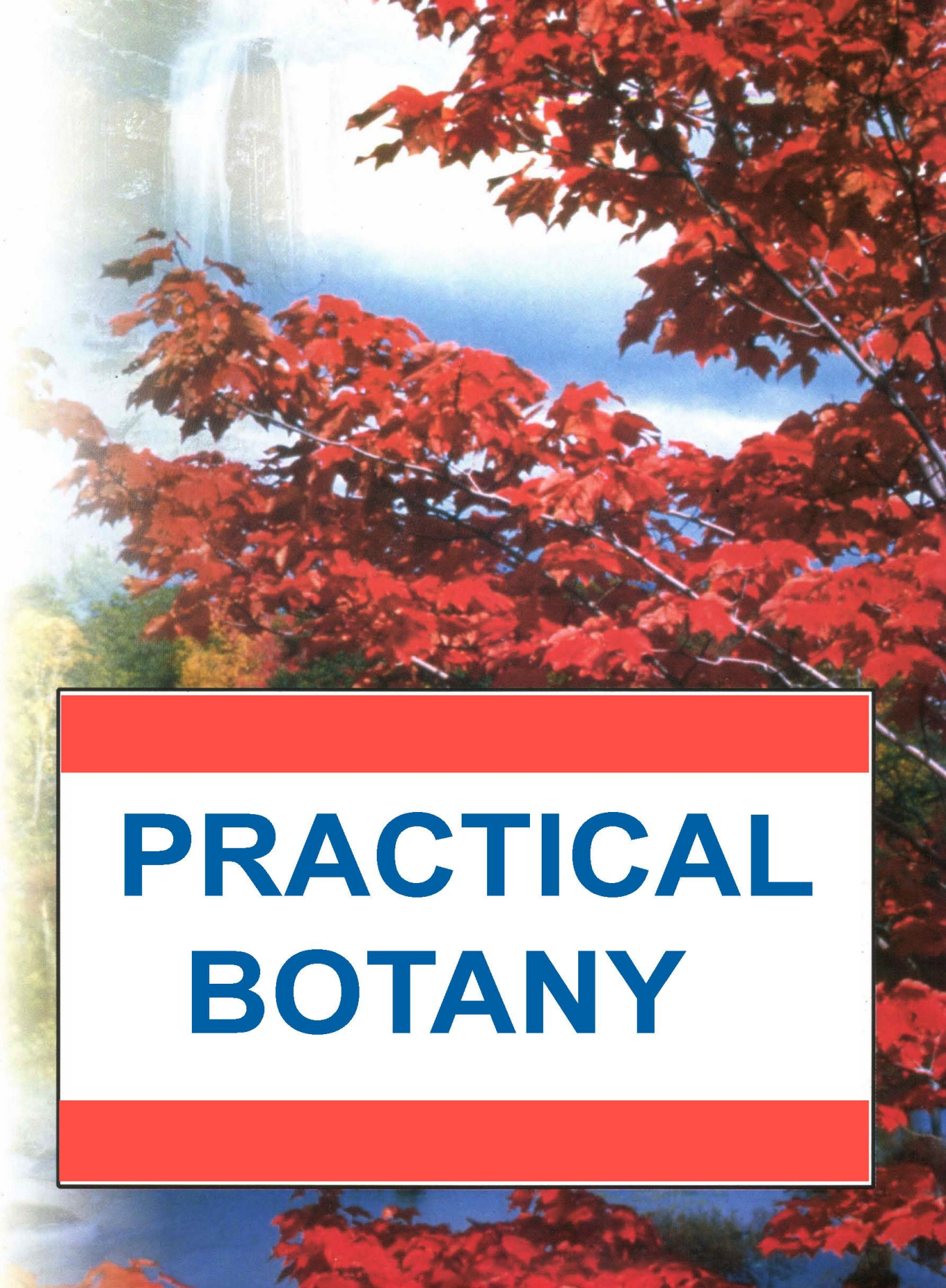
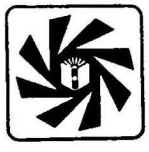


PRACTICAL BOTANY

Practical Botany



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1

Introduction to Laboratory Work

Laboratory work forms a very important aspect of scientific studies. The bookish knowledge needs to be provided with a natural vision that allows clear picture of the subject matter appearing in the book. Laboratory work also helps in understanding the descriptions otherwise difficult to realize by simple reading. It also sharpens the scientific skills of observation and analysis which would be valuable in life as well. Hence, laboratory work should be done with utmost sincerity and integrity.

In a botany laboratory one is expected to follow certain norms and etiquettes so that maximum benefits could be derived. The following tips would prove helpful

1. The facilities provided in the laboratory should be properly used.
2. Laboratory provisions should be handled with care.
3. The assigned work should be completed within stipulated period of time.
4. The laboratory working place and the equipments provided should be cleaned and rearranged in the same order in which these were provided to you.
5. It is also essential to read the exercises to be performed before attending the laboratory and performing the exercise.

The Work Schedule

Once inside the laboratory, it is expected that one keeps busy with one's own work. It is equally necessary that a definite plan of work is followed.

1. Listen to the instructions given by the teacher-in-charge at the start of the session.
2. Plan and assemble the apparatus as per the directions given to you.
3. Let the experiment run for the specified period of time.
4. Observe the changes or results when experiment is going on.
5. Note down the observations and the results.
6. If sections are to be cut, follow the methods used in your anatomy classes.
7. Keep the record of work in your note-book.
8. Write down the experiments in methodological manner.
   (a) Object
   (b) Materials required
   (c) Procedure or method
   (d) Observations
   (e) Results
   (f) Discussion/Conclusions
9. If diagrams are required, draw them using a sharp pencil and label the important aspects. The diagrams should be proportionate, neat, clean and without shading unless required.
10. Proper notes or description should also be written at appropriate places. These should be just sufficient for reproducing at the time of examination and hence should be precise.

Necessary Instruments

The variety of instruments required depends upon the nature of work. It has, however, been found convenient to prepare a small kit in suitable containers such as a pencil box containing the following —

1. A pair of forceps,

2. two fine, long handle, dissecting needles,
3. glass droppers,
4. good and sharp razor,
5. safety blade,
6. a fine hair brush,
7. a pair of sharpened pencils,
8. pencil eraser,
9. a clean and soft handkerchief and
10. practical record with cover file and spare pages, etc.

Microscope

It is the most indispensable instrument in a biology laboratory, so much so that it comes to be called 'The primary instrument of the biologists'. It helps to increase the resolving power (property to distinguish objects lying very close as separate bodies) of human eye which fails to recognise objects lying closer between 0.01 to 0.25 mm.

Some common types of microscopes are listed below—

1. dissecting microscope,
2. compound microscope,
3. binocular microscope,
4. phase contrast microscope and
5. electron microscpe, etc.

Of these, dissecting microscope and compound microscope are very commonly used by the students.

[I] Dissecting microscope

It is used for dissection, specially during taxonomic studies, embryo separation, etc.

Construction. It consists of basal foot, a vertical limb, stage and a lens. The basal foot is a stand. The limb has an attached stage made of glass plate. A folded arm which can be moved vertically holds the lens. A mirror is attached at the base of the limb.

Mechanical operation. The following are some steps while operating the microscope.

1. Move the lens and adjust it over the object.
2. Illuminate the object suitably by adjusting the mirror.
3. Focus the object by using adjustment screw.

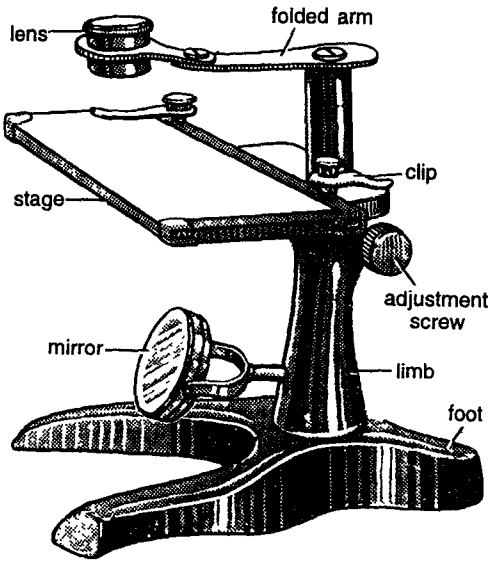


Fig. 1. A dissecting microscope.

[II] Compound microscope

It is one of the most commonly used and by far the most suitable microscope in the Botany Laboratory. At one time, it employs one ocular (eye piece) and one objective, in working position. As such, it is also known as monocular-mono-objective microscope.

Construction. The microscope is built around a strong basal foot and a vertical limb. The foot supports the vertical limb.

A round, rectangular or square stage is fixed to the limb. It is provided with spring clips to hold the slide in position.

A movable or fixed sub-stage is situated directly below the stage. It is provided with an iris diaphragm and condenser lens. Iris diaphragm is a wheel-shaped metal disc to regulate the aperture, through which light rays reach the condenser and are passed to an object. Condenser is a system of two or more lenses under the stage which receives parallel light rays from mirror and converge them at the level of stage.

A movable concave mirror is fixed at the lowermost part of the limb to focus a converging cone of rays at the level of specimen. Whether day or artificial light is used as a source, concave mirror converges the light if there are no condensing lenses.

Body of the microscope is composed of tube. At the upper end of the tube, is an ocular (eye piece) which can be changed for lower or higher values of magnifications. At the lower end of this tube is a revolving nose-piece with about three objectives viz. low power, high power and oil immersion. The magnifications of these lenses range from 3.2x to 100x. The conventional low power objective is 10x.

Tube of the microscope is vertically movable with the help of coarse and fine adjustment screws on the limb, operated by a rack and pinion system. Coarse adjustment moves the tube rapidly while fine adjustment screw does it gradually.

Mechanical operation. The following procedure is adopted while using the microscope.

1. Microscope is placed in maximum diffuse light. Direct sunlight is harmful for the eyes. The northern light is most suitable. If light source is artificial, filter (preferably blue coloured) is used.
2. Light is adjusted by turning the mirror towards the source of light and also by moving the sub-stage up and down, as well as with the help of iris diaphrragm.
3. A prepared slide is placed on the stage. Object is adjusted just over the stage aperure.
4. The object is located and focussed with a low-power objective using coarse adjustment.
5. If higher magnification is desired, nosepiece is turned to next higher power. Fine adjustment can be used freely at this stage, while the use of coarse adjustment is avoided.
6. High power objective and subsequent higher powers are used only when object is properly mounted under coverslip.
7. The object should always be observed with both eyes open.

Care. The following points should be kept in mind so that microscope is not spoiled and continues to serve the purpose for longer period.

1. Before and after the use, all the lenses and metal parts including stage should be cleaned. The lenses are cleaned with tissue paper, muslin cloth or clean and soft handkerchief.
2. Microscope is kept covered when not in use. Proper cases, plastic bags, bell jars or even a clean cloth can be used.

