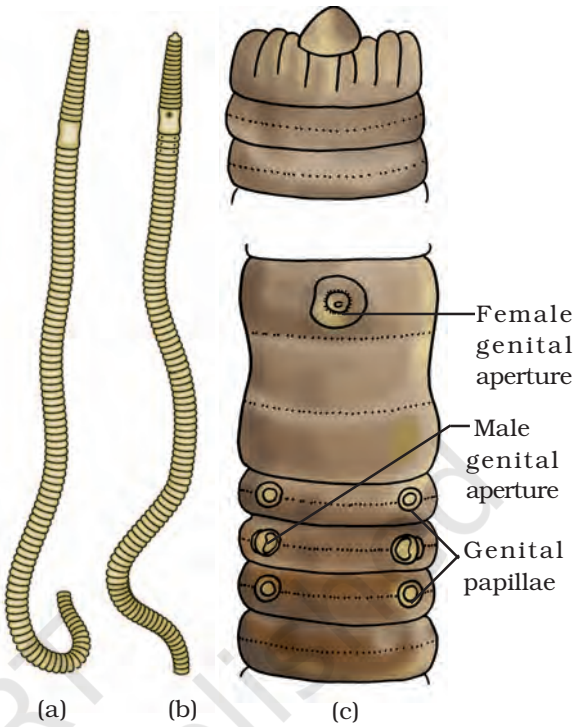


Fig. 14.1 External morphology of earthworm (a) Dorsal (b) Ventral (c) Details in magnified view

Cockroach

The following external features may be observed in the model:

(i) Body is dorsoventrally flattened, and bilaterally symmetrical. The body is covered externally by chitinous plates called **sclerites**. The dorsal sclerites are called ***tergum,*** while the ventral ones are called ***sternum***.

(ii) Body is distinctly divisible into three parts, i.e., head, thorax and abdomen (Fig. 14.2).

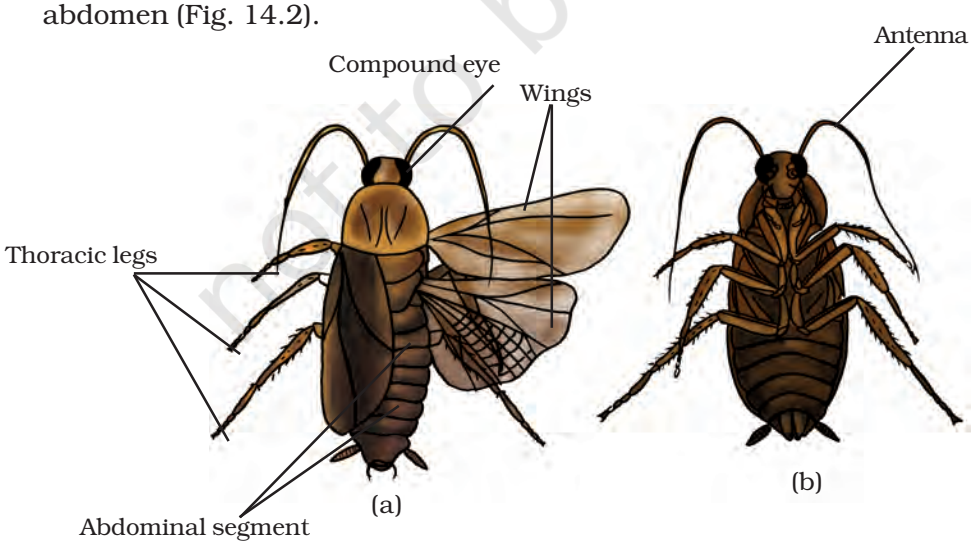


Fig. 14.2 External morphology of cockroach (a) Dorsal view (b) Ventral view

(a) Head: It is a triangular structure attached movably with the thorax, and is oriented perpendicular to the body axis. Head consists of six chitinous plates, all fused together. A pair of large compound eyes, and segmented long antennae (singular: antenna) are situated laterally on the head. The antennae are present very close to the eyes. Ventrally, an opening called mouth is present on the head that remains surrounded by the mouth parts consisting of a pair of **mandibles**, **first maxillae**, **labium or fused second maxillae, hypopharynx** and **labrum** (Fig. 14.3).

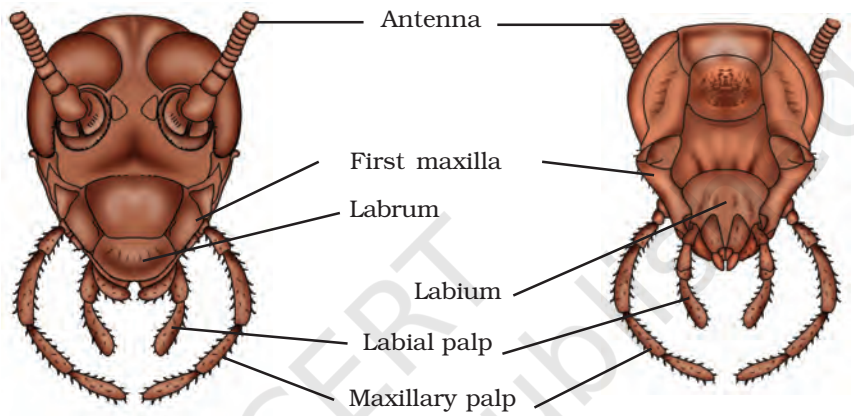


Fig. 14.3 External morphology of cockroach

(b) Thorax: It has three segments, i.e., **prothorax, mesothorax** and **metathorax.** The prothorax is larger than the rest. From each of the thoracic segment a pair of jointed legs arises ventrally. The leg segments are **coxa, trochanter, femur, tibia** and **tarsus**, which consist of five jointed tarsomeres (Fig. 14.4). Two pairs of wings are present, of which one pair is attached to the mesothoracic and the other pair to the metathoracic segments. Both pairs of the wings together cover the entire body segments starting from mesothorax. The forewing called **tegmina** is thick as well as feathery and it covers the thin and membranous hind wing while at rest.

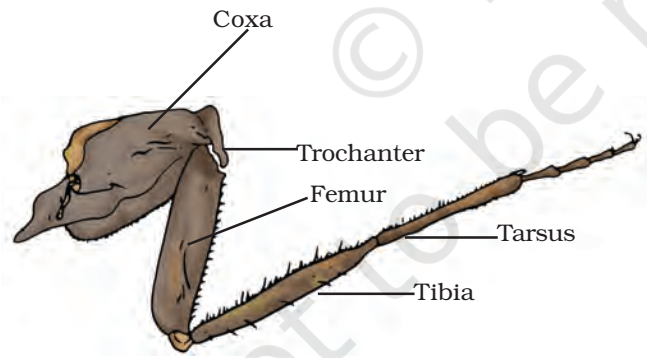


Fig. 14.4 Leg of cockroach showing different parts

(c) Abdomen: It consists of ten segments. The last four segments are slightly narrower; hence the posterior end of the abdomen looks tapering. Dimorphism of male and female is distinct in the posterior abdominal segments. In females, the seventh sclerite of abdominal segment overlaps the eighth and the ninth sclerites and together

form a boat shaped genital pouch ventrally. On the other hand in males, only the seventh segment overlaps the eighth segment. A pair of long and jointed **anal cerci** is present laterally on the tenth segment of both male and female. The males have an additional pair of filamentous **anal style,** attached ventrally to the ninth segment (Fig. 14.5).

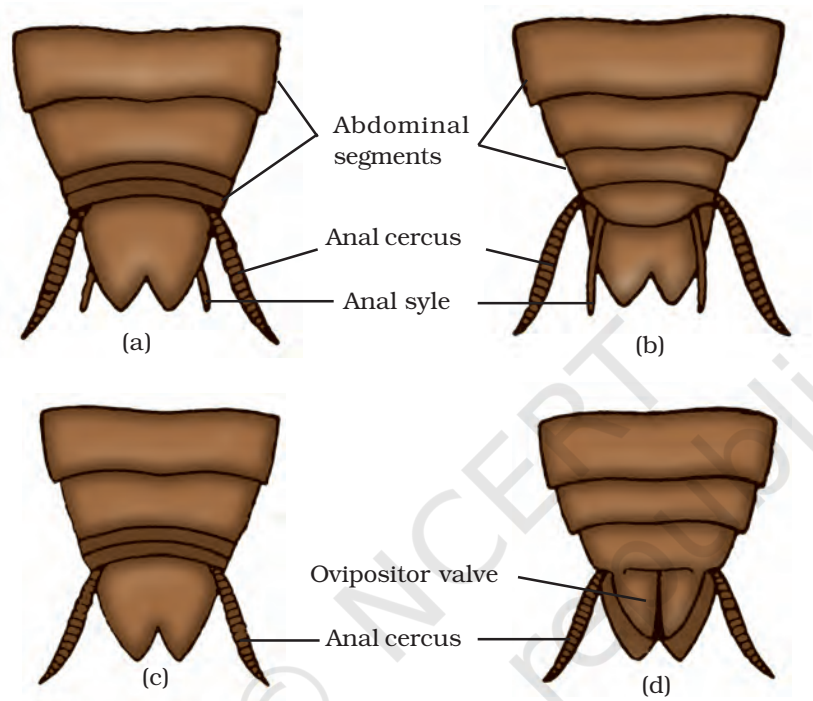


Fig. 14.5 Posterior abdominal segments of cockroach
(a) Male dorsal view (b) Male ventral view
(c) Female dorsal view (d) Female ventral view

Frog

The following external features may be observed in the model.

(i) Body is bilaterally symmetrical and streamlined (Fig. 14.6). Head is triangular in shape. On the dorso-lateral margins of head, is present a pair of bulging eyes. Nictitating membrane is present to cover the eyeball. Behind the eyes a distinct pair of circular patch of skin, called **tympanic membrane**, is present. Mouth is beset with upper and lower jaws. Mouth opens into the buccal cavity in which a posteriorly attached

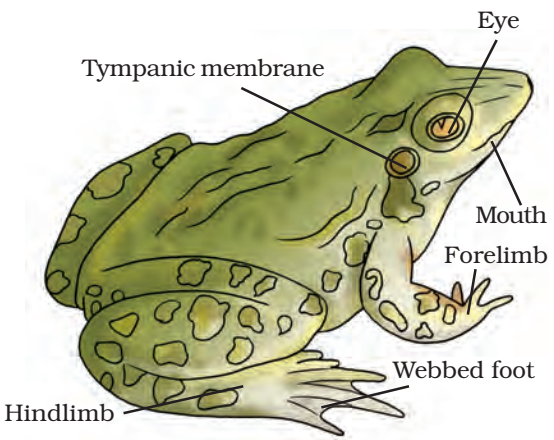


Fig. 14.6 External morphology of frog

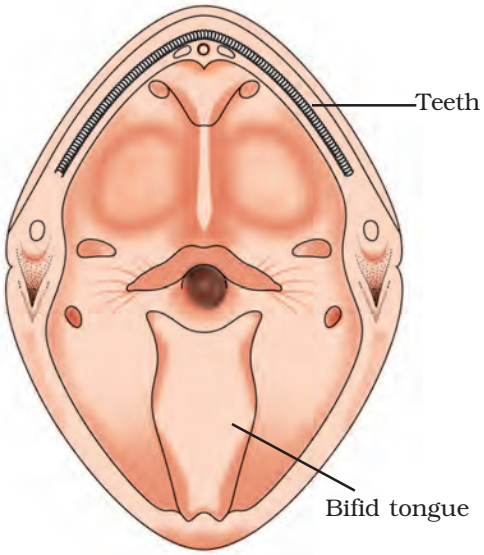


Fig. 14.7 Buccal cavity of frog

bifid and highly muscular tongue is present. Upper jaw contains rows of pointed teeth. Lower jaw is toothless. Dorsally on the snout, a pair of nostrils are present (Fig. 14.7).

(ii) Behind the head, a wide and long trunk is present. Neck is absent. At the posterior end of the trunk a **cloacal** aperture is present. There is no tail. Trunk is provided with two pairs of limbs i.e. the forelimb and the hindlimb. The forelimbs are smaller in length than the hind limbs. Forelimb is differentiated into upper arm, forearm, and a hand with four small digits without claws. The hindlimb consists of a thigh, shank and a foot with five long and webbed digits (Fig. 14.8).

(iii) There is a distinct sexual dimorphism between male and female frog. Males are comparatively smaller in size and the base of the first digit of the forelimb becomes thick and pad like during breeding season only. This is called **nuptial pad** (Fig. 14.8), which helps in holding the females during mating. Males also have a pair of prominent vocal sacs on the ventral surface of lower jaw to produce croaking sound.

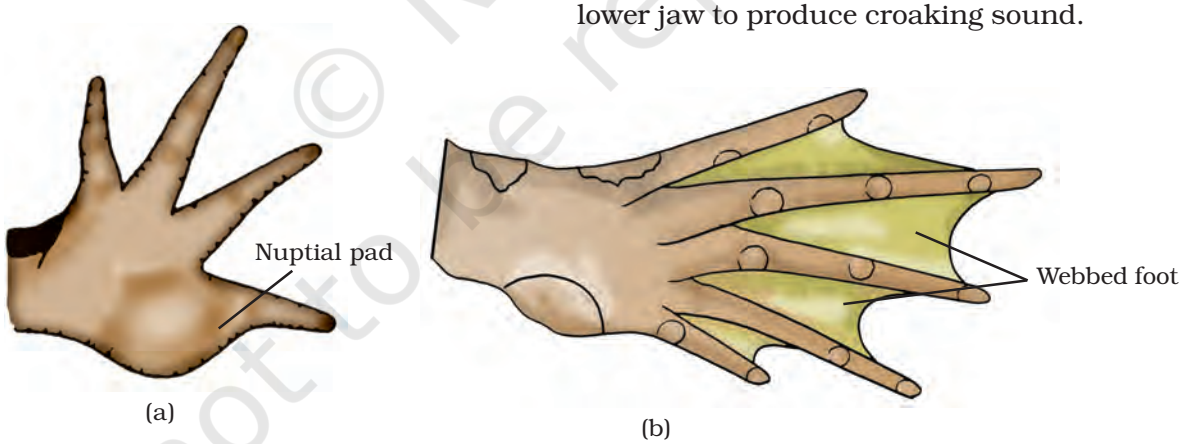


Fig. 14.8 Digits of the limbs of frog (a) Nuptial pad of fore limb (b) Webbed foot of hind limb

Questions

1. What are important significances of study of an organism through its model?

Exercise 15

Aim: To demonstrate osmosis by potato osmometer.

Principle: Osmosis is a common physical process observed in living cells and tissues of all organisms. It is defined as the movement of molecules of solvent from a region of its higher concentration to a region of its lower concentration across a selectively permeable membrane, such as the plasma membrane.

Requirement: Fresh large sized potato tuber, beaker, 20% sucrose solution, water, petridish blade/scalpel, bell pin needle marked with waterproof ink.

Procedure

- Cut the potato tuber into two equal halves with a razor blade or scalpel. Peel off the outer skin. As the shape of the tuber is irregular, shape the two halves in squares.
- Scoop from the centre of the tuber the soft parenchyma to make a small cavity of circular or square shape. The cavity prepared by scooping should have minimum thickness at the bottom.
- Fill half the cavity with 20% sugar solution. Fix a pin into cavity in such a way that the mark is in line with the sucrose solution layer as shown in the Fig. 15.1.

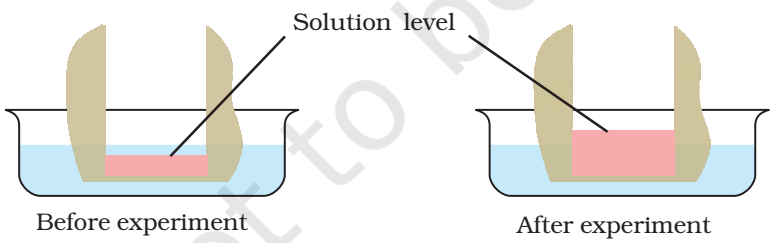


Fig. 15.1. Potato osmometer showing osmosis

- Place the osmometer in a beaker/petridish filled with water in such a way that 2/3rd of the potato osmometer is dipped in water.
- Leave the set up undisturbed for about an hour.
- Observe the level of sugar solution in the osmometer at the end of experiment.
- Repeat the experiment using water in tuber cavity and sucrose solution in beaker/petridish.

Discussion

The volume of sucrose solution inside the osmometer increases due to entry of water from the beaker as a result of endosmis. A water potential gradient is established between the sucrose solution present in the osmometer and the external water. Although living cells of potato tuber separate these two liquids, they permit entry of water into sugar solution. Interpret the results you observed when water has been used in place of sucrose solution in the osmometer (potato tuber).

Questions

1. Is there any difference in water potential of sugar solution and water?
2. Why is the water potential of sugar solution more negative?
3. What will be the rate of osmosis if we use 5% sugar solution in the osmometer?
4. How does water from outside enter the osmometer? Does it pass through cells of the tuber?

Exercise 16

Aim: Study of plasmolysis in epidermal peel of leaf.

Principle: Living cells are generally turgid due to the presence of water. When cells are immersed in hypertonic solution, shrinkage of protoplasm takes place with visible separation of plasma membrane from the cell walls. This is called plasmolysis and occurs due to exosmosis, a phenomenon in which water from the cells moves into the surrounding medium which is hypertonic, that is more concentrated than the cell sap.

Requirement: Leaves of *Rhoeo/Tradescantia*; 20% sucrose solution, slide, cover slip, needle, petri dish /watch glass, microscope.

Procedure

- Carefully prepare thin and transparent epidermal peels from the coloured epidermis by tearing the leaf or with the help of a blade. Select four thin peels, keep them in water in a watch glass to avoid dehydration.
- Mount peel 'A' in a drop of water on a glass slide, place a cover slip and observe under microscope. Observe the peel after 5, 15, 20 and 30 minutes.
- Place the other peels B, C, D and E in 20% sucrose solution.
- Observe the peels B, C, D and E after 5, 15, 20 and 30 minutes

	Time (minutes)	Total no. of cells in a field of microscope (M)	No. of Cells showing plasmolysis (N)	% of plasmolysis $\frac{N}{M} \times 100$
Peel A (control)	**5** **15** **20** **30**			
Peel B	**5**			
Peel C	**15**			
Peel D	**20**			
Peel E	**30**			

respectively under microscope. Observe the changes in cells. Record the observations in the table.

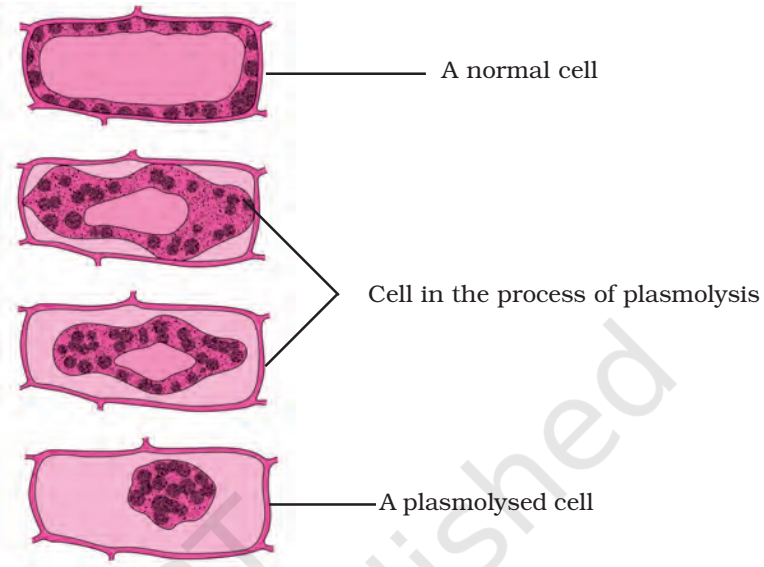


Fig.16.1. Different stages of plasmolysis in a plant cell

- Restrict your observations to cells with coloured protoplasts only.
- Draw diagrams of the cells you observe in each of the peel (Fig.16.1).

Observations

Tabulate your observations in the given table.

Discussion

Cells in the peels B, C and D will exhibit plasmolysis.

Questions

1. Why do cells of peel A kept in water remain turgid?
2. Why is plasmolysis observed in cells of peels kept in sucrose solution?
3. What will happen if the plasmolysed peels are transferred to water?
4. Name the hypertonic and hypotonic solutions used in the experiment?

Exercise 17

Aim: Study of imbibition in raisins or seeds.

Principle: The first indication shown by seed that will germinate is that it swells in volume by absorbing water, by a process called **imbibition.** Imbibition is a special type of diffusion where water is absorbed by solids (colloids) causing them to increase enormously in volume. The tissues of seeds contain several hydrophilic colloidal biomolecules. Water molecules from the external medium get attracted towards them and enter in seeds/raisins.

Requirement: Raisins (dry grapes), dry seeds of gram, green moong, 2 measuring cylinders of 100ml volume, water, balance and weights.

Procedure

- Take two measuring cylinders of 100ml capacity, mark them A and B.
- Take 30ml of water in each cylinder.
- Weight 25 g each of raisins and dry seeds and put them separately in cylinder A and B; and note the volume by rise in water level, as initial volume (X).
- Allow the raisins and seeds to soak in the same cylinder for 1-2 hour.
- Remove the raisins and seeds from measuring cylinder and blot the water by blotting paper.
- Remove the water from the cylinders and take 30ml of water again in each. Again put the soaked raisins and seeds, in the respective cylinders. Note the volume by rise in water level, as final volume (Y).
- Calculate the increase in the volume of raisins or seeds by taking the difference of X and Y (Y–X).
- Remove the raisins and seeds again and take the final weight and calculate the increase in weight by taking the difference of final and initial weight.

Observation

Record your observation in the given table:

Measurement of Weight

Material	Initial Weight (M)	Final Weight (N)	Difference of Weight (N–M) = O	% of water imbibed $\frac{O}{M} \times 100$
Raisins (A)	25g			
Seed (B)	25g			

Measurement of Volume

Material	Initial Volume (Volume of the dry raisins or seeds)			Final Volume (Volume of the soaked raisins or seeds)			Increase in volume (Y–X) = Z	% of water imbibed $\left(\frac{Z}{X} \times 100\right)$
	Initial Reading	Final Reading	Difference X	Initial Reading	Final Reading	Difference Y		
Raisins (A)								
Seeds (B)								

Discussion

Raisins/seeds swell gradually with passage of time.