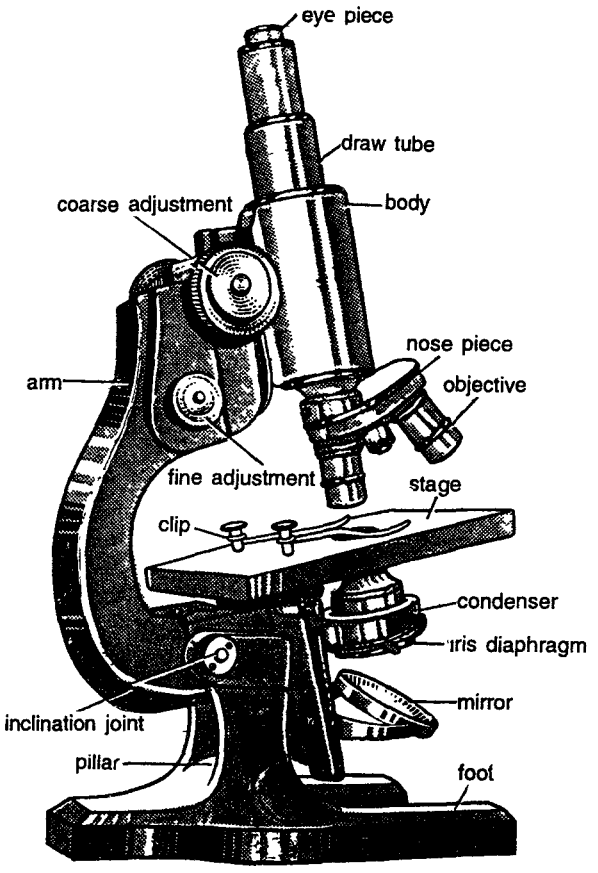


Fig. 2. A compound microscope.

3. Objectives should not be ordinarily removed from the nosepiece.
4. Operating screws, condenser, iris diaphragm, mirror and stage or stage clips should always be handled carefully.

Other Laboratory Provisions

Some other provisions available in the laboratory include staining rack, dropping bottles, slides, cover glasses, watch glasses, petri dishes, beakers, enamel trays, wash bottles, spirit lamp, hone, strop, dusters, etc. Some of these are described below.

1. Staining rack. The stains, chemicals, mounting media, etc., are stored in these bottles. This glass bottle has a narrow mouth fited with a slotted cock. Cock is provided with a beak that permits the liquid to flow out in drops.

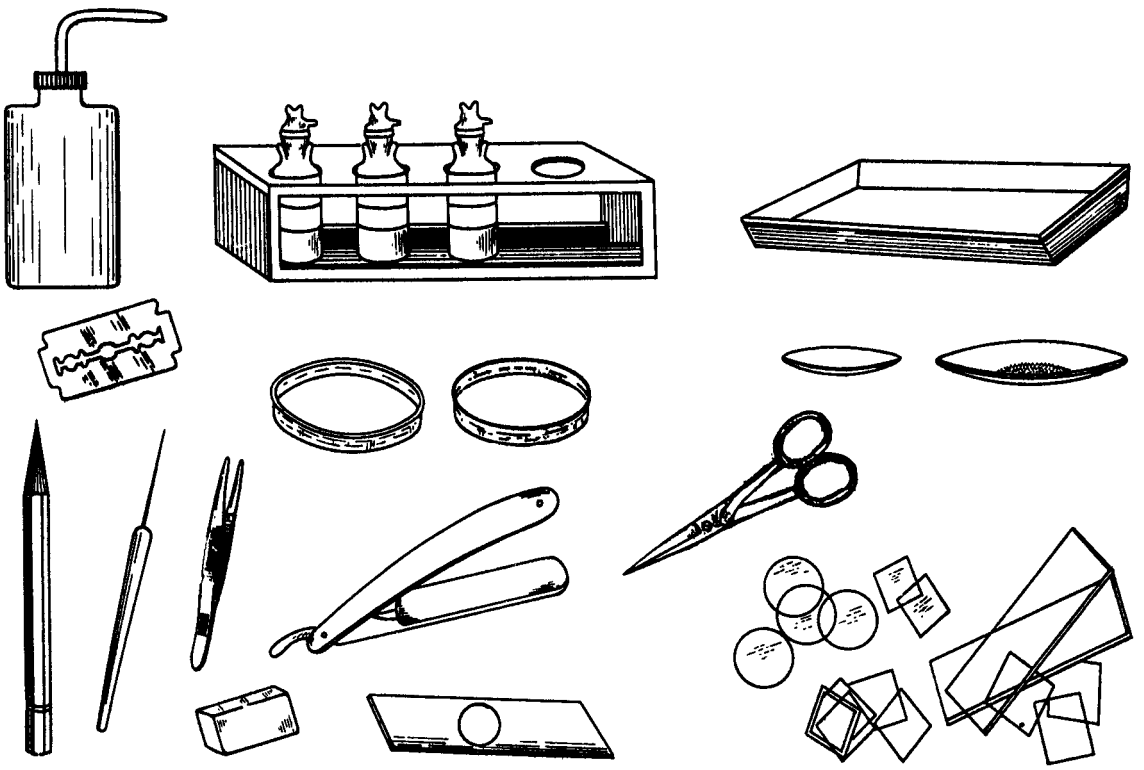


Fig. 3. Some laboratory provisions and necessary instruments

2. Dropping Bottle. The stains, chemicals mounting media, etc., are stored in these bottles. This glass bottle has a narrow mouth fitted with a slotted cock. Cock is provided with a back that permits the liquid to flow out in drops.

3. Slides. The size of slide is mostly 3" × 1" (25 mm × 75 mm). It is about 1 mm thick. These are used to mount the material under study.

4. Cover glasses. The cover glasses are mounted on the object when the preparation is finally ready. These may be either square or round shaped. The standard thickness of the coverslip is 0.17 mm.

Laboratory Techniques

[I] Section cutting

Sections of preserved material are cut in suitable planes for histological and ecological studies. Razor is suitable for cutting the sections in laboratory.

1. Planes. The following are a few commonly needed planes :

In case of cylindrical organs : (e.g. stems, roots, etc.)

Transverse. The section is cut by passing razor's edge at right angles to the longitudinal axis.

Longitudinal. The section is cut by passing razor's edge at right angles to the transverse axis. Two sections are possible in this plane.

(i) Radial Longitudinal section (R.L.s.) if it passes along one of the radii.

(ii) Tangential Longitudinal section (T.L.s.) if section is cut along one of its tangents.

In case of dorsiventral organs (e.g. leaf, thallus of livewort, etc.), transverse section is cut. It is known as vertical transverse section (being cut in vertical plane).

2. Method. Following steps would be useful for section cutting.

1. Soft, thin and small materials are placed in pith either by piercing a hole with a needle or by splitting it logitudinally with a blade. The piths used include carrot and radish roots, potato tubers, etc.

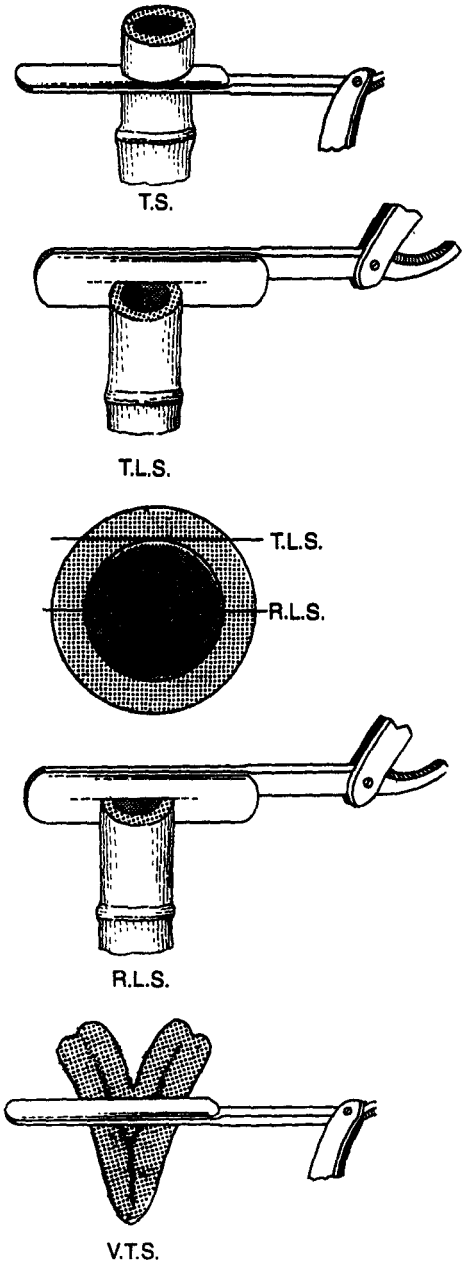


Fig. 4. Planes for section cutting.

2. A razor must be held properly to cut the section. The handle and the blade of the razor should be at right angles to one another. The handle should remain free while the index finger is placed on the hooked end of the razor; 1st, 2nd and 3rd fingers pressed against the thick back edge of the razor and thumb against the milled surface of the thick shank of blade.
3. The material or the pith with embedded materials is held between the thumb and the fingers of the left hand.
4. The material in the left hand and the razor's edge should form right angle.
5. The razor is now moved quickly over the material and the stroke is completed in one action only.
6. More and more uniform strokes are used till desired quality and number of sections are obtained. Care is taken to keep the material and the razor flooded with water.

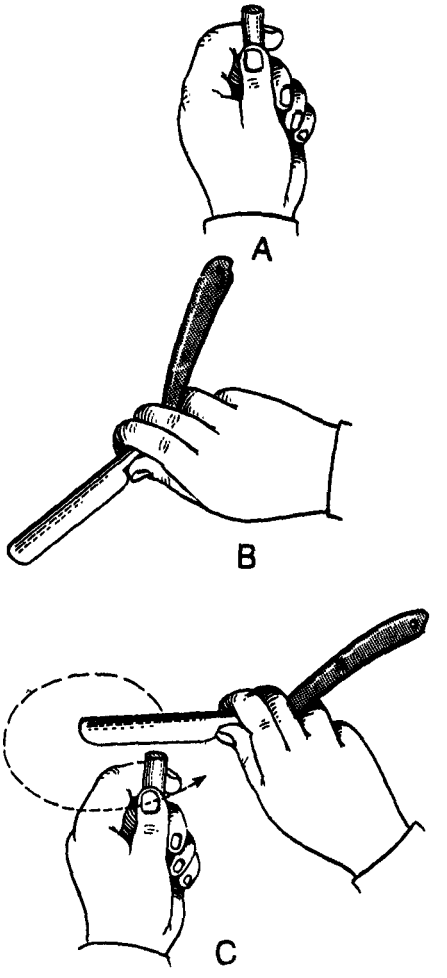


Fig. 5. Method of section cutting. **A.** holding the material. **B.** correct way of holding the razor, **C.** holding the material and stroke of the razor.