Questions

1. It becomes difficult to shut wooden window panes and doors during rainy seasons. Why?
2. Why do we store grains/seeds in moisture free containers?
3. Though both osmosis and imbibition are associated with movement of water molecules, the two are different processes. Explain.
4. Will imbibition occur if seeds/raisins are kept in a beaker containing alcohol?

Exercise 18

Aim: To study the distribution of stomata on the upper and lower surfaces of leaves.

Principle: Stomata are tiny microscopic structures present in leaves of all flowering plants. Number and distribution of stomata per unit area is variable in leaves of different plants.

A typical stoma consists of a pair of guard cells enclosing an aperture in the center called the stomatal aperture. Stomata perform two important functions; that of, transpiration and exchange of gases.

Requirement: Leaf samples - (*Hibiscus*/Balsam/*Bougainvillea*/*Petunia*/*Cassia*/*Solanum*/any broad-leaved dicots and grass) microscope glass slides, cover slips, water, needle, brush, and petridishes/watch glasses.

Procedure

- Prepare thin peels of upper and lower epidermis of a grass leaf and of any two dicot leaves by tearing the leaf or with the help of a razor blade and keep the peels in separate watch glasses/petridishes.
- Mount the upper epidermal peel in a drop of water taken on a slide. Carefully cover the peel with cover slip so as to avoid air bubbles.
- Focus the peel under the high power of microscope. Note the presence/absence of stomata seen in the field of microscope. Count the number of stomata seen in the microscope field. Draw figure of stomata giving details.
- Now repeat the same with peels of lower epidermis.

Observations

Record your observations in the table given below.

	Name of the plant	No. of stomata in the microscopic field		Shape of guard cell
		Upper epidermis	Lower epidermis	
Dicot leaf	Sample A			
	Sample B			
Monocot leaf	Sample C			

Discussion

Carefully examine the results recorded for the leaf samples. Is the number of stomata more in lower epidermis or in the upper epidermis? Correlate the number of stomata with rates of respiration and exchange of gases.

Questions

1. Name the processes that will be affected if stomata were not present in leaves.
2. Draw the figure of a stoma you have observed in a monocot leaf and compare it with the shape of stomata in dicot leaf.
3. In land plants, stomata are generally more on the lower epidermis than on upper epidermis. What is the advantage?

Exercise 19

Aim: To demonstrate difference in rate of transpiration between two surfaces of leaf.

Principle: Transpiration is loss of water in the form of vapour from the leaves and other aerial parts of the stem. About 85% of transpiration takes place through the stomata. Some amount of water is also lost through cuticular transpiration. Rate of transpiration depends upon several factors like light, temperature, wind, humidity and also on the size, type, number and distribution of stomata on the leaves. You have noticed in Experiment No.18, the distribution and number of stomata on the two surfaces of leaf varies. In majority of plants, especially in dorsiventral leaves, number of stomata is more on the lower epidermis than the upper epidermis.

Transpiration can be easily demonstrated by cobalt chloride paper test. Cobalt chloride is blue coloured in anhydrous (dry) form but turns pink when it comes in contact with water. This property of cobalt chloride is used to demonstrate that water is lost during transpiration. We can use the time taken for change of colour from blue to pink to measure the rate of transpiration as effected by various external factors mentioned above.

Requirement: A herbaceous broad leaved plant, filter paper, 5% cobalt chloride solution, hot plate/oven, wire gauze, cellotape, dessicator, slides, rubber bands.

Procedure

- Prepare 100 mL of 5% cobalt chloride solution by dissolving 5g of cobalt chloride in 100 mL distilled water.
- Cut filter paper into small strips and immerse them in cobalt chloride solution taken in a petridish for 3-5 minutes.
- With forceps, transfer the soaked strips on to the wire gauge and allow excess $CoCl_2$ solution to drain off.
- Dry the filter paper strips on hot plate/oven taking care not to burn or char the paper. The anhydrous cobalt chloride coated strips will be blue in colour. Store them in a dessicator.
- Select a leaf of a potted plant growing in sunlight. If water droplets are seen, blot the leaf dry with cloth/blotting sheet.

Fig. 19.1 Experimental leaf with cobalt chloride paper

- Keep one dry (strip) cobalt chloride paper on the upper surface of leaf and stick it with cellotape. Similarly stick another strip of $CoCl_2$ on the lower surface. The $CoCl_2$ strips can be held in position with the help of two slides and rubber bands (Fig. 19.1).
- Place the potted plant in sunlight.

Name of	Time taken for change of the colour from blue to pink (in minutes)									
the plant		2	4	6	8	10	12	14	16	18	20
	Upper epidermis										
	Lower epidermis										

Observation

Observe the colour of cobalt chloride paper attached to both surfaces of leaf at regular intervals and note down your observations in the table provided.

Note: Put (√) mark for change in colour and (X) mark if there is no change in colour.

Discussion

It will be observed that the filter paper attached to the lower surface turns pink much faster than the strip on the upper surface. This is because the number of stomata are generally more in the lower epidermis than in the upper surface. As a result, the amount of water vapour lost by transpiration from the lower surface is more than the upper surface.

Questions

1. What is the reason for the colour change observed cobalt chloride paper?
2. What result would you observe if the cobalt chloride experiment is carried out in dark conditions?
3. Why is midday or afternoon the ideal time to perform the experiment to study rate of transpiration?
4. Name any two external factors, which affect the rate of transpiration.
5. Does transpiration take place in aquatic plants?

Exercise 20

Aim: To detect the presence of carbohydrates like glucose, sucrose and starch.

Principle: Carbohydrates with the free aldehyde or ketone groups (i.e., simple sugars) reduce copper sulphate of Benedict and Fehling's reagent to cuprous oxide forming a yellow or brownish-red coloured precipitate depending on the concentration of sugar.

Colour	Approximate amount of reducing sugar
No change of blue colour	Absence of reducing sugar
Blue changes to green precipitate	0.1-0.5 % of reducing sugar
Blue changes to yellow precipitate	to 0.5 to 1.0% of reducing sugar
Blue changes to orange-red precipitate	to 1.0-2.0 % of reducing sugar
Blue changes to brick-red precipitate	Over 2.0 % of reducing sugar

Requirements: Glasswares - test tubes, funnel, beaker; Chemicals - Benedict's solution, Fehling's solutions A and B; concentrated HCl, saturated solution of NaOH, dilute iodine solution; Equipments - water bath; Miscellaneous - test tube holder, test tube stand, spirit lamp.

Sample for test: Fruit juice of apples/banana/leaves of onion/sugar cane extract, milk, etc.

Preparation of reagents

(i) Benedict's reagent: Mix 173g of sodium citrate and 100g of anhydrous sodium carbonate in 600mL of water in a beaker and warm gently (solution A). Dissolve 17.3g of hydrated $CuSO_4$ in 100mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make the volume upto the mark with water.

(ii) Fehling's reagent A: Dissolve 6.93g of copper sulphate in 100mL of distilled water.

(iii) Fehling's reagent B: 20g of KOH and 34.6g of sodium-potassium tartarate (Rochelle's salt) dissolved in 100 mL of distilled water.

A. Test for glucose

Procedure

Take small pieces of banana, apple and grapes. Crush them separately and strain their juices in different test tubes. Take milk in another test tube. Cut

fresh leaves of onion bulb into small pieces and boil for 2 to 3 minutes in a test tube and filter it.

(a) Benedict's test

- Take 2 mL of juice (fruit extract) / milk / onion leaf juice in a test tube.
- Add 2 mL of Benedict's reagent to it and boil. Direct heating of test tube should not be done. It should be carried out in a water bath at the boiling point of water.
- The colour changes from blue to green and finally to orange or brick red indicating the presence of simple sugar (glucose).

Benedict's Test

Name of the food items	Colour of the Precipitate	Inference drawn
1. Apple juice	1. _____	1. _____
2. Banana extract	2. _____	2. _____
3. Grapes juice	3. _____	3. _____
4. Onion juice	4. _____	4. _____
5. Milk	5. _____	5. _____

(b) Fehling's test

- Mix equal volume of Fehling's solution A and B (1-2mL) in a test tube.
- Add equal volume of the fruit juice or onion leaf juice or milk and place it in water-bath at the boiling of water.
- The initial blue colour turns green to yellow and finally a brick-red precipitate is formed.

Fehling's Test

Name of the food items	Colour of the Precipitate	Inference drawn
1. Apple juice	1. _____	1. _____
2. Banana extract	2. _____	2. _____
3. Grapes juice	3. _____	3. _____
4. Onion juice	4. _____	4. _____
5. Milk	5. _____	5. _____

B. Test for Sucrose

Procedure

Collect sugarcane juice or cut sugarbeet into small pieces. Crush them and strain their juice.

(a) Benedict's and Fehling's test

- Perform Benedict's and Fehling's test with cane sugar or sugar beet juice as described above.
- No change of colour indicates the absence of simple sugars (i.e., monosachharides).

(b) Hydrolysis test

- Take 10 mL of sample juice in a beaker, add few drops of concentrated HCl and boil.
- After cooling, add 4 mL of saturated solution of NaOH or Na_2CO_3 to neutralize the solution. Now divide the solution into two parts. Transfer one part in test tube 'A' and the other in test tube 'B'.
- Perform Benedict's test with sample in test tube 'A'. Do you observe any change in colour? If there is a change, it indicates the presence of simple sugar or monosaccharides.
- Perform Fehling's test with sample in test tube 'B'. Do you observe any change in colour? If there is change, it indicates the presence of simple sugar or monosaccharides.

C. Test for starch (Iodine test)

Principle: Iodine specifically makes a blue-coloured complex with starch.

Procedure

- Add 1 or 2 drops of dilute iodine solution to 2 to 3mL of extract.
- A blue-black colour shows the presence of starch.
- The blue colour disappears on heating and reappears on cooling.

Discussion

The composition of blue coloured substance is not well defined. This may be an adsorption complex of starch with iodine rather than a definite compound.

Questions

1. What is the use of HCl in the test for carbohydrate?
2. Why does the blue colour disappear on boiling and reappear on cooling in the test for starch?
3. Sucrose solution gives a negative test with Benedict's test. Why?
4. Will Iodine test give a positive result with glucose, fructose or sucrose solution?
5. Why are monosaccharides called reducing sugars?

Exercise 21

Aim: To detect the presence of proteins.

Principle: Proteins respond to some colour reactions due to the presence of one or more radicals or groups of the complex protein molecule. All proteins do not contain the same amino acids, and hence they do not respond to all colour reactions. Nitrogen atoms in the peptide chain form a complex (violet colour) with copper ions in the Biuret test. (Biuret test is for peptide bond in the molecule of a protein.) Xanthoproteic test is specific for protein containing aromatic amino acids. The benzene ring in the amino acids is nitrated by heating with nitric acid and forms yellow nitro-compounds which turns to orange colour with alkali.

Requirement: Glasswares: test tube, spirit lamp; Chemicals: 40% NaOH, 1% $CuSO_4$ solution, Concentrated HNO_3, 20% NaOH solution; Miscellaneous: test tube holder, test tube stand.

Procedure

(a) Biuret test

- Take 2 mL of protein solution (milk, albumin of egg or gram seed extract) in a test tube.
- Add 1 mL of 40% NaOH solution and 1 or 2 drops of 1% $CuSO_4$ solution.
- A violet colour indicates the presence of proteins. Care must be taken that excess of copper sulphate is not added otherwise there will be blue colour instead of violet colour.