7. Sections float in water on the razor's edge. These are carefully lifted by a fine camel hair brush and then transferred to a watch glass containing water.
8. After the section cutting is over, the razor is wiped dry and clean without disturbing the edge. It is honed, stropped and encased.
9. The sections which float on water in the watch glass are considered to be thin
10. These sections are lifted by a hair brush, placed on a slide in a drop of water and observed through microscope. A thin and uniform section is selected for staining.

[II] Stains and staining

The selected sections need to be stained. The stains help to distinguish different tissues, cells or inclusions from one another by developing specific colours. Acetocarmine, Aniline blue, Crystal violet, Erythrosine, Hematoxylins, Fast green Light green and Safranin are some of the commonly used stains.

1. Specificity. Most of the stains are specific in reaction and are purposely used so that definite structures or substances are stained. The following are some of the stains used for staining different structures.

Achromatic figure
Aniline blue
Erythorosine
Fast green
Light green
Cellulose cell wall
Aniline blue
Delafield hematoxylin
Fast green
Light green
Lignified cell wall
Crystal violet
Safranin
Suberised cell wall
Safranin
Cytoplasm
Aniline blue
Erythrosine
Fast green
Light green
Cutinised cell wall
Crystal violet
Erythrosine
Safranin
Callose
Aniline blue
Chitin
Safranin
Proteins
Safranin
Mitochondria
Crystal violet
Plastids
Crystal violet
Iron hematoxylin
Nucleus
Crystal violet
Hematoxylin
Safranin
Chromosomes
Hematoxylin
Safranin

2. Single stains. Safranin or fast green is used alone to stain filaments of algae, fungi, sections of bryophytes, spores of pteridophytes, pollen grains of gymnosperms etc. Aniline blue or safranin is suitable for algae.

Following is the common method of staining.

1. The material is kept in a watch glass. A few drops of stain are added so that the material is immersed in the stain.
2. The material is allowed to remain so for a few minutes and allowed to take stain. The time required varies with materials.
3. After the stain is taken up, the excess of stain is washed off in water. The washing is repeated till stain stops coming out.
4. In some cases, excess stain is removed by acid water or acid alcohol if water alone fails to do so.
5. The stained material is ready for mounting.

3. Combinations. Commonly two or more stains are employed wherever tissue differentiation is found. Combination of acidic and basis dyes of contrasting colours is of general use. This permits the distinction of woody tissue from non-woody tissue. The following few combinations are commonly recommended —

1. hematoxylin and safranin,
2. safranin and fast green,
3. safranin and aniline blue,
4. safranin and crystal violet and
5. crystal violet and erythrosine.

4. Staining procedures. There are two types of preparations — semi-permanent and permanent. The procedure for semi-permanent and temporary preparations is given below. Certain preparations are made for temporary use. The material is studied and the slide is then discarded. The method for staining them is given below.

1. The selected sections are transferred from watch glass containing water to another watch glass containing principal stain (e.g. hematoxylin, safranin or crystal violet).
2. The sections are allowed to remain in the stain for sometime (for about 4-5 minutes).
3. Excess amount of stain is removed by washing the sections repeatedly with water. This can be seen under the microscope. The stain should be taken either by lignified or non-lignified tissues. Otherwise the section should be washed till the stain disappears from one type of tissue.
4. If destaining is not achieved, sections are washed with acid alcohol. In this case, further

washing with water is necessary till traces of acid are removed.

5. This is followed by transfer of sections to a watch glass containing counter-stain (e.g. safranin, fast green, eryhrosine). This stain acts on the tissue more rapidly than the principal stain. Therefore, section is kept in this stain for shorter duration (about a minute or two).
6. Excess of stain is removed by washing stained sections with glycerine (15-20%). The section should distinctly bring out demarcation between tissue system while preserving the colour of the stain.
7. The section is now ready for mounting.

[III] Mounting an object

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

1. Mounting media. Following are some of the common media used for temporary preparations.

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

(b) Glycerine. Pure glycerine diluted to 15-20% is widely used. Semi-permanent and temporary preparations are mounted in glycerine.

(c) Glycerine jelly. Jelly is also used for mounting. It is made of gelatin 1 : glycerine 7 : water 6.

Warm the gelatin for two hours by adding water. Phenol (1%) is added later. Add crystals of safranin if desired. Allow the solution to cool and settle into jelly.

Many other mounting media like canada balsam, cedar oil, dammar, balsam, venetian turpentines and synthetic resins are also used.

2. Care. Following care should be taken during mounting.

1. Object should be mounted in the centre of the slide. A simple method may prove suitable for this purpose. Take a piece of thick and white cardboard sheet larger than the size of the slide. Place the slide over it. Draw lines along all the four edges. Join all the four corner

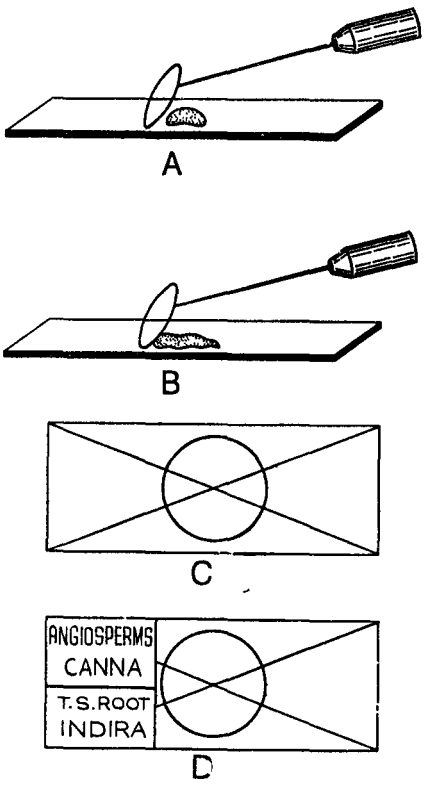


Fig. 6 Method of mounting coverslip.

Specific schemes for staining combinations
(For temporary and semi-permanent preparations)

1. Hematoxylin & safranin	2. Safranin & fast green or aniline blue
Select a section ↓ Stain with hematoxylin ↓ Wash with water ↓ Wash with ammonia water till stain turns blue (tap water is suitable if alkaline). ↓ Wash with water ↓ Stain with safranin ↓ Wash with glycerine ↓ Mount in glycerine	Select a section ↓ Stain with safranin (for 4-5 minutes) ↓ Wash with water ↓ Destain with acid alcohol if necessary ↓ Wash repeatedly with water ↓ Stain with fast green or aniline blue (for about a minute) ↓ Wash with glycerine ↓ Mount in glycerine

points diagonally by two lines. The point, where these two lines meet, gives the centre of the slide. While mounting an object, place the slide over this drawn sheet and an object on the central point.

2. No air bubbles should enter the medium while mounting. This results in drying of medium and preparation is spoiled. To avoid air bubbles, touch one side of the coverslip to the drop of mounting medium on the slide. Support the coverslip by needle and lower it gradually before finally removing it.
3. Use the necessary small quantity of mounting medium so that it does not flow on to the slide. If so, use little lesser quantity for the next preparation. The extra amount can be soaked by touching a piece of blotting paper to the edge of the coverslip.
4. Preparation should be clean, hence the edges of slide and the coverslip alone should be held between the fingers.
5. Labels are pasted uniformly on one side of the prepared slide. It should carry the name of the division or generic and specific names, the part mounted and the section's plane. At the bottom, the name of the student who had prepared the slide be written.

3. Sealing the coverslip. Temporary preparations can be sealed with Canada balsam, gum, dammar, nail polish, etc. Such a preparation is called a semi-permanent preparation.

Sealing is done by simply painting the edges of the coverslip with sealing agent in such a way that the space between the slide and the coverslip gets filled with the agent. It should prevent mounting medium from drying.

Similarly ringing table should be used for sealing the round coverslips. The use of Canada balsam in ringing is more convenient.

Record of Work

After the preparations are ready, these should be carefully observed, salient features noted and drawn on a practical record sheet. The following suggestions would prove useful.

1. Punched holes should be on the left hand side of the drawing sheet.
2. Always use a sharp and pointed pencil for thin and uniform lines.
3. Diagrams of the entire plant or its various aspects are drawn on the same page. The diagrams of other specimens should in no case be drawn on the same page.
4. The sequence of the diagrams should always be - external features, anatomy and then reproduction.
5. For anatomical studies an outline diagram followed by a cellular sketch of its suitable sector are drawn one above the other on the same page.
6. All the parts of the diagram must be labelled. Capital letters are used for labelling. The labels are arranged one below the other in a row.
7. Labelling lines should never cross one another. Beautification and shading is not required until specific effects are to be produced.
8. Every diagram must have caption at its bottom (e.g. T. s. stem).
9. Date is written in the left hand corner of the page.
10. Classification and name of the plant are given in the right hand corner of the sheet.
11. The description is written either on the reverse side of the drawing sheet or on a new facing page.
12. During description only technical terms are used. The points of identification are added in the end.
13. Anatomical studies are described as others. A section should be described starting from epidermis to the central region; give thickness of layer (how many cells deep), shape and size of the cells constituting it. Also give details of the structure of stele and vascular bundle.

Collection

Field work is one of the most essential part in Botanical study. It permits to see many types of plants, otherwise not seen and available in the laboratory. It is, therefore, advisable to go round many localities and explore their vegetation. Organised excursions or outings, led by experienced persons, add to the knowledge of common plants in nature.

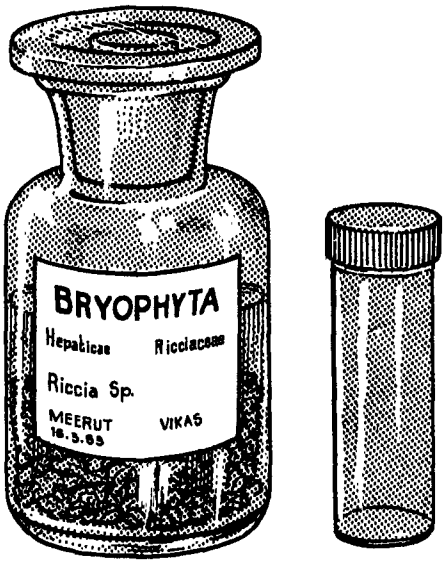


Fig. 7. Collection bottles.

While on a collection trip, (local or outstation) following things should be included in your kit.

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

2. Preservatives. Formalin-Acetic-Alcohol (FAA) or Alcohol 70% or Alcohol 90% and/or Formalin 6%-10%.

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

After collecting the plant, it should be immediately killed and preserved or pressed to avoid its rotting and dehydration. Plants are either sprinkled or immersed with a little of the killing agent at the spot. On return to the laboratory collected material should be transferred to new and suitable containers with fresh preservative. The plants should be completely immersed in the preservative.

Materials if collected in large quantities, are preserved in containers. But if materials are collected in lesser quantities a herbarium sheet is prepared. Even if large quantity of such plants is available, one plant with fertile parts be preserved in the form of a herbarium sheet, while others should be packed in a container.

Every tube should be labelled. It is desired to write the name of the specimen, place and date of collection. The place of collection and date should also be written on a small piece of white card with a pencil on the spot and inserted in the container. On return to laboratory, material is identified with the help of standard books. A label bearing name of the division and class to which the material belongs, the name of the material, date and place of collection and also the name of student is pasted on the container. All the containers should be of uniform size as far as possible.

Herbarium

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

[I] Preparation of herbarium sheets

1. Equipment. On excursion for the collection of plants, following items should to be carried.

1. Trowel or pick,
2. Collecting can (vasculum) or field plant press,
3. Heavy laboratory plant press,
4. Blotting papers or old newspapers,
5. Collecting sheets,
6. Mounting sheets,
7. Gum, gummed tape, labels, notebook, pen and pencil, etc.

Trowel or pick is used to dig out the plant as a whole wherever possible. A light-weight field press is most practical. It is made by taking two pieces of plyboard or heavy binder's board of 12" × 17" size. These are held together by two pieces of heavy cord or straps tied or buckled together. The press can be carried over the shoulders. A heavy plant press carries sheets of size at least 11.5 × 17 inches. It is made of iron and tied and tightened by iron chain and screws. This is used for pressing specimen after they are brought to the laboratory. Vasculum may be used in case only a small number of plants are to be brought back.

2. Collection. Collected plants are placed in the collecting sheets. The most practical size is 16.5 × 23 inches; when folded 16.5 × 11.5 inches. Old newspapers serve this purpose to an appreciable extent and a large supply should always be included in the kit.

A collected specimen should indude root, stem, leaves and flowers. The plants are placed between the sheets or newspapers in such a way that relation between different organs is maintained. Herbaceous plants, 2 feet or less higher, may be collected entire. These can be bent to V or N shape whenever necessary. The most desirable is to collect a branch, about one foot high, containing leaves and flowers. In cases, where entire plant or branch cannot be folded to the size of herbarium sheet, only reproductive and fruiting parts and a stem bearing a few leaves are collected.

Delicate reproductive parts collapse even if pressed fresh. These can be pressed perfectly by applying bits of moist paper to the fresh reproductive structures and spreading them when plants are placed in the press. If parts of the herbaceous plant are thick and difficult to dry, split them before placing on the collecting sheet.

Water plants collapse if dried by usual method. These should be rolled up in wet paper when in the field and brought to the laboratory. On return to the laboratory, these plants are placed in water and floated out on sheets of white paper. The sheets are taken out of water carefully, so that the various parts do not cohere. The white sheets are placed in the blotting paper and then dried as usual.

After specimen has been collected and placed in collecting sheet, it is kept in plant press. This collecting sheet be placed in between blotting papers, one on either side.

While on collection, it is important to note date, locality, habitat, height, method of branching, colour of reproductive parts, common name, etc. This should be noted separately in a field-book.

3. Pressing. The collecting sheets should be transferred to a heavy laboratory press. It must be remembered that after pressing specimen would acquire the same shape, as on collecting sheet. The press is securely tightened. It may also be equally useful if field press is kept under heavy weight. The press should be placed in a warm, well-aired place to dry.

After 24 hours, press is taken out and opened. The old newspapers and blotting sheets are replaced by new unused ones. At least such 3-4 changes are given at an interval of 2-3 days. An average specimen takes about a week for complete drying.

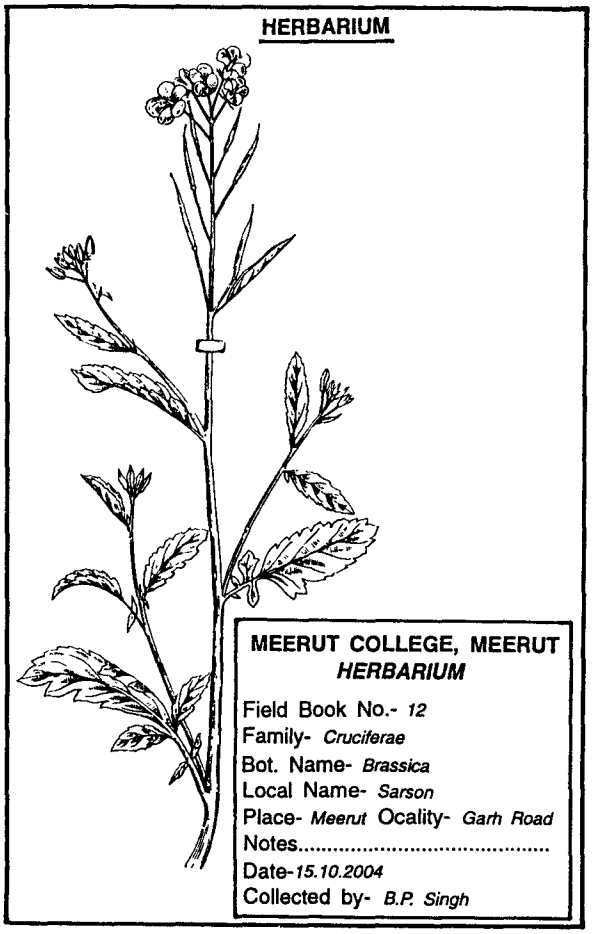


Fig. 8. A typical herbarium sheet.

Sometimes to hasten the process of drying plant press may be placed near the source of heat.

4. Mounting. The specimen are ready for mounting once they are completely dry. The standard size of the sheet is 16.5 × 11.5 inches. However, 16 × 10 inches size also has been used. The paper should be of good weight and not thin and flexible. The quality should be so, that is does not turn yellow even with a considerable lapse of time.

To mount the dried plant specimen, one of the following methods would be convenient —

1. The gum is spread on a glass plate and specimen is laid on it. As soon as all the parts come in contact with gum, it is lifted and then placed in a position on a mounting sheet.

2. The specimen is inverted and painted with gum by a brush and then transferred to a mounting sheet.
3. The specimen is placed on a herbarium sheet and small strips of gummed tape or cellulose tape are pasted at suitable places, so that most of the part remains loose.

After mounting the specimen, a label is pasted in the right hand lower corner of the sheet. This carries information regarding botanical name of the plant, common name, date, collector's name, place of collection, etc.

5. Arrangement of sheets. The sheets, are finally arranged in accordance with standard classification (preferably Bentham and Hooker's for Angiosperms or the most accepted ones for other groups of plants). The sheets are arranged into groups according to their class of utilization such as fibres, oils, beverages, durugs, etc. or species, genera, families, classes, orders, series and sub-divisions, etc. Each group is placed in a separate envelope, slightly larger than the herbarium sheets (e.g. 17 × 12 or 17 × 11 inches). Each of such envelopes must be labelled and a proper index be written or pasted over it.

6. Care of sheets. Herbarium sheets are often attacked by museum pests, fungi, etc. To guard against them, specimen are fumed with carbon bisulphide, 3-4 times a year. Mounted specimen may also be treated with mercuric bichloride or copper sulphate. To prevent them from attack, powdered naphthalene balls or gamaxene powder be also spread from time to time. This ensures durability and long life of the herbarium sheet.

2

Experiments in Plant Physiology

The Procedure

In a physiology laboratory student either watches a demonstration or performs the experiment himself. He is expected to follow certain instructions so that laboratory work becomes useful. .

The following are some of the useful hints.

1. A student should first read the procedure of the experiment from the book and then attend the laboratory.
2. The details and the instructions given by the teacher in the laboratory should be patiently heard and carefully understood.
3. The theoretical aspects involving the experiment may also please be already read and kept in mind while conducting or observing the experiment.
4. It is important to remember the equipment required and how to assemble it.
5. After procuring the necessary equipment assemble it and then experiment should be started.
6. The expected observations may be carefully noted.
7. Once the experiment is over, write the various details under following heads —
   (a) Object
   (b) Materials required
   (c) Procedure or method
   (d) Observations or results
   (e) Conclusions
8. See that conclusions drawn are compatible with the theoretical background.

Water Relations

Exercise 1

***Object :* To study the permeability of plasma membrane using organic solvents.**

Requirements

Beet root, distilled water, test tubes, test tube holder, test tube stand, razor/cork borer, 5% ethanol, etc.

Procedure

1. Cut slices or cylinders of beet root by razor or cork borer.
2. Repeatedly wash the slices or cylinders with distilled water till pigment stops diffusing into the water.
3. Fill the test tubes — (A) with distilled water and another (B) with 5% ethanol.
4. Place a few slices or beet root cylinders in each tube.
5. Allow the test tubes to remain as such for about 30 minutes.

Results

The red anthocyanin pigment diffuses in test tube B containing 5% ethanol but not in test tube A containing distilled water.

Conclusion

The appearance of red colour in the test tube containing ethanol is due to diffusion of anthocyanin pigment from inside the cell to external medium. In a living plant cell selectively permeable cell membrane prevents outward diffusion of anthocyanin Toxic substances like ethanol kill the cell and, therefore, the cell membrane also which looses its selective permeability and allows anthocyanin to diffuse out.

Note : Organic compounds like chloroform, formalin, etc. can also be used in place of ethanol.

Exercise 2

***Object :* To study the effect of temperature on permeability of plasma membrane.**

Requirements

Beet root, razor or cork borer, distilled water, test tubes, test tube holder, test tube stand, tripod stand, beaker, wire gauze, etc.

Procedure

1. Cut slices or cylinders of beet root using razor or cork borer.
2. Wash the slices/cylinders repeatedly with distilled water till pigment stops diffusing into the water.
3. Fill the test tube with distilled water and place the already washed slices/cylinders of beet root.
4. Place a beaker containing water on tripod stand. The test tubes with beet root cylinder are placed in the beaker.
5. The beaker is heated by using spirit lamp.
6. Allow the heating till red coloured anthocyanin diffuses into the water of the test tube.

Results

The water in test tube appears red coloured.

Conclusion

The diffusion of anthocyanin is the result of death of cell membrane due to higher temperature.

Exercise 3

***Object* : To demonstrate the osmosis by using potato osmometer.**

Requirements

Potato tubers, sugar solution, water, beakers, petri dishes, capillary tubes, thread, etc.

Procedure

1. Peel off the outer skin of the potato tuber. Cut one end-flat. Make a hole or cavity in the centre of the potato almost up to the bottom. Following three (2,3 and 4) conditions are created.
2. Fill the cavity with sugar solution. Fit an airtight cork at the mouth of the cavity. Insert a capillary tube in the hole of the cork. Mark the level of sugar solution, if any, in the capillary tube. Put this assembly in a petri dish filled with water. Allow the experiment to remain as such.
3. The same experiment is done in the following way also. Fill the cavity of the potato tuber with sugar solution. Mark the level in the cavity by piercing a pin. Place this tuber in a petri dish containing pure water.