(b) Xanthoproteic test

- Add carefully 1mL of concentrated HNO_3 to 2mL of protein solution (albumin of egg, milk or gram seed extract).
- A white precipitate is formed.
- Boil the solution and the colour changes to yellow.
- Cool the test tube and add 2mL of 20% NaOH (or ammonia solution) to make it alkaline.
- The colour changes to orange indicating the presence of proteins.

Discussion

A yellow stain is often observed on skin when it comes in contact with nitric acid. The reason of yellow stain is xanthoproteic reaction.

Questions

1. Why does the skin turn yellow when it inadvertently comes in contact with HNO_3?
2. Why are only few drops of $CuSO_4$ solution added during the biuret test?

Exercise 22

Aim: To detect the presence of fats (lipid) in different plants and animal materials.

Principle: Lipids (fats) are usually insoluble in water but soluble in organic solvent. Sudan III reagent gives a specific red colour with fats.

Requirement: Glassware; test tubes; Chemicals: Sudan III, ethyl alcohol, chloroform, ether, benzene; Miscellaneous: test tube holder, test tube stand, oil/ghee/butter.

Procedure

(a) Solubility test

- Arrange five dry test tubes in a test tube stand.
- Add 2 mL of water, ether, chloroform, benzene and ethyl alcohol to each test tube followed by one drop of mustard oil, ghee or butter.
- Shake thoroughly.
- Oil, ghee or butter is broken into small droplets in water which float at the surface indicating that fat is insoluble in water. But in acetone, benzene and ethyl alcohol no such droplets are formed as the oil, ghee or butter dissolves in these organic solvents.

(b) Sudan III test

- Take 2mL of mustard oil in a test tube.
- Add equal volume of water to it and shake.
- Add a pinch of sudan III and shake again.
- As the layers separate out, the lipid layer is seen to be stained red, whereas water layer remains uncoloured. This indicates the presence of lipids.

Questions

1. What is the simplest form of fat?
2. Will fat dissolved in organic solvent give a positive result with sudan III?

Exercise 23

Aim: Separation of plant pigments (chloroplast pigments) by paper chromatography.

Principle: The chloroplasts contain photosynthetic pigments - Chlorophyll a, Chlorophyll b, Carotenes and Xanthophylls. Pigments absorb solar radiation at different wavelengths of the visible spectrum for photosynthesis. These pigments differ in their chemistry, and hence in their physicochemical properties, such as molecular weight, solubility in the solvent etc.

Paper chromatography is a popular technique widely used for separating various chlorophyll pigments from a mixture. In chromatography, pigments move to different distances, depending on their solubility in the solvent system, on a fine quality cellulose paper (Whatmann No.1 chromatography paper). Movement of pigments on the chromatography paper is governed by the principle of adsorption and capillary action. The solvent system components vary in density and thus move at different rates due to wick action through the chromatography paper. Lighter components move faster than the heavier components. Differential solubility of pigments in the solvent system and the differential rates of mobility of solvent system components is used for separation of pigments.

Requirement: Mucilage-free leaves of locally availabe herbaceous plants, test-tubes (6"x1"), boiling tube with split rubber cork, chromatography chamber or wide mouth test tube, 100 ml and 10 ml measuring cylinders, 50 mL beaker, capillary tube, mortar and pestle, acetone, petroleum ether, muslin cloth, sand, scissors, pins, Whatmann No. 1 filter paper, ordinary blotting sheet.

Procedure

Preparation of pigment extract

- Select tender, herbaceous, mucilage free leaves for the experiment. Remove the petiole and mid rib from 20-30 mature leaves.
- Cut the leaves into smaller bits and transfer them into a mortar. Add a pinch of sand, a few mL of acetone and grind thoroughly until the leaf extract becomes dark green in colour.
- Filter extract through a muslin cloth and collect the filtrate in 50 ml beaker.
- Prepare 9:1 solution of petroleum ether and acetone. Transfer 3-5 ml of this mixture into the chromatography chamber or wide mouthed test tube. Close the chamber or the test-tube with the split cork tightly as the solvents are volatile and keep it in standing position.

Fig. 23.1 Loading of pigment extract

- Cut a strip of Whatmann No. 1 chromatography paper (6" X 1/2"), which can easily fit into the chamber or the test-tube.
- Using scissors make one end of the chromatography paper into a triangular shape with a pointed end.
- Using the capillary tube, load the pigment extract on the lower side of the chromatography paper in the spot (labelled S in Fig. 23.1) till the spot is dark green in colour. Make sure that the diameter of the loaded spot is not more than 3-5mm. Dry the spot by blowing warm air on it. Never use ink/ ball pen for marking on chromatography paper.
- Suspend the loaded chromatography paper into the boiling tube with the help of a split rubber cork or in chromatography chamber (Fig. 23.2) or wide mouth test tube.
- Make sure that the loaded spot is clearly above the solvent.
- Keep the apparatus straight and leave the set up in a test tube stand undisturbed for 40 minutes. Note the rise of solvent and pigments.
- Remove the chromatography paper after the four different coloured pigments appear.
- Cover the chromatogram with black paper to prevent photo oxidation.

Fig. 23.2 Experimental setup of the chromatography

Fig. 23.3 A chromatogram of chlorophyll

Observation

Identify the pigments on the basis of colours. Sequentially from the bottom, the four colours visible are: greenish yellow (chlorophyll b;) bluish green - (chlorophyll a), yellow (carotene) and orange (xanthophyll) (Fig. 23.3).

Discussion

The four major photosynthetic pigments have been separated by chromatography and identified. This process of separation is called monodimensional ascending chromatography since the solution and pigments move upwards in one direction only.

Questions

1. Which photosynthetic pigment moves farthest and why?
2. Leaves in general appear green although they possess yellow and orange pigments. Comment.
3. Why do we add sand while preparing the pigment extract?
4. Among the two solvent system components in chromatography, which moves farthest and why?

Exercise 24

Aim: To study the rate of respiration in flower buds/germinating seeds.

Principle: Respiration is a vital process in living organisms and generates energy through break down of food materials in presence/absence of O_2. The released energy is used for all life processes. Rate of respiration depends on internal and external factors (age, physiological status and type of cell, temperature, and availability of oxygen).

Requirement: Flower buds/germinating seeds, boiling tube, single bore rubber cork fitted with a pipette, cotton, stand with burette clamp, KOH solution, black paper and filter paper.

Procedure

- Take about 10-15 buds or 10-15g germinating seeds in a boiling tube or wide mouth test-tube. Introduce a wad of cotton. Dip a 2 ×1cm strip of filter paper in KOH solution and place it in the tube ensuring that it does not touch the cotton swab or seeds.
- Dip the tip of the pipette in water and slowly suck-in water in such a manner that a small air bubble is trapped in it. Now insert the attached rubber cork (along with pipette) into the tube as shown in Fig. 24.1. The test tube should be fixed in horizontal position with burette clamp.
- Note the position of air bubble in the pipette.
- Record the distance travelled by the bubble at 2 minute intervals for a period of time.
- Now shift the set up to bright sunlight (outside the laboratory). After a few minutes, note the distance travelled by the bubble at 2 minute intervals for the same period of time.

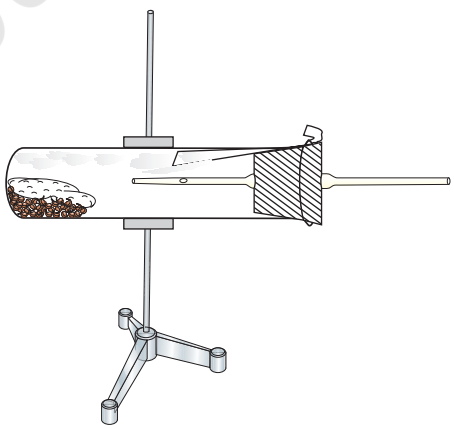


Fig. 24.1 Experimental setup for the study of rate of respiration

Observation

Compare the two sets of values obtained in the experiments. It is likely that in the experiment conducted in bright sunlight the bubble moves much faster indicating higher rate of respiration. One of the factors that is responsible for increase in rate of respiration is temperature. Can you think of a reason?

Discussion

Notice that the rates of respiration are not same in different materials and under different conditions. Respiration is an enzymatic process where food materials are broken down to release energy. Light and temperature affect the process. Young meristematic cells show high rate of respiration.

Questions

1. What is the role of KOH strip used in the experiment?
2. Why does the air bubble move in the pipette towards the boiling tube?
3. What will happen if the setup is not air tight?

Exercise 25

Aim: Observation and comment on the setup.

A. ***Anaerobic Respiration***

Principle: Breakdown of food substances to yield energy in the absence of oxygen is called anaerobic respiration. It is observed in several soil anaerobic microorganisms, yeast and certain types of tissues in human body. Anaerobic respiration yields much less energy per mole of glucose as compared with aerobic respiration. In germinating seeds/flower buds the equation for anaerobic respiration is $C_6H_{12}O_6 \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} CO_2 + C_2H_5OH$.

Requirement: Germinating seeds (gram/urad/moong), flower buds, a small test tube/ glass vial, petridish, a plastic tray slightly bigger than the size of petridish, mercury, forceps, KOH pellets, burrette stand with clamp.

Procedure

- Take a test tube and completely fill it with mercury. Invert it over a petridish which is also filled with mercury. There must be a continuous column of mercury in the test tube.
- Tilt the test tube slightly and with the help of forceps introduce 3 - 4 healthy germinating gram seeds.
- Gently tap the test tube with your finger nail/forceps so that the seeds move upwards in the mercury column.
- With a clamp fix the test tube to a stand and keep the setup undisturbed for two hours.
- Observe the setup.
- Introduce 3-4 KOH pellets in the same way as seeds were introduced. Observe the changes.

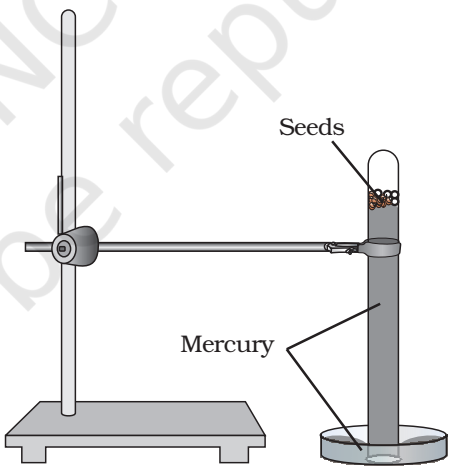


Fig. 25.1 Experimental setup for the study of rate of anaerobic respiration

Observation

A space is formed at the top of the test tube due to downward displacement of mercury. When KOH pellets are introduced, the gap slowly disappears and mercury again fills up the entire tube.

Discussion

The germinating seeds respire in a situation when these are completely cut off from air in the presence of a continuous column of mercury. The carbon dioxide gas released gets collected at the top of the tube and displaces mercury. The CO_2 released disolves in KOH and the mercury level rises again. This establishes the fact that seed/buds have respired anaerobically.

B. *Phototropism*

Principle: Light is responsible for inducing many responses and physiological processes in plants. Light induced growth response in plants is called phototropism. Shoot is positively phototropic. Light controls the distribution of auxins in the shoot tip and a greater quantity of auxin accumulation occurs in the shaded portion leading to rapid cell division resulting in faster growth and the bending or curvature of the stem towards light.