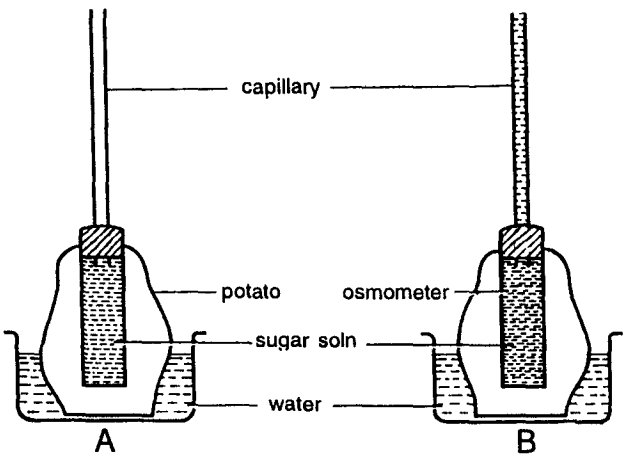


Fig. 1. Demonstration of osmosis by using potato osmometer. **A.** Experiment at the beginning. **B.** Experiment at the end. Note the change in level.

4. The cavity of the other tuber is filled with water and the level is marked by piercing a pin. This tuber is placed in a petri dish containing concentrated sugar solution.

Results and conclusions

In case 2, the level in the capillary begins to rise and becomes stable after sometime. This is due to osmosis where water moves from the petri dish into the cavity of the tuber through semi-permeable tuber cells.

In case 3, the initial level A rises to level B after some time. The increase is due to the movement of water from the outside (*i.e.* petri dish) through semi-permeable membranes of the potato tuber. Since the osmosis shows the movement of water from outside into the tuber, the process is endosmosis. There is an increase in the volume whenever endosmosis takes place.

In case 4, the initial level A falls to B, after sometime. The decrease or fall in the level is due to the movement of water from inside the tuber to the outside. The outer solution in this case is hypertonic and hence the water moves out of the cavity of tuber. This phenomenon of osmosis is called exosmosis.

Demonstration of exosmosis and endosmosis can also be repeated by placing fresh grapes, potato slices, garden beet, etc. in sugar solution and water, respectively.

Exercise 4

***Object* : To study the phenomenon of plasmolysis.**

Requirements

Tradescantia/Rhoeo discolor leaf, safety blade, sugar solutions of different concentrations, cover-slips, slides, water, microscope, etc.

Procedure

1. Peel off a small segment from the lower leaf surface. This can be done by tearing the leaf obliquely with a single jerk or scraping it with safety blade.

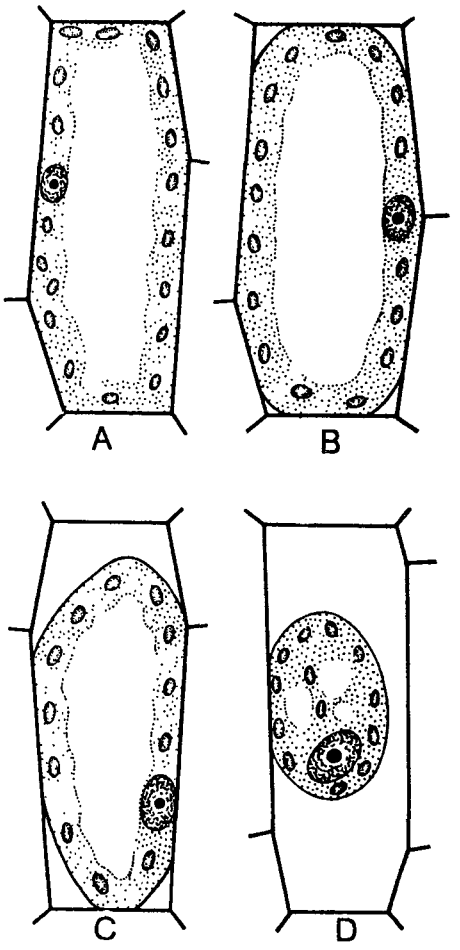


Fig. 2. Cells showing plasmolysis. **A.** Normal cell, **B.** Cell showing withdrawl of contents from the wall, (incipient plasmolysis), **C.** Further contraction of cell contents, **D.** A plasmolysed cell.

2. Mount the peel in a drop of water on a slide and then place a cover-slip. Observe under the microscope. Draw the protoplasm and let the preparation be called as A.
3. Take another peel, cut into pieces and similarly mount pieces in a drop of sugar solutions of different concentrations (0.25 M, 0.5 M and 0.75 M). Observe each preparation under the microscope. Draw the boundaries of protoplasm and let the preparations be called as B, C and D respectively in order of increasing concentrations.

Results

In condition **A** the cell structure can be seen clearly. The cells are turgid and protoplasm is closely pressed against the cell wall.

B. When slightly concentrated sugar solution (0.25 M) is used for mounting, the cell contents withdraw a little from the cell wall. Colourless space between cell wall and the coloured cell sap, is distinct.

C. When a little more concentrated sugar solution (0.5 M) is used, the cell contents move appreciably away from the cell wall, leaving a considerable space between the wall and the sap.

D. When the peel is mounted in a drop of concentrated sugar solution (0.75 M) the cell contents withdraw from the cell wall and shrink into a small, round and ball-like form.

Conclusions

Preparation **A** shows normal condition where cell sap presses the protoplasm against the cell wall which is slightly inflated. The cell is called turgid.

B. The withdrawal of the cell contents is due to the loss of water from the cell (exosmosis). The small space between the cell wall and the contents indicates the beginning of plasmolysis, and is known as incipient plasmolysis.

C and **D**. With the increase in the concentration of sugar solution outside water from the cell comes out and the space between cell wall and the contents increases. Finally, due to continued exosomosis the cell contents shrink and collect on one side. Such a cell is called plasmolysed.

In a plasmolysed cell, the space between the hypertonic solution cell wall and the contents is filled with the same solution that is placed outside the cell. The incipient plasmolysis can be stopped if such a cell is placed either in pure water or hypotonic solution. The cell then attains its normal structure (turgidity) due to endosmosis. The process is known as deplasmolysis. But if the plasmolysis continues, the cells show desiccation and plant becomes permanently wilted.

(This experiment can also be done by using petals of *Thunbergia* and *Luffa*, leaves of *Zebrina* and *Hydrilla*, staminal hairs of *Tradescantia*, and vegetative and large filaments of *Spirogyra*).

Exercise 5

***Object* : To determine the osmotic potential of vacuolar sap by plasmolytic method.**

or

To determine osmotic pressure of onion scale/ *Rhoeo* leaf peel by plasmolytic method.

Requirements

Onion bulb, *Rhoeo* leaves, 6 petri dishes, 6 test tubes, test tube stand, 2 pipettes, 2 beakers, hair brush, distilled water, 1 M sucrose solution, slides, cover-slips, microscope, graph paper, razor/blade/ sharp scalpel, pencil, marker, etc.

Procedure

1. Take 6 petri dishes and 6 test tubes.
2. Label them appropriately for each of the following sucrose solutions :
   0.3 M, 0.35 M, 0.40 M, 0.45 M, 0.50 M, 0.60 M
3. Using suitable pipettes, a beaker of distilled water, and a beaker of 1 M sucrose solution (342.30 gm in 1000 cc) prepare 20 cc of sucrose solution of the required concentrations in each test tube.
4. Mix the solutions thoroughly by shaking. Pour the solutions to appropriately marked petri dishes.
5. Take onion leaf. Cut the inner epidermis into 6 squares of approximately 5 mm, using sharp razor or blade. Place one square piece of epidermal tissue into each petri dish. Ensure that the piece is completely immersed in the solution.

***Table 1.* Sucrose dilution table.**

S. No.	Concentration of sucrose solution	Volume of distilled water (cc)	Volume of 1 M sucrose solution (cc)
1	0 30 M	14	6
2.	0 35 M	13	7
3.	0 40 M	12	8
4.	0.45 M	11	9
5.	0 50 M	10	10
6	0.60 M	8	12

Table 2. **Solute potentials of given sucrose solutions at 20°C.**

Concentration of sucrose solution	Solute potential (Kpa)	Solute potential (atm)
0.25	– 680	– 6.7
0.30	– 820	– 8.1
0.35	– 970	– 9.6
0 40	– 1120	– 11.1
0.45	– 1280	– 12.6
0.50	– 1450	– 14.3
0.55	– 1620	– 16.0
0.60	– 1800	– 17.8
0.65	– 1980	– 19.5

6. Allow the epidermal peelings to remain undisturbed for about twenty minutes.
7. Remove the epidermal peel from 0.60 M solution and mount it on a slide in sucrose solution of the same concentration. Add a cover-slip and examine using microscope.
8. Select a suitable area of cells using low power. Change to medium or high power objective and observe to record plasmolysed and unplasmolysed state of a fixed number of cells (such as 10, 50, 100 .....). Cells showing protoplast pulled away from the protoplast should be counted as plasmolysed.
9. Repeat the above procedure for all other pieces of epidermal peels by mounting them in their respective solutions.
10. Count the total number of cells observed and the number of plasmolysed cells counted amongst them.
11. Read the graph to determine the molarity of sucrose solution which causes 50% of the cells to plasmolyse.
12. Determine the percentage of plasmolysed cells (vertical axis) against molarity of sucrose solution (horizontal axis) using the data thus collected (Use table 2).
13. From this graph determine the solute potential (osmotic potential) of the solution which caused 50% plasmolysis. This is equal to the mean solute potential of the cell sap.

Results

The graph shows that solution of 0.40 M caused plasmolysis in 50% of cells. Thus solute potential of the cell sap would be – 1120 kPa or osmotic potential would be – 11.1 atm.

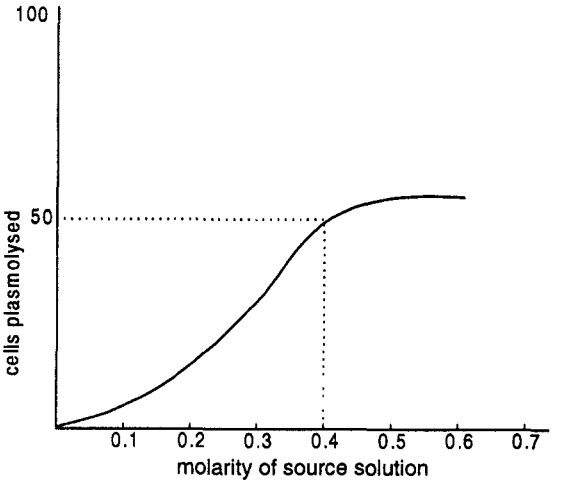


Fig. 3. Percentage of epidermal cells plasmolysed in different concentrations of sucrose solution.

Conclusion

The solute potential is determined by using following relationship.

ψ of the cell = $\psi_s + \psi_p$ ∴ $\psi_p = 0$, then $\psi = \psi_s$

At this stage there is no pressure potential or turgor pressure. Since such a stage is difficult to observe, incipient plasmolysis or stage at which plasmolysis has just begun is considered.

Exercise 6

***Object* : To determine the water potential of plant tissues.**

Requirements

Fresh potato tuber, 6 petri dishes, 5 test tubes, test tube stand, 2 pipettes, 2 beakers, distilled water, 1 M sucrose solution, scalpel/knife, cork borer, beakers, graph paper, pencil, marker, etc.

Procedure

1. Label six petri dishes, one for each of the following : distilled water, 0.1 M, 0.25 M, 0.50 M, 0.75 M and 1.0 M sucrose solutions. Label 5 test tubes similarly for sucrose solutions only.
2. Using graduated pipette, a beaker of distilled water, and a beaker of 1 M sucrose solution (342.30 gm in 1000 cc), prepare 20 cc of

Table 3. **Plasmolysed and unplasmolysed cells observed during experiment.**

Serial No.	Sucrose Conc. (M)	No. of Observations	Total No. of Cells	No. of Plasmolysed cells	No. of Unplasmolysed cells
1	0 30	1			
		2			
		3			
2.	0 35	1			
		2			
		3			
3	0 40	1			
		2			
		3			
4	0 45	1			
		2			
		3			
5	0 50	1			
		2			
		3			
6	0.60	1			
		2			
		3			

sucrose solution of the required concentration in each test tube.

3. Shake the tubes to mix the solutions thoroughly.
4. Pour the solutions in the appropriately labelled petri dishes. Place them on graph paper making sure that the bottom is dry.
5. Add 20 cc of distilled water to the remaining petri dish.
6. Using a cork-borer of approximately 1 cm diameter obtain 12 cylinders from a single potato tuber.
7. Cut all the 12 cylinders into measured and uniform length (about 5 cm) with a razor blade giving a clean transverse cut.
8. Complete preparation of cylinders as quickly as possible so that there is no loss of water due to evaporation.
9. Completely immerse two cylinders in each petri dish and immediately measure their lengths against the graph paper seen through the bottoms of petri dishes.
10. Agitate the contents of each dish.
11. Allow the cylinders to remain immersed for at least one hour. Make sure that petri dishes are covered during this period.
12. Measure the lengths again and calculate the mean percentage change in length. Plot a graph of the mean percentage change in length (vertical axis) against the molarity of sucrose solution (horizontal axis). Changes in length are proportional to changes in volume.
13. Determine the molarity of sucrose solution that causes no change in length by reading the graph.
14. Plot a graph of solute potential (vertical axis) against molarity of sucrose solution (horizontal axis) using the data provided in table.
15. From this graph determine solute potential of the solution which caused no change in length. The water potential of the tissue is determined by the following formula.

Table. 4. **Solute potentials of given sucrose solutions at 20°C.**

Concentration of sucrose solution (molarity)	Solute potential (kPa)	Solute potential (atm)
0.10 M	– 260	– 2.6
0.25 M	– 680	– 6.7
0.50 M	– 1450	– 14.3
0.75 M	– 2370	– 23.3
1.00 M	– 3510	– 34.6

$$\psi \text{ cell} = \psi \text{ external solution} = \psi_s \qquad \textit{(B-52)}$$

Table. 5. **Lengths of potato tuber cylinders left in distilled water and different concentrations of sucrose solutions to determine water potential.**

Serial No.	Molarity of sucrose solution	Length of cylinder (in cm)			
		at the start		after 1 hour	
		1	2	1	2
1.	Distilled water	4.8	5.0	5.0	5.3
2.	0.10 M	5.1	4.8	5.3	4.9
3.	0.25 M	5.2	4.8	5.2	4.9
4.	0.50 M	4.9	5.0	4.9	5.0
5.	0.75 M	4.9	4.9	4.6	4.7
6.	1.00 M	4.8	4.9	4.7	4.6

Results

1. In this case there was no change in length at 0.50 M solution of sucrose.
2. The solute potential of this sucrose solution is – 1450 kPa (= – 14.3 atm). Therefore, the water potential of the potato tuber tissue is – 1450 kPa or – 14.3 atm.

Exercise 7

***Object* : To demonstrate imbibition by plaster of Paris Method.**

Requirements

Plaster of Paris, water, petri dishes, etc.

Procedure

1. Take a small amount of plaster of Paris.
2. Add proportionate amount of water to wet the POP powder.
3. Mix thoroughly and prepare a ball.
4. Add a few more drops of water.
5. Allow it to remain as such for 10-15 minutes.

Results

The ball of POP appears swollen.

Conclusion

The increase in the volume is due to a special type of diffusion called imbibition. The substances high in colloidal material are very good imbibants.

Table 6. **Osmosis, various External media and the Result.**

Cell Sap	External Medium	The Result
1. Concentration more i.e. (a) cell sap concentrated or (b) less solvent mols. Cell sap **Hypertonic**	1. Concentration less i.e. (a) solution dilute or (b) more solvent mols. Solution **Hypotonic**	(i) external solution hypotonic, (ii) water moves into the cell, (iii) cell volume increases, (iv) Process is **endosmosis.**
2. Concentration less i e. (a) cell sap dilute or (b) more solvent mols Cell sap **Hypotonic**	2. Concentration more i.e (a) solution concentrated or (b) less solvent mols Solution **Hypertonic**	(i) external solution hypertonic, (ii) water moves out of the cell, (iii) cell volume decreases, (iv) process is **exosmosis**
3 Concentration same in both. same number of solvent mols.	3. Solution **Isotonic**	(i) both solutions **isotonic** (ii) no movement of water, (iii) no change in volume

Transpiration

Exercise 1

***Object* : To study different types of stomata.**

Requirements

Leaves of *Citrullus/Capsicum/Tridax/Tagetus/Sedum/ Brassica/Vigna/Dianthus/Ocimum,* etc., slides, coverslips, microscope, water, safranin, glycerine, needles, forceps, etc.

Procedure

1. Tear the leaf suddenly with force keeping the lower epidermis upwards.
2. A thin membranous lower epidermis gets separated near the broken edges. Pull this membranous part into a strip with forceps or fingers.
3. The strip is stained with 1% aqueous safranin, washed in water and then mounted in glycerine.

Observations

1. The stomata are generally present on the lower leaf surface.
2. A typical dicotyledonous stomatal apparatus consists of guard cells and the surrounding accessory or subsidiary cells.
3. The guard cells are bean-shaped or kidney-shaped. The walls are unevenly thickened. The outer convex surface is thin and elastic while the inner concave surface is thick and non-elastic.
4. Each guard cell has a prominent nucleus. Chloroplasts are discoid and are arranged centrifugally (near the wall).
5. The subsidiary cells or accessory cells are epidermal cells associated with guard cells. These are generally morphologically distinct from the other epidermal cells.

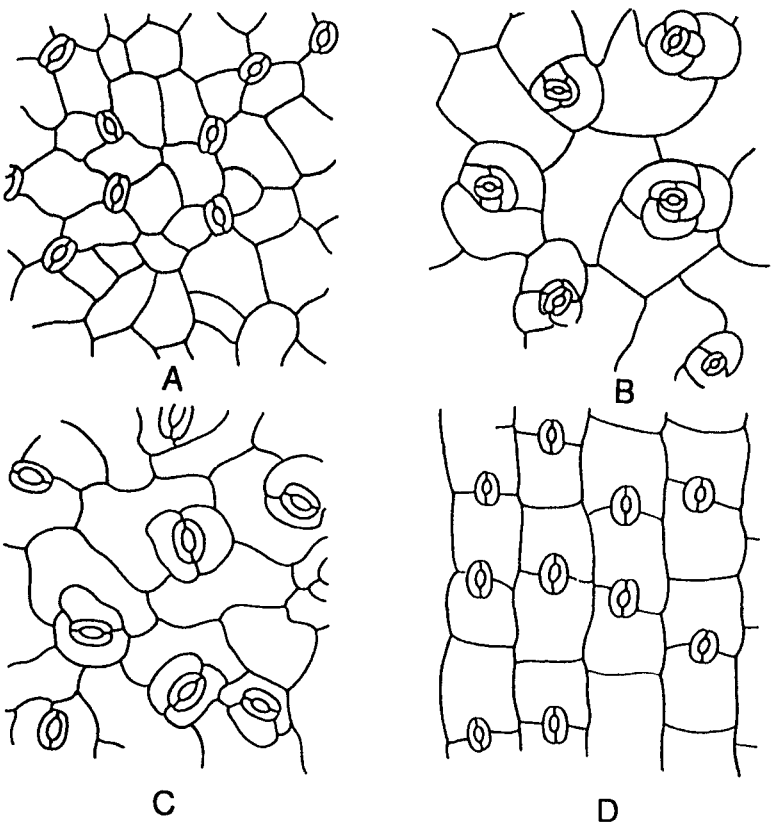


Fig. 1. Different types of stomata **A.** Anomocytic, **B.** Anisocytic, **C.** Paracytic, **D.** Diacytic

6. On the basis of number and arrangement of subsidiary cells, Metcalfe and Chalk (1950) proposed following four types of stomata in dicotyledons.

(a) Anomocytic. (Irregular-celled type; formerly Ranunculaceous type). Subsidiary cells are not present and several ordinary epidermal cells irregularly surround stomata, e.g. *Citrullus* (Eng.— Watermelon, Hindi — Tarbooz), *Capsicum* (Eng. — Chillies, Hindi — Hari mirch), *Tagetes* (Eng. — Marigold, Hindi — Genda), *Tridax*, etc.

(b) Anisocytic. (Unequal-celled type; formerly Cruciferous type). There are three subsidiary cells surrounding the stoma. Of these, one is distinctly smaller than the other two; e.g.; *Brassica* (Eng. — Mustard, Hindi — Sarson), *Sedum*, etc.

(c) Paracytic. (Parallel-celled type, formerly Rubiaceous type). In this type one or more subsidiary cells flank the stoma, parallel to the long axis of the guard cells, e.g., *Vigna mungo* (Eng. — Black gram, Hindi — urd), *Vigna radiata* (Eng. — Green gram, Hindi — Mung), etc.

(d) Diacytic. (Cross-celled type, formerly Caryophyllaceous type). In this type a pair of subsidiary cells with their common walls at right angles to the long axis of the guard cells, surround the stoma, e.g., *Dianthus* (Eng. — Carnation, Pink), *Ocimum* (Eng. — Basil, Hindi — Tulsi); etc.

Exercise 2

***Object* : Demonstration of the stomatal and cuticular transpiration by four leaves method.**

Requirements

Four fresh dorsiventral leaves, thread, stands, grease, etc.

Procedure

1. Take four similarly grown leaves of a plant. Smear grease at the cut end of the petiole.
2. Apply grease on both the surfaces of leaf A, lower surface of leaf B, and on upper surface of leaf C. Leave leaf D without grease.
3. Note the changes in the leaves after some time.

Results

Following results are obtained at the end of the experiment.

Leaves	Surface with grease	Results
D	no grease	first to dry
C	upper	second to dry
B	lower	third to dry
A	both	little change

Conclusion

The dorsiventral leaves possess stomata on both the surfaces. The frequency of stomata on the lower surface is more than the upper surface. Hence following conclusions are drawn.

Leaf A. The grease applied on both the surfaces completely checks loss of water and, therefore, the leaf does not wilt.