Requirement: Two herbaceous potted plant of same species, or seeds of mustard, an earthen pot filled with sandy soil, water.

Procedure

- Keep one potted herbaceous plant on a table near a window for a week and the other in the open space (Fig. 25.2). Water the plant, regularly and observe.

OR

- Germinate about 20-30 mustard seeds each in two pots. Keep one pot on a table near a window and the other in exposed open space. Water them daily. Observe the curvature of seedlings after a week in both setups.

Fig. 25.2 A potted plant showing phototropism

Observation

You will observe that the pots with plant/seed kept near window exhibit a distinct bending towards the light.

Discussion

The curvature or bending of shoot towards the source of light is due to the redistribution of auxins in the apical meristem of plants kept near the

window. The shoot/seedling kept in open space do not show any curvature. Shoots always exhibit positive phototropism.

C. *Apical bud removal (Apical dominance)*

Principle: Stem grows due to divisions of the apical meristematic cells situated at the tip of stem. Plants possess axillary (lateral) buds in the axil of leaves. The axillary buds are generally dormant and are not expressed because the apical bud imposes a natural inhibition over the growth of lateral buds. This influence exerted by the apical bud over the axillary buds is called apical dominance.

Requirement: Two potted plants of same species with sparse branching- *(Chenopodium/Croton/ Tecoma/Petunia/Hibiscus)*, knife/scalpel/blade.

Procedure

- Using a blade cut the tip of the stem of the experimental plant (plant A). Keep another plant of the same species without cutting its tip as control (plant B).
- Water both the plants regularly and observe.

Observation

After about 7-10 days, several lateral (axillary) branches will be seen in plant A as compared to plant B.

Discussion

When the apical bud is removed in plant A, the apical dominance no longer exists, as a result the lateral buds are activated and give rise to lateral branches.

D. *Suction due to transpiration (Transpiration pull)*

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

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

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Procedure

- Insert a graduated 1 mL pipette to one end of a rubber tube so that it fits tightly.
- Select a twig of appropriate diameter and size and make an oblique cut with a knife taking care not to rip off the bark. Moisten the cut edge with water.
- Fill the rubber tube and pipette with water by suction.
- Insert the twig into the other end of the rubber tube and tie a thread to make it air tight (Fig. 25.3).
- Fix the set up with the help of burette stand and clamp as shown in the Fig. 25.3.
- Take a glass tube/beaker and fill it with coloured water (using eosin/methylene blue/blue ink) and keep the nozzle of the pipette in it.
- Note the level of coloured water in pipette. Keep the setup in sunlight/under a fan for an hour and note the final level of coloured water in the pipette.

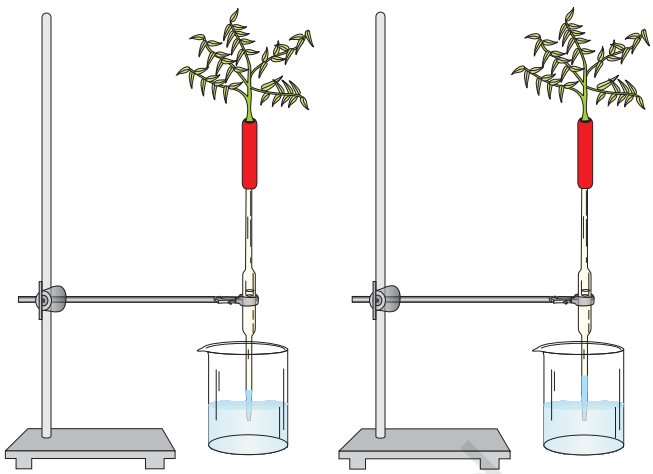


Fig. 25.3 Experimental setup showing suction due to transpiration

Observation

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

Discussion

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

In nature this suction force is transmitted into the root xylem and root hair, which enables plants to absorb water from the soil.

Questions

1. What other material could be used for demonstrating anaerobic respiration?
2. Would the same plant kept near the window respond similarly to artificial light?

Exercise 26

Aim: To study the enzymatic action of salivary amylase on starch.

Principle: An enzyme is a biological catalyst. The enzyme salivary amylase or ptyalin present in saliva initiates the breakdown of starch, which hydrolyzes it into disaccharide maltose, isomaltose and short chain glucose polymers called - dextrins. Enzymatic activity of salivary amylase can be tested by detecting the presence or absence of starch.

Requirement: *Glass wares:* Test tubes, cavity blocks, beakers, dropper, and funnel; *Chemicals:* NaCl, Na_2HPO_4, KH_2PO_4, Iodine crystals, potassium iodide; *Equipments:* water bath or incubator thermometer; *Miscellaneous* - cotton, rubber, distilled water, test-tube stand, test-tube holder.

Preparation of reagents

(i) 1% starch solution: Add 1 g of soluble starch to 10ml of distilled water and mix them. Boil 90 ml of distilled water and to it add 10 mL of starch solution already prepared by stirring. Leave the solution overnight and then filter to get 1% starch solution.

(ii) 1% NaCl solution: Dissolve 1 g of NaCl in 100 mL of distilled water.

(iii) Iodine solution (Lugol's): Dissolve 1 g of iodine crystals and 2g of potassium iodide in 100 mL of distilled water.

(iv) Preparation of buffer solution at pH 6.8: Buffer solution can be prepared by dissolving one buffer tablet of 6.8 or 7 pH in 100 ml distilled water or prepare M/15 Na_2HPO_4 solution (9.67g Na_2HPO_4 in 1000 ml of distilled water) and M/15 KH_2PO_4 solution (9.06g of KH_2PO_4 dissolved in 1000 ml of distilled water). Mix equal volume as per requirement to get buffer solution.

Procedure

- Take cotton soaked in distilled water. Remove the excess water by pressing and then spread the moistened cotton over the mouth of a funnel in such a way that it acts as a filter. After cleaning mouth, chew a piece of rubber/cotton and pour the saliva into the funnel. Saliva filtered through wet cotton will be collected in the test tube. Avoid using filter paper for filtering saliva. Take 1 ml of saliva and add 19 ml of distilled water to get saliva solution.
- Take two sets of test tubes (8-10 test tubes in each set) in two separate test tube stand each containing 1 mL of iodine solution to

act as indicator tubes. Mark them 1, 2, 3 . . .. in both test tube stands.

- Switch on the electric water bath or oven. Set the temperature at 37°C. Maintain uniform temperature (37°C) of the water in water bath or water in a beaker inside incubator throughout the experiment.
- In a test tube, take 10 ml of starch solution, 2 ml of 1% NaCl solution and 2 ml of buffer solution. Mix them well and transfer half of the solution into another test tube. Mark one test tube as experimental tube and the other as a control tube.
- Now transfer both experimental and control test tubes to the water bath or keep them in the beaker containing water inside incubator for about 10 minutes so that temperature of solutions reaches 37°C.
- Add 1 ml of saliva solution to the experimental tube and 1ml of distilled water to control tube. Keep both tubes in water bath/ incubator throughout experiment.
- With the help of a dropper, take a drop each from experimental and control tubes and pour it into two separate indicator tubes (marked 1) containing iodine (from two series of indicator tubes - one for experimental and other for control). Record the time of mixing as zero minute reading and note the change in colour of iodine in both tubes.
- After two minutes, again transfer a drop each from experimental and control tubes to indicator tubes (marked 2) and note the colour of iodine. Repeat the step at interval of every two minutes till the colour of iodine solution does not change any further (achromatic point). Always take the same amount of solution throughout the experiment to be added to iodine tubes.
- Compare the series of experimental tubes with the control iodine tubes.

Time (Min) Indicator tube	**Control Indicator tube**	**Experimental**
0	Blue colour	Blue colour
2	_____	_____
4	_____	_____
6	_____	_____
___	_____	_____
___	_____	_____
___	_____	_____

Observation

(i) Note the colour in control indicator tubes.

(ii) Note the colour change in both test tubes and the time taken for the change.

(iii) Perform the Benedict's test for confirmation of the presence of reducing sugar.

Discussion

On the basis of following questions draw your conclusion:

- Did the colour change occur in both sets?
- Which set showed colour change and why?
- Which set did not show colour change and why?
- Which set showed positive Benedict's reaction and what does it confirm?
- How much time did it take to reach the achromatic point (no change in the colour of the indicator)?

23-04-2018

Exercise 27

Aim: To study the effect of temperature on the activity of salivary amylase.

Principle: Most of the enzymes are proteinaceous in nature. Over a limited range of temperatures, the rate of enzyme catalyzed reactions increases as temperature rises. The rate of many biologic reactions roughly doubles with a 10°C rise in temperature and is halved if the temperature is decreased by 10°C. There is an optimal temperature at which the reaction is most rapid. Above and below this, the reaction rate decreases sharply.

Requirement: Glass wares: test tubes, cavity block, beakers, dropper, funnel, test-tube stand, test-tube holder; Chemicals: NaCl, Na_2HPO_4, KH_2PO_4, Iodine crystals, potassium iodide, Prepared Reagents as in Experiment 26; Equipments: water bath or incubator, thermometers; Miscellaneous - cotton, rubber, distilled water,

Procedure

The first four initial steps are the same as in previous Experiment 26.

- In a beaker, take 15 mL of starch solution, 3 mL of 1% NaCl solution and 3 mL of buffer solution. Mix them thoroughly. Divide the solution into three test tubes and mark them as A, B and C. All three test tubes are experimental tubes.
- Now transfer experimental tube A into a beaker containing ice and a thermometer for recording temperature. Temperature of this beaker is to be maintained at 5°C. Transfer the second experimental tube B into water bath set at 37°C and the third experimental tube (C) into the beaker maintained at 70°C.
- Without taking them out add to the three experimental tubes (A, B,and C), 1 mL of saliva solution.
- Take a drop from each of the experimental tubes with the help of a dropper and add it to the corresponding indicator tubes containing iodine solution. Note this time as zero minute reading.
- At intervals of every 2 minutes keep on repeating the above step and note the change in colour of iodine solution. Continue this till the colour of iodine does not change.
- Note the time taken for different experimental tubes till they do not give any colour with iodine.

Observation

To digest completely 5 mL of 1% starch solution, 1 mL of diluted enzyme takes ------- minutes at 5^{0}C, ------- minutes at 37^{0}C and -----minutes at 70^{0}C.

Time minute	Reaction with iodine from experimental tube A	Reaction with iodine from experimental tube B	Reaction with iodine from experimental tube C
0 2 4 6 8 10 12 ____ ____ ____	Blue colour	Blue colour	Blue colour

Discussion

On the basis of the following questions draw your conclusion:

- At which temperature the reaction is optimum?
- Did all three sets of tubes reach achromatic point? If not, why so?
- What inference do you draw about enzyme activity from your experiment?

23-04-2018

Exercise 28

Aim: To study the effect of pH on the action of salivary amylase.

Principle: Optimal activity for most of the enzymes is generally observed between pH 5.0 and 9.0. However, a few enzymes, e.g., pepsin are active at pH values well outside this range. Above and below this range, the reaction rate reduces as enzymes get denaturated.

Requirement: Glass wares: test tubes, beakers, dropper, funnel; Chemicals: NaCl, Na_2HPO_4, KH_2PO_4, iodine crystals, potassium iodide, Buffer solutions of pH 4 and 9, Equipments: water bath or oven, thermometer; Miscellaneous: cotton, rubber, distilled water.

Preparation of reagents

- Buffer solutions of pH 4 and 9 can be prepared by dissolving buffer tablets in appropriate amount of distilled water as indicated on the paper.

Procedure

The first four initial steps are the same as in previous Experiment 26.

- Prepare three sets of indicator test tubes (8 to 10 in each set) in three separate test tube stands. Label test tube stands as A (for 6.8 pH), B (for 4 pH) and C (for 9 pH). In each test tube take 0.5 ml of iodine solution.
- In a test tube, take 5 ml of 1% starch solution, 1 ml of 1% NaCl solution and 1 ml of pH 6.8 buffer solution, mark it as control tube or A. In a second test tube, take 5 ml of 1% starch solution, 1 ml of 1% NaCl solution and 1 ml of pH 4 buffer solutions, mark it as experimental tube 'B'. In a third test tube, take 5 ml of 1% starch solution. 1 ml of 1% iodine solution and 1 ml of pH 9 buffer solution. Mark it as experimental tube 'C'.
- Transfer 1 ml of dilute saliva into each test tube and mix the two thoroughly. Place all three test tubes in water bath set at 37^0C.
- Take a drop from each of the experimental tubes with the help of dropper and add to the corresponding indicator tubes containing iodine solution. Note this time as zero minute reading.
- At intervals of every 2 minutes repeat the above steps and note the change in colour of iodine solution. Continue this till the colour of iodine does not change.

- Note the time taken for different experimental tubes till they do not give any colour with iodine.

Time minute	Tube 'A'	Tube 'B'	Tube 'B'
0	Blue colour	Blue colour	Blue colour
2	_____	_____	_____
4	_____	_____	_____
____	_____	_____	_____
____	_____	_____	_____

Calculate the time taken to reach the achromatic point in tubes A, B and C. Find out whether in any of the three tubes achromatic point was not observed.

Discussion

On the basis of following questions draw your conclusion:

- At which pH is the reaction optimum?
- Did all three sets of tubes reach achromatic point? If not, why so?
- What inference do you draw about enzyme activity from your experiment?

Questions

1. How many pairs of salivary glands are found in human beings?
2. What is an enzyme?
3. Why are enzymes mentioned as biocatalysts?
4. Why is NaCl solution added in the starch solution while testing salivary amylase activity?
5. What are the end products of salivary amylase activity?
6. What is achromatic point?
7. What is the optimum temperature and pH for salivary amylase action?
8. What is the need for secretion of pancreatic amylase into the intestine?
9. What do you mean by optimum temperature, pH and denaturation of enzyme?
10. How will you confirm that there is complete digestion of starch?

23-04-2018

Exercise 29

Aim: To detect the presence of urea in the given sample of urine.

Principle: Urea is mainly excreted into urine via kidneys. The nitrogen of amino acids is removed as urea. Normally a healthy adult person excretes about 15g of nitrogen per day; 95% of this nitrogen is excreted as urinary urea. The amino groups of amino acids are ultimately removed as ammonia (NH_3). This NH_3, is highly toxic, and is ultimately converted into urea. Normally urine is acidic. If the urine is kept exposed to atmosphere, it splits and ammonia gets released and thus the stored urine becomes alkaline.

At optimum pH and temperature urease enzyme decomposes urea into ammonia and carbon dioxide which form ammonium carbonate (an alkaline substance). which changes the slightly acidic solution to alkaline solution. When phenol red is used as indicator in this reaction mixture, the colour of solution changes from yellow to pink.

Requirement: Glasswares: test tubes, Chemicals: 2% Na_2CO_3 solution, 2% acetic acid, sodium hypobromite, sodium hydroxide, 1% acetic acid, urease tablet, phenol red, dilute NaOH solution, 1% $CuSO_4$ Solution Equipments: test tube holder, test tube stand, spirit lamp.

Procedure

(a) Urease test

- Take 2 mL of urine in one test tube and 2 mL of water in the other.
- Add a drop of phenol red indicator to each tube.
- Add 2% Na_2CO_3 solution drop by drop till the pink colour develops in both test tubes (just alkaline).
- Now add 2% acetic acid to each test tube drop by drop till the pink colour disappears (just acidic).
- Add a pinch of soybean powder (contains the enzyme urease) or a pinch of urease enzyme powder to each test tube and rotate the tubes between the palms or warm both the tubes to about 60°C. Overheating should be avoided to prevent denaturation of enzyme.
- The pink colour appears in the tube containing urine but not in the other tube containing water.

Discussion

The enzyme urease acting on urea releases ammonia as shown in the following reaction:

$(NH_2)_2CO \xrightarrow[Na_2CO_3]{Urease} (NH_4)_2CO_3 \xrightarrow{heating} 2NH_3 + H_2CO_3$

Urea (neutral) — Ammonium carbonate (alkaline)

$+H_2O \downarrow$

NH_4OH

This test is a specific test for urea because the enzyme urease shows its specificity for the substrate urea. The optimum pH (just acidic) and temperature (60°C) must be maintained for the activity of the enzyme urease. Urea is formed in the liver from ammonia and carbon dioxide. Ammonia is the product of deamination of amino acids. Therefore, urea excretion in urine is dependent on the amount of protein ingested.

Note: In place of Soyabean powder or urease enzyme, the aqueous extract of *Cajanus cajan* (Arhar) can also be used as a source of urease.

(b) Biuret test

- Place a small amount of urea in a dry test tube and heat it on a low flame. Urea melts with the liberation of ammonia.
- On further heating it solidifies (in case of urine, the urine is heated till it is completely evaporated).
- Cool the tube. Add 3mL of water and shake.
- Add to it 1mL of dilute NaOH and 1 or 2 drops of 1% $CuSO_4$ solution. The pink colour develops indicating the presence of urea. Excess drops of $CuSO_4$ should not be added, otherwise $CuSO_4$ will form $Cu(OH)_2$ with NaOH forming a blue colour. This is sometimes mistaken for a positive Biuret test.

Discussion

Urea when heated decomposes with the liberation of ammonia and the formation of biuret. The biuret is dissolved in water and develops a pink/violet colour forming a complex with the alkaline copper sulphate solution.

(c) Sodium hypobromite test

- To the 2 mL of the given sample of urine in a test tube, add 2 drops of alkaline sodium hypobromite solution.
- Brisk effervescence of nitrogen appears in the test tube which indicates presence of urea in the sample.

Discussion

When urine is treated with sodium hypobromite solution containing excess of sodium hydroxide, the carbon dioxide and nitrogen gas are evolved. CO_2 after reacting with NaOH gives Na HCO_3.

$(NH_2)_2$ CO + NaH Br ⟶ CO_2 + N_2 + NaBr + H_2

urea

+NaOH

NaHCO3

Exercise 30

Aim: To test the presence of sugar in the given sample of urine.

Principle: In normal urine, practically there is no glucose. Presence of glucose in urine is called glucosuria. To detect reducing sugars, such as glucose, fructose etc. in urine Benedict's or Fehling's tests are done.

$CuSO_4$ present in Benedict's solution or Fehling's solution is reduced on boiling by the reducing substances (glucose, fructose etc.) to form the coloured precipitate of cuprous oxide. The light green, green, yellow and brick red precipitates of cuprous oxides depend on the concentration of reducing substances present in urine.

Glucose reduces the blue cupric sulphate of Benedict's reagent or Fehling's reagent to a coloured insoluble precipitate.

$$CH_2OH(CHOH)_4\,CHO + 2Cu^{++}\,SO_4^{--} + 2H_2O \xrightarrow{\text{heating}}$$

Glucose (reducing sugar) — Cupric sulphate (blue solution)

$$CH_2OH(CHOH)_4\,COOH + Cu_2O + 4H^+ + SO_4^{--}$$

Gluconic acid (oxidised sugar) — cuprous oxide (red precipitate)

Requirement: Glasswares: Test tubes, beakers, spirit lamp, pipette; Chemicals: Benedict's solution, Fehling's solution A and B, Seliwanoff's reagent, Miscellaneous: Test tube holder, test tube stand, urine sample.

Preparation of Reagents

(i) Benedict's reagent Mix: 173 g of sodium citrate and 100 g of anhydrous sodium carbonate in 600 mL of water in a beaker and warm gently (solution A). Dissolve 17.3 g of hydrated $CuSO_4$ in 100 mL of distilled water (solution B). Add solution B to solution A with constant stirring. Cool and transfer to a one litre flask and make upto the mark with water.

(ii) Fehling's reagent A: Dissolve 6.93 g of copper sulphate in 100 mL of distilled water.

(iii) Fehling's reagent B: 20 g of KOH and 34.6 g of sodium-potassium tartarate (Rochelle's salt) dissolved in 100 mL of distilled water.

(iv) Seliwanoff's reagent: Dissolve 50 mg of resorcinol in 33 mL of concentrated hydrochloric acid and dilute it to 100 mL with distilled water.

(v) In absence of appropriate samples containing abnormal components of urine, these components (glucose, albumin) can be added in the normal urine sample.

Procedure

(a) Benedict's test

- Take 5 mL of Benedict's reagent in a test tube. Add 0.5 mL (8 drops) of freshly passed urine to it.
- Boil for 2 minutes holding the test tube firmly with a test tube holder (during boiling, the contents of the test tube get a tendency to spurt out. Hence, it is wise to keep shaking the test tube after holding it in the inclined position near the flame to avoid overboiling).
- A light green, green, yellow and brick red precipitate indicates the presence of reducing substances in urine.
- The various coloured precipitates depend on the concentration of reducing sugars in urine which gives a rough estimate of the concentration given below:

Colour of precipitate	% of reducing suger present
Light green -----	0.1 to 0.5
Green ------	0.5 to 1.0
Yellow ------	1.0 to 2.0
Brick red ------	above 2

(b) Fehling's test

- Take equal volumes (2 mL) of Fehling's solution A and B in a test tube. Mix them well.
- Add the above solution drop wise to 1 mL of urine sample taken in a test tube. Heat the test tube after each drop is added.
- A yellow or orange or brick red precipitate is formed which indicates the presence of reducing sugar in urine.

Note: Benedict's and Fehling's test are not necessarily indicative of only glucose in urine but it may also indicates the presence of other reducing sugars, such as lactose (in case of pregnant woman and lactating mothers), fructose (in fructosuria), galactose (in galactosuria), homogentisic acid (in alkaptonuria), glucuronates and mucin.

(c) Seliwanoff's test

The qualitative Benedict's test is not very specific test for glucose, since other reducing sugars also give positive tests, such as fructose, galactose, lactose, maltose, pentose. Other urinary constituents, drugs and contaminants in the urine may give false positive results. For detecting whether glucose or fructose is present in the urine, Seliwanoff's test should be performed.

This test is to be performed when urine sample gives positive test for Benedict's or Fehling's test.

- Take 3 mL of Seliwanoff's reagent and add 1 mL of urine sample.
- Boil for two minutes. Appearance of red to orange colour indicates the presence of fructose.
- If no colour appears in 2 minutes, continue boiling for 5 minutes.
- If faint orange or no colour appears, then it indicates the presence of glucose.

Discussion

Test is sensitive between 50-80 mg glucose/100 mL urine. Less than this amount in the urine will not be detected by this test.