Leaf B. The grease applied on the lower surface blocks most of the stomata. Thus, there is no transpiration from the lower surface. The transpiration from the upper leaf surface continues at a low rate, number of stomata being very less.

Leaf C. The grease on the upper surface blocks only a few stomata. The lower surface with large number of stomata continues to loose water. Thus, this leaf dries faster than leaf A (with no transpiration) and leaf B (with transpiration from upper surface only).

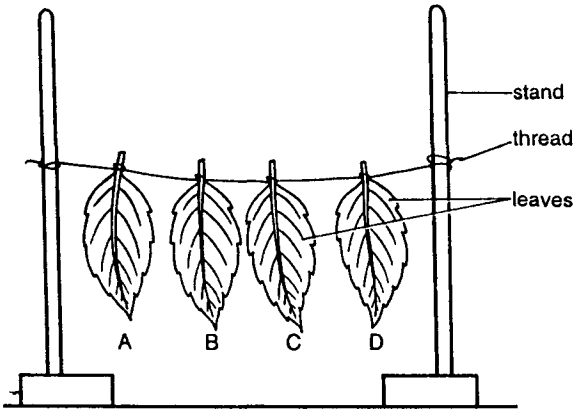


Fig. 2. Four leaves experiment to demonstrate stomatal and cuticular transpiration. **A.** Both surfaces with grease, **B.** Lower surface greased, **C.** Upper surface greased, **D.** Both surfaces without grease.

Leaf D. The first leaf to dry is, however, D. This is because the leaf transpires water from both upper and lower surfaces.

Exercise 3

***Object* : To study the relative rates of water-vapour loss (transpiration) from the leaf surfaces of different plants by cobalt chloride method.**

Requirements

A potted plant, stop-watch, filter papers, cobalt chloride solution (3%), glass slides, clips, desiccator, punching machine, etc.

Procedure

1. Dip filter papers in 3% cobalt chloride solution. Squeeze out excess solution. Dry the filter papers and cut discs of suitable sizes or punch holes with punching machine. Store these discs in a desiccator.
2. Take a potted and well watered plant. Place the dried discs of cobalt chloride filter paper, one each on the upper and lower leaf surfaces. Press them closer to surfaces by glass slides. Clip the slides together.
3. Note the time taken by the filter paper discs to change its blue colour to pink.
4. Repeat the experiment under different conditions and with different types of plants.

Results

Observations should be tabulated in the following form.

Plants : (Different plants can be used.)
Condition : Exccessive light/shade/wind/etc.

Sr. No.	Time taken by cobalt chloride paper to turn pink	
	Lower Surface	Upper Surface
1	25 seconds	30 seconds
2	22 seconds	30 seconds
3	20 seconds	28 seconds
4.	24 seconds	30 seconds
5	18 seconds	26 seconds

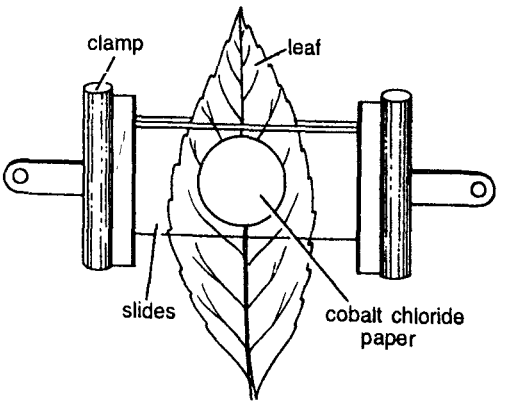


Fig. 3 Cobalt chloride method to study the relative rates of transpiration from leaf surfaces

The time taken for change in colour from blue to pink on the lower leaf surface is less as compared to the upper surface.

Conclusion

The quick change in the colour of cobalt chloride paper on the lower surface indicated more loss of water vapours from this surface than the upper one.

This is due to more stomata per unit area (frequency) on the lower surface than the upper one. In hypostomatic leaves (stomata only on the lower surface), the change in the cobalt chloride paper is faster on the lower surface than the upper where only cuticular transpiration takes place.

Exercise 4

***Object* : To demonstrate the rate of transpiration by using potometer(s).**

Requirement

Choose any one of the following potometers — Simple potometer/Farmer's potometer/Ganong's potometer/Improved type large capacity potometer/ Bose's potometer; water, plants, grease, beaker, stop-watch, etc.

Procedure

Since there are many types of potometers, the construction of each one of them is described separately.

1. Simple potometer. It consists of a glass tube (U-shaped) with a side arm. The upper end of tube is fitted with one-holed cork while the side arm is completely closed. The lower end of the tube is fitted with one holed cork through which passes a capillary tube. The capillary tube is either graduated or a scale is fixed to it (fig. 4A).

A plant is inserted through a single-holed cork, into the side tube, allowing it to dip into the water. The transpiration begins and the water in the capillary tube rises. At this time, the lower end of capillary is dipped into a beaker containing water, thereby introducing an air bubble. The rate of movement of bubble is measured by allowing it to move a definite distance and the time taken by it is noted by a stop-watch.

2. Farmer's potometer. It is made up of a wide mouthed bottle which is closed by a rubber cork with three holes. A twig is inserted through one of the holes, thistle funnel (with a stop-cock acting as a reservoir) through another and a bent tube of narrow diameter through the third. This tube is either graduated or a scale is fixed to it. (fig. 4B).

This bottle, reservoir and the bent tube are filled with water. An air bubble is introduced into the bent tube. The rate is determined by the movement of an air bubble over a definite distance and time taken is noted by a stop-watch.

3. Ganong's potometer. This potometer also works on the same principle, however, the construction of the apparatus is different (as shown in the figure 4C).

4. Improved type of potometer. This type of potometer is designed for plants with a comparatively smaller root system. The construction is shown in the figure 4D.

5. Boses's potometer. It consists of a wide-mouthed bottle filled with water, closed by a two-holed rubber cork. Through one of the holes a twig is inserted, allowing it to dip in the water. A bent tube with two bulbs is introduced into second hole. A drop of non-volatile oil is placed in the outer bulb (fig. 4E).

During transpiration, water is absorbed by the plant pulling the oil drop towards the inner oil bulb through the arm. It bursts after reaching the inner bulb. Sooner it bursts, once again it moves back into the horizontal capillary arm. The movement from the horizontal arm into the inner bulb is repeated. The time taken for this movement (two consecutive bursts) is noted by the stop-watch and the rate of transpiration is determined.

The rate of transpiration using different plants kept under the following conditions can be noted.

A. in darkness,
B. atmosphere with higher relative humidity,
C. under high temperature,
D. under fan (wind movement) and
E. decreasing amount of soil water.

Results

A. When the plants are kept in darkness, the air bubble does not move.

B. When the plants are kept in atmosphere with higher relative humidity, the air bubble moves slowly.

C. When the plants are kept in place with high temperature, the air bubble moves faster.

D. When the plants are kept under fan, the air bubble moves faster.

E. When the plant is not properly watered, the air bubble moves slowly.

Conclusions

A. When the plants are kept in darkness.

The air bubble does not move, indicating that transpiration does not take place in the dark.

The stomata remain closed in the dark. Absence of photosynthesis in the dark results in accumulation of CO_2 from respiration. The cytoplasm becomes acidic and pH is lowered to 5. Under this condition sugar (osmotically active) in the guard cells is converted to starch (osmotically inactive). The osmotic concentration of the guard cells is lowered, exosmosis takes place, turgor pressure is reduced and walls of the guard cells return to normal position, closing the stomatal pore.

$$\text{Sugar} \xrightarrow[\text{phosphorylase}]{\text{pH5}} \text{Starch}$$

B. Plants are kept in atmosphere with high relative humidity.

The air bubble then moves slowly, indicating low rate of transpiration.

Normally, the internal atmosphere of the leaf is saturated with water vapours, while the external atmosphere is generally less humid. Therefore, a

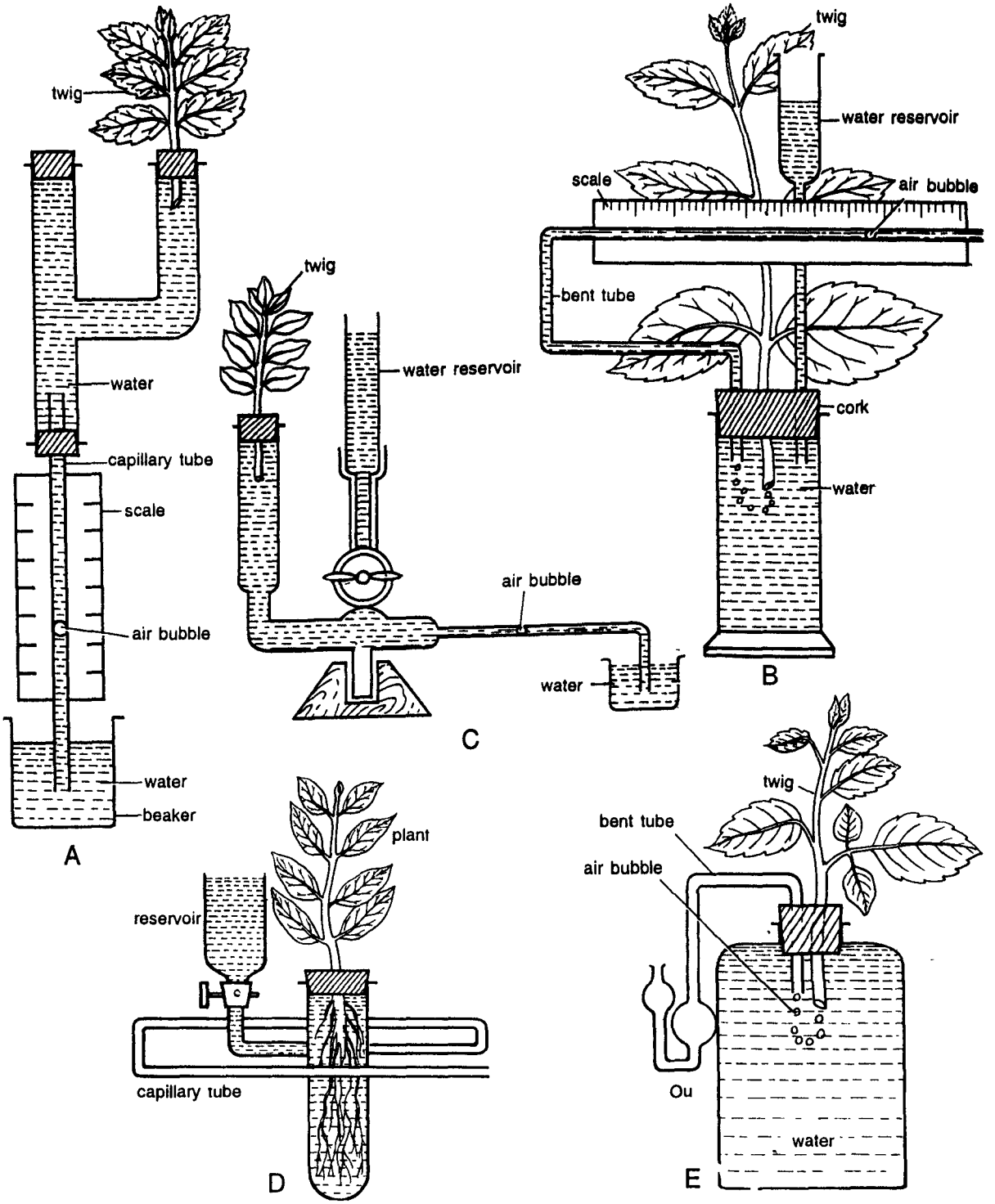


Fig. 4 Different types of potometers used for demonstration of transpiration rate. **A.** Simple potometer, **B.** Farmer's potometer, **C.** Ganong's potometer, **D.** Improved type of potometer, **E.** Bose's potometer.

vapour pressure gradient exists between the internal and the external atmosphere. The vapours diffuse from inside the leaf to external atmosphere. The lesser is the relative humidity of the external atmosphere, more rapid will be the diffusion from inside the leaf. However, in the present case external atmosphere being more humid (i.e. more amount of water vapours) the difference between the internal leaf atmosphere and the external atmosphere is lesser. At this stage, diffusion of vapours from saturated internal atmosphere to almost saturated external atmosphere is very less. The rate of transpiration is very low. It shall show a higher rate if the external atmosphere is less humid i.e. when air is dry.