23-04-2018

Exercise 31

Aim: To detect the presence of albumin in the given sample of urine.

Principle: Nitric acid causes the precipitation of albumin. When heated or treated with sulphosalicylic acid, albumin undergoes coagulation.

Requirement: Glasswares: Test tubes, graduated pipette (5 mL capacity), spirit lamp; Chemicals: Concentrated nitric acid, acetic acid, Robert's solution, sulphosalicylic acid or a solution containing 13% salicylic acid and 20% sulphuric acid; Miscellaneous: test tube stand, test tube holder.

Procedure

(a) Nitric acid ring test

- Take 5 mL of concentrated nitric acid in a test tube.
- Incline the tube and add the urine sample with a dropper, so that the latter flows down slowly along the side of the test tube to form a separate layer.
- A white ring develops at the junction of the two liquids which indicates the presence of albumin in the urine sample.

OR

- Take about 5 mL of Robert's solution in a test tube.
- Now incline the test tube and add 2 to 3 mL of the given sample of urine by means of a dropper along the inner side of the test tube so that it forms a layer over the Robert's solution.
- The presence of white ring at the junction of two layers indicates the presence of albumin in the sample.

(b) Heat coagulation test

- Take about 6 to 8 mL of urine in a test tube.
- Incline the test tube at an angle and heat the upper one-third of the test tube by a low flame.
- Turbidity develops in the heated portion of the urine.
- Add 1% acetic acid drop by drop and boil or simply add a drop of 33% acetic acid.
- If the turbidity persists it confirms the presence of albumin in the urine sample (disappearance of turbidity, confirms the presence of phosphates).

(c) Sulphosalicylic acid test

- Take 3 mL of urine in a test tube.
- Add a few drops of sulphosalicylic acid and heat it gently.
- A whitish or cloudy turbid solution or precipitate (coagulation) in the solution indicates the presence of albumin in the urine sample.

Discussion

A trace of protein which is less than 250 mg (in 24 hours urine) is found in normal urine. Under pathological conditions like albuminuria, albumin is found in urine above normal level. This amount is so negligible that it escapes detection by any of the simple test. In kidney disturbance and in high blood pressure, albumin level in urine is significantly high.

Questions

1. What is the colour of the urine and name the pigments responsible for this characteristic colour?
2. In which organ of our body, highly toxic ammonia is converted into urea?
3. Name the disorder that shows presence of excess urea in the urine.
4. Name the disorder in which glucose level is high in urine.
5. What do you call those animals that eliminate nitrogen mainly in the form of urea?
6. Which other organ of our body also excretes urea in small amount?
7. Which reagents will demonstrate presence of protein in urine?
8. What is the significance of appearance of different colours while performing Benedict's test?
9. What is the significance of performing Seliwanoff's test?

23-04-2018

Exercise 32

Aim: To detect the presence of bile salts in the given sample of urine

Principle: Old and damaged RBCs are removed from the circulation mostly in the spleen and to some extent in the liver by macrophages. Hemoglobin of the RBCs is broken down in the cytoplasm of macrophages. When iron is removed from heme component of hemoglobin, the iron-free portion of heme is converted to biliverdin, a green pigment, and then into bilirubin, a yellow orange pigment. Bilirubin enters the blood and is transported to the liver from spleen. In the liver, bilirubin is secreted by liver cells into bile, which passes into the small intestine and then into the large intestine. Bilirubin is detected in urine in certain pathological conditions only.

Requirement: Test tubes, measuring cylinders (10 ml), funnel, dropping pipette or drop bottle, Lugol's iodine solution, barium chloride solution (10%,) Fouchet reagent, sulphur powder, concentrated nitric acid, test tube holder, test tube stand, cotton, filter paper, distilled water.

Preparation of reagents

(i) Lugol's iodine solution: Dissolve 1g of iodine crystals and 2 g of potassium iodide in 100 mL of distilled water.

(ii) Fouchet reagent: Dissolve 25 mL of trichloroacetic acid (TCA) in 75 mL of distilled water. Now add 1g of ferric chloride to this solution and mix.

(iii) 10% Barium chloride solution: Dissolve 10 g of $BaCl_2$ in 90 ml of distilled water and make up the solution to 100 mL with water.

Procedure

(a) Lugol's Iodine Test

Pour 4 ml of urine sample into a test tube. Add 4 drops of lugol's iodine solution to this tube. Shake the tube well and observe. A faint yellow to brown colour indicates absence of bile pigments while light to dark green colour indicates the presence of bile pigments.

(b) Gmelins Test

Take 5 ml of concentrated nitric acid in a test tube. Add an equal volume of the given urine sample to it slowly along the sides of

the test tube. Formation of a green, blue, yellow or red ring at the junction of the two solutions indicates the presence of bile pigments.

Procedure

Bile pigments present in the urine react with concentrated nitric acid and induces formation of a coloured ring at the junction between the urine and acid layer.

(c) Fouchets Test

Take 5 mL of the given urine sample. Add 2-5 mL of $BaCl_2$ to this test tube and mix the two solution. A precipitate will appear. Now filter the mixture. The precipitate containing the bile pigments remains on the filter paper. Add 2 drops of Fouchet reagent to the precipitate on the filter paper. If the precipitate turns green, it shows the presence of bile pigments.

Discussion

The colourless bilirubin is oxidised by the ferric ion of ferric chloride (present in the Fouchet Reagent) to green biliverdin.

$$\underset{\text{(colourless)}}{\text{Bilirubin} + Fe^{+++}} \longrightarrow \underset{\text{(green)}}{\text{Biliverdin} + Fe^{++}}$$

Questions

1. Give the names of the pigments found in bile.
2. Which organ of the body produces bile pigments?
3. Which pigment provides colouration to the bile?
4. What are the functions of bile pigments?
5. How are the bile pigments produced?
6. Mention the name of the diseases during which excretion of bile pigments occurs in urine.
7. What are the different tests to detect the presence of bile pigments in urine?

23-04-2018

Exercise 33

Aim: To study the human skeleton

Principle: Human skeleton in adults is composed of 206 bones. It is divisible into two categories: Axial and appendicular skeleton. The axial skeleton consists of the bones of the skull, vertebral column, sternum and ribs. The appendicular skeleton consists of the bones of the limbs along with their girdles.

Requirement: Specimen of human skeleton

Procedure

(i) Observe the different types of bones and joints present in a human skeleton.

(ii) Draw labeled diagram of your observations.

Observation

(a) Human Skull

(i) It is composed of two sets of bones - cranial and facial (Fig. 33.1).

(ii) Cranial bones are occipital, parietal, frontal, temporal, sphenoid and ethmoid bones.

(iii) Corresponding to their location in the body, the cranial bones have strong bone case for eyes called orbit.

(iv) Facial bones form the front part (i.e., face) of the skull.

(v) A single U-shaped bone called hyoid is present at the base of the buccal cavity.

(vi) A nasal passage formed by nasal bones is present just below the orbit.

(vii) Maxilla and pre-maxilla bones form the upper jaw, and the mandible bone forms the lower jaw. These two bones also form the face, and into them are lodged teeth in special sockets. Teeth are not bones.

(viii) Distinct sutures in zig-zag fashion are present at the junctions of the frontal with the two parietals, as well as between the two parietals.

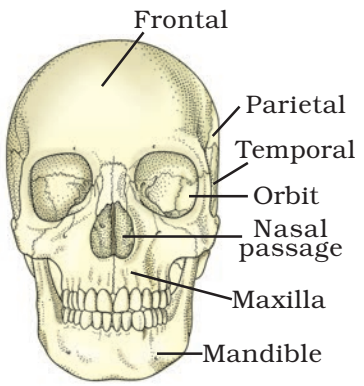


Fig. 33.1 Human Skull

(ix) The occipital bone has a very big foramen at its posterior base, the foramen magnum, through which the brain is continued posteriorly as a spinal cord.

(x) The skull is dicondylic, i.e., it has two occipital condyles for articulation with the first cervical vertebra.

Note: The cranium forms the hard protective outer covering for the brain. All the bones of the cranium are articulated by fibrous or fixed or immovable joints. Mandible is the strongest bone of the body.

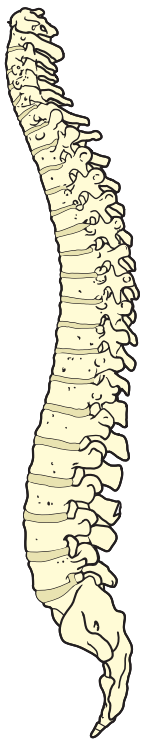


Fig. 33.2 Vertebral column

(b) Vertebral Column

(i) It consists of 26 serially arranged units (Fig. 33.2) called vertebrae (singular: vertebra).

(ii) Each vertebra has a central hollow portion called neural canal through which the spinal cord passes. The first vertebra is the atlas and it articulates with the occipital condyles of skull.

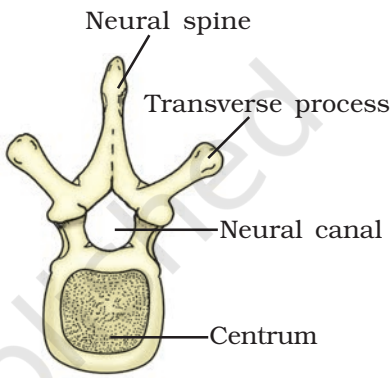


Fig. 33.3 A typical vertebra

(iii) Vertebral column has several types of vertebrae: cervical (7), thoracic (12), lumbar (5), sacral (1 which is fused), and caudal or coccygeal (1which is fused).

(iv) A typical vertebra (Fig. 33.3) has a — (i) centrum, the modified notochord (ii) two laterally projecting transverse process (iii) a neural canal through which passes the spinal cord (iv) a mid dorsal neural spine formed by the union of neural arch. Depending upon their location in the body, secondary modifications are seen in the length of transverse process and the length of neural spine. The two neighbouring vertebrae articulate with each other through their anterior and posterior zygapophyses. Intervertebral discs are present between the centra of two neighbouring vertebrae.

Note: The vertebral column forms the central axis of the body and keeps it erect. It encloses and protects the spinal cord and provides surface for the attachment of skull, ribs, pectoral and pelvic girdles, as well as several muscles in the neck, thorax, abdomen and waist.

(c) Rib Cage and Sternum

(i) Sternum forms the floor of branchial basket. It bears 7 (seven) notches for articulation with ribs. It has hexagonal disc at the top called manubrium. Lower end has a reduced bone called xiphoid process (Fig. 33.4).

(ii) Ribs can be put under two classes: the thoracic ribs, and the sternal ribs. The thoracic ribs articulate with the thoracic vertebrae, and the sternal ribs do so with the sternum. Some (7) of the thoracic ribs are attached to the sternal ribs with the help of ligaments, enabling the increase and decrease in volume of the thoracic chamber during respiration.

(iii) There are 12 (twelve) pairs of thoracic ribs. Each rib is a thin flat bone and is carried ventrally from the vertebral column. It has a head articulating with the centrum, and tubercle articulating with transverse process of vertebrae (Fig. 33.4).

(iv) 7 (seven) pairs of thoracic ribs are attached to the sternal ribs.

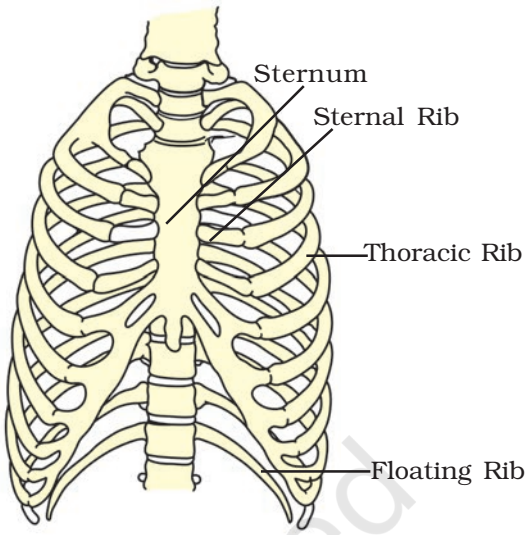


Fig. 33.4 Rib cage and sternum

(v) Last 5 (five) pairs of thoracic ribs do not articulate with sternal ribs, and are called false ribs. Among these, the last 2 (two) pairs of false ribs are free and are called floating ribs.

Note: Rib cage and sternum covers and protects the vital organs such as heart and lungs. The ribs are also helpful in breathing.

(d) Pectoral Girdle

(i) It consists of a clavicle and a scapula (Fig. 33.5).

(ii) Scapula is a large triangular flat bone with a slightly elevated ridge called spine. The spine projects as a flat, expanded process called the acromion.

(iii) The clavicle is a long slender bone with two curvatures. The clavicle articulates with the acromion.

(iv) Below the acromion is a depression called the glenoid cavity, for articulation of the head of the humerus to form the shoulder joint.

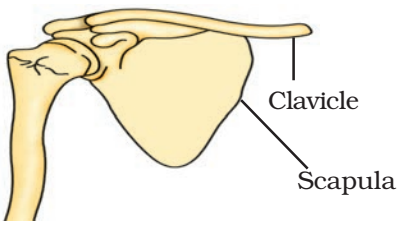


Fig. 33.5 Pectoral Girdle

Note: Pectoral girdle is formed of two halves. Each half consists of a scapula and a clavicle. The clavicle is commonly called collar bone. The third element of vetebrate pectoral girdle, the coracoid is highly reduced in man, and is present only by a small projection over the glenoid cavity.

(e) Pelvic Girdle

(i) It consists of two halves.

(ii) Each half is formed by the fusion of three bones - ilium, ischium and pubis (Fig. 33.6).

(iii) At the point of fusion of the above bones is a cavity called acetabulum to which the thigh bone articulates.

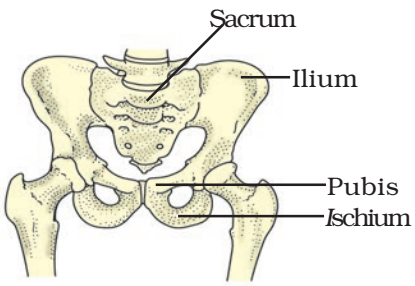


Fig. 33.6 Pelvic Girdle

(v) The two halves of the pelvic girdle meet ventrally to form the pubic symphysis.

Note: Pelvic girdle is commonly called hip bone. The ilium articulates anteriorly with the flat transverse process of sacral vertebrae.

(f) Bones of the Hand or Fore Limb

(i) It is made up of bones consisting of humerus, radius and ulna, carpals, metacarpals and phalanges (Fig. 33.7).

(ii) Humerus is a straight bone with a long shaft, and forms the upper arm. The head of the humerus fits into the glenoid cavity of the pectoral girdle. It has a crest at its proximal end in the form of deltoid ridge for the attachment of arm muscles. The distal end has a foramen and a trochlear process, which forms elbow joint with radius and ulna.

(iii) Radius-ulna consists of 2 (two) separate bones of the forearm namely radius and ulna. Ulna is more developed and has olecranon process at its proximal end, which forms elbow joint with humerus.

(iv) Carpals consist of 8 (eight) small bones arranged in two rows. It forms the wrist (Fig. 33.8).

(v) Metacarpals are made up of 5 (five) long bones forming the palm of hand.

(vi) Phalanges consist of 2 (two) in the thumb and, 3 (three) bones in the remaining four fingers, thus totalling 14 (fourteen) bones.

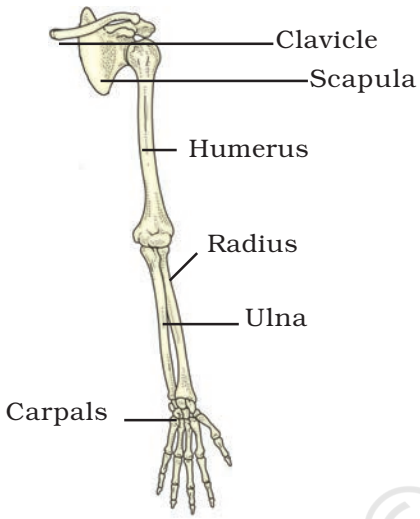


Fig. 33.7 Forelimb

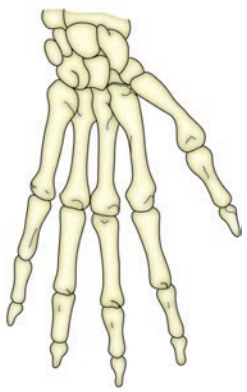


Fig. 33.8 Carpals, Metacarpals and Phalanges

(g) Bones of the Leg or Hind Limb

(i) It is made up of femur, tibia and fibula, patella (knee cap) tarsals, metatarsals, phalanges (Fig. 33.9).

(ii) The femur is the longest bone. The head of femur fits into the acetabulum of the pelvic girdle. The proximal end has trochanters for attachment of thigh muscles. The distal end has two condyles, which articulate with triangular shaped patella and proximal part of tibia to form knee on the ventral side.

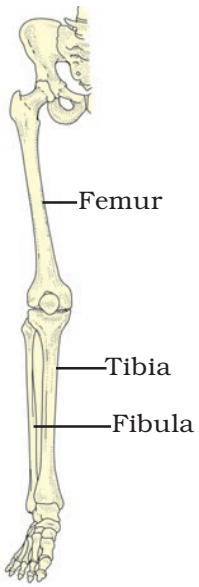


Fig. 33.9 Hind limb

(iii) Tibia-fibula consists of two separate bones namely tibia and fibula and is present in the shank region of leg. Tibia is more developed than fibula. Its proximal end articulates with femur and patella and forms knee.

(iv) There are 7 (seven) tarsal bones, which are arranged in two rows to form the ankle. The largest bone of these is calcareous which form heel (Fig. 33.10).

(v) Metatarsals consist of 5 (five) bones and form foot.

(vi) Phalanges consist of 2 (two) bones in big toe and three bones in each of the remaining toes thus totaling 14 (fourteen) bones.

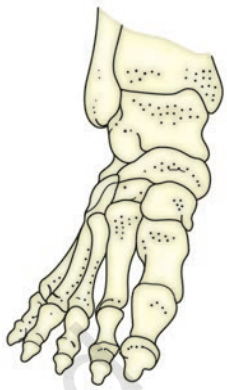


Fig. 33.10 Tarsals

Exercise 34

Aim: To study different types of joints in human skeleton.

Principle: Bones may be movable, slightly movable or immobile depending on the nature of joints. Joints are defined as regions/surfaces of contact between bone and cartilage.

Requirement: Specimen of human skeleton, charts and models of skeleton.

Procedure

(i) Observe the different types of bones and joints present in a human skeleton.

(ii) Draw labeled diagram of your observations.

Observation

(a) Gliding Joints

(i) These are flat joints, which allow back and forth or side-to-side movement of all or a few joining elements. However, twisting is not possible.

(ii) These joints are found between bones of tarsals and carpals (Fig. 34.1).

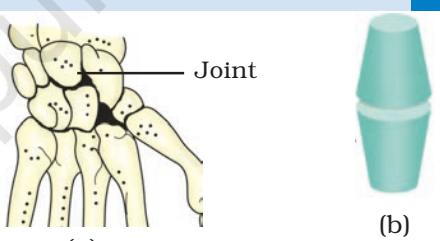


Fig. 34.1 Gliding Joints (a) hind limb bones showing the joint (b) Diagramatic representation

(b) Pivot Joints

(i) These joints allow rotational movement.

(ii) These joints are found between the atlas and axis vertebrae of backbone. It is the odontoid process of the axis vertebra over which the atlas along with the skull rotate (Fig. 34.2).

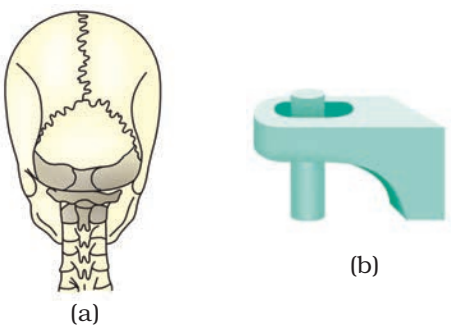
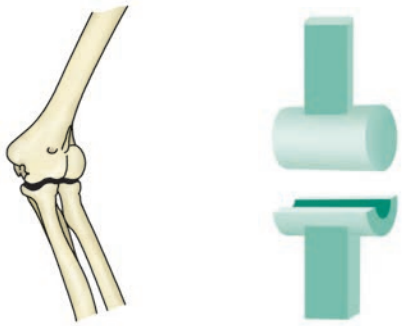


Fig. 34.2 Pivot Joints (a) Skull and vertebral column showing the joint (b) Diagramatic representation



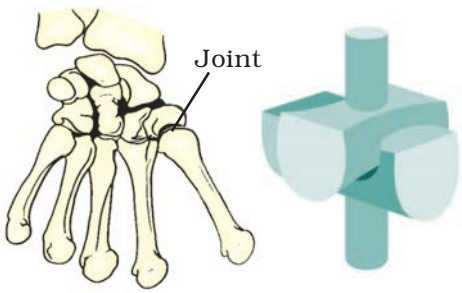
(a) (b)

Fig. 34.3 Hinge Joints (a) elbow bone showing the joint (b) Diagramatic representation

(c) Hinge Joints

(i) These joints allow movement in one plane only.

(ii) These joints are present in elbow and knee (Fig. 34.3).



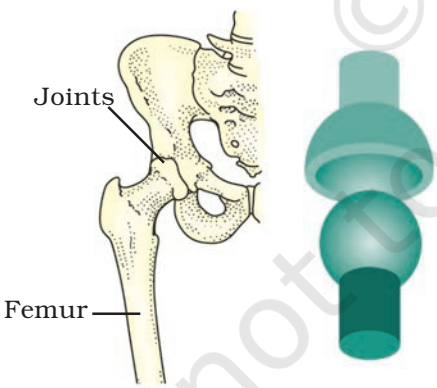
(a) (b)

Fig. 34.4 Saddle (a) carpals and thumb bone showing the joint (b) Diagramatic representation

(d) Saddle Joints

(i) These joints allow movement in two planes.

(ii) These joints are found in bones of metacarpals and carpals of thumb (Fig. 34.4).



(a) (b)

Fig. 34.5 Ball and socket Joints (a) Femur and pelvic girdle showing the joint (b) Diagramatic representation

(e) Ball and Socket Joints

(i) These joints allow movement in more than two planes (Fig. 34.5).

(ii) These joints are present between humerus with pectoral girdle, femur with pelvic girdle, and maleus with incus (in ear ossicles).