C. The plants are kept in a region of high temperature.

With increase in temperature of the external atmosphere, air bubble moves faster, thus indicating increase in the rate of transpiration. This is due to

(i) stomatal movement,
(ii) change in the vapour pressure gradients and
(iii) increased evaporation.

(i) Higher temperatures increase the stomatal opening. Temperatures approaching 0°C result in stomatal closure while there is gradual increase in stomatal opening up to about 30°C. Thus higher is the temperature, stomatal opening is wider. The rate of transpiration increases.

(ii) The vapour pressure changes with the temperature. Higher is the temperature, the external atmosphere becomes less denser and thus contains lesser water vapours. The internal leaf atmosphere being saturated, vapours diffuse outward to the external atmosphere (containing less vapours). Thus, higher temperature steepens the vapour pressure gradient.

(iii) The higher is the temperature, more would be the heat, hence more would be the evaporation of water from the leaf. Thus, rate of transpiration is higher.

D. If plants are kept under fan (air movement).

The rate of transpiration increases under fan (or high velocity of wind). Saturated air surrounding the leaf is removed by wind.

The air surrounding the plants is generally saturated with water vapours due to transpiration. The vapour pressure gradient becomes less steeper. The rate of transpiration is lowered. However, the wind carries away water vapours accumulated around the leaf. The concentration of vapours becomes lower. The vapour pressure gradient becomes sharper. The transpiration increases.

E. When amount of available soil water is less.

Transpiration depends upon the absorption of soil water. As long as an equilibrium between the water absorbed from the soil and the water lost by transpiration is maintained, the rate of transpiration does not fall. The higher rate of transpiration reduces amount of soil water. The water absorption is reduced and so also the rate of transpiration. Continued transpiration would finally result into wilting.

Exercise 5

***Object* : To demonstrate transpiration pull.**

Requirements

Stand, beaker, a glass tube or capillary tube with a wide mouth, cork, oil cloth, plant or twig, water, mercury, beaker, etc.

Procedure

1. Fill beaker (or petri dish) with mercury. Invert a capillary over the mercury and fill it with water.
2. Insert the twig through hole into the cork in a way that the cut end of the plant is dipped in the water. Make the cork and hole airtight by applying vaseline or tie oilcloth securely around it.
3. Note the initial level of mercury in the capillary.
4. Allow the experiment to stand in open and sunny place. Note the level of mercury at the end of the experiment.

Results

The level of mercury rises.

Conclusions

The plant transpires and absorbs water to make up the deficit thus created. This results in pull or suction, called as transpiration pull.

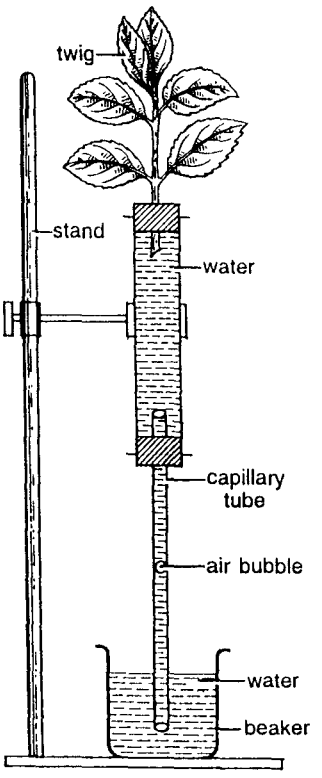


Fig 5 Apparatus set up to demonstrate transpiration pull

The transpiration pull exerts a tension on the water present in conducting tracheary elements. This tension is passed down to the roots. Thus, the whole column of water is lifted. The mercury column is similarly pulled upward exhibiting transpiration pull.

Exercise 6

***Object* : To demonstrate the root pressure.**

Requirements

A well-watered potted plant, a razor, rubber tubing, manometer, mercury, water, stand, etc.

Procedure

1. The plant is watered heavily. The shoot of the potted plant is cut off a few inches above soil level just below the first leaf.
2. The cut end of the stump is connected to a manometer by rubber tubing. The tube above the stump is filled with water, and the bent tube of the manometer is filled with mercury.
3. The initial level of the mercury is noted. The apparatus is kept as such (Preferably in a humid chamber) for a few hours and the level of mercury is noted again.

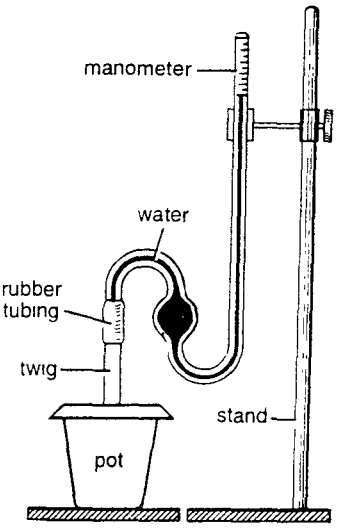


Fig 6 Apparatus used for demonstration of root pressure

Results

The rise of mercury level in the manometer tube is observed.

Conclusions

The rise in level is due to water from the cut end of the stump. The water is forced into the stem due to root pressure. Root pressure is a hydrostatic pressure developed due to water absorbed by the roots.

If plant is not heavily watered, the water level falls down, because water is used by the cut end of the 'stump'. This is called negative root pressure. It occurs if absorption of water is less than the rate of transpiration. The conditions which do not allow proper absorption of water such as poor aeration, cold or dry soil, high concentration of solutes in the soil or presence of toxic substances, as well as those conditions which allow high rate of transpiration, either reduce or prevent root pressure. Hence the water from the water reservoir (or plant)

shall be absorbed by the twig. The positive root pressure is seen early in the morning because all the favourable conditions are available to the plant during night.

The root pressure is said to be responsible for the ascent of sap. However, the magnitude of root pressure is very low that can help transport water only to a few feet.

Photosynthesis

Exercise 1

***Object* : To observe the rate of photosynthesis under varying carbon dioxide concentrations.**

Requirements

Wilmott's bubbler, water, twigs of *Hydrilla*, distilled water, sodium bicarbonate, stop watch, etc.

Procedure

Wilmott's bubbler can be prepared in the laboratory as described below.

1. A wide-mouthed bottle is taken. It is completely filled with pond water. A cork is then fitted, through which a glass tube (glass reservoir) wide at its open end is passed in a way, that its lower end dips in the water.
2. Another narrow glass tube open at both the ends is made into a bent jet and introduced into the glass reservoir. The twigs of *Hydrilla* are tied at the lower end of this narrow glass tube inside the bottle.
3. For studying the rate of photosynthesis under varying carbon dioxide concentrations, different amounts of sodium bicarbonate are added (see table e.g. 0·5g, 1·0g, 2·0g, 3·0g, 3·5g, etc.

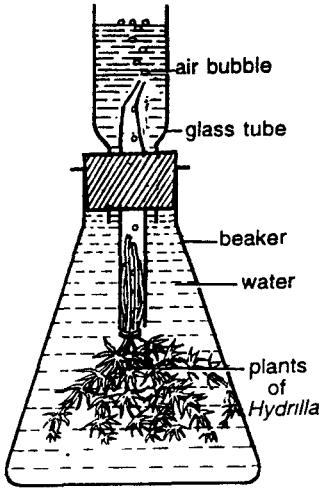


Fig. 1. Wilmott's bubbler to compare the rate of photosynthesis under different carbon dioxide concentrations

Resuits

The following is a table of results.

Effect of CO_2

Sr. No.	Conc. of $NaHCO_3$	Time taken for 5 bubbles of oxygen
1	Tap water	No bubbles
2.	0.5 g	10.1 sec
3.	1.0 g	4.9 sec
4.	2.0 g	3.0 sec
5.	3.0 g	3.0 sec
6.	3.5 g	no bubbles

Conclusion

1. In tap water there would be no evolution of oxygen bubbles because tap water contains negligible amount of carbon dioxide.
2. When sodium bicarbonate is added to water the rate of photosynthesis gradually increases with increased supply of carbon dioxide.
3. At certain higher amount of sodium bicarbonate, more of carbon dioxide is supplied. Therefore, some other factor becomes limiting and the rate of photosynthesis does not increase further. This can be observed by constant rate of evolution of oxygen bubbles.
4. When still higher amount of carbon dioxide is supplied in the form of sodium bicarbonate, it becomes toxic, photosynthesis stops and there is no evolution of oxygen bubbles.

Exercise 2

***Object* : To observe the effect of light on oxygen evolution during photosynthesis using Wilmott's bubbler.**

Procedure

Wilmott's bubbler can be prepared in the laboratory as given below.

1. A wide-mouthed bottle is taken. It is completely filled with pond water. A cork is then fitted, through which a glass tube (water reservoir) wide at its open end is passed in a way, that its lower end dips in the water.
2. Another narrow glass tube open at both the ends is made into a bent jet and introduced into the glass reservoir. The twigs of *Hydrilla* are tied at the lower end of this narrow glass tube inside the bottle.

For studying the rate of photosynthesis following different conditions are provided to the apparatus.

A. The bubbler is kept in sunlight and shade alternately.

B. The bubbler is covered with red, green and blue cellophane papers.

In each case the number of bubbles and time taken for their evolution are noted—

Results

The observations are tabulated in the following form.

A. Effect of light and shade

Sr. No.	Sun/shade	Time taken for 5 bubbles
1.	Sun	21.0 sec
2.	Shade	36.0 Sec
3.	Sun	19.0 sec
4.	Shade	43.0 sec

Conclusion

The rate of evolution of oxygen bubbles is a measure of photosynthetic rate.

A. Effect of light and shade. In shade the rate of photosynthesis slows down as compared to in sunlight.

Normally, light is not a limiting factor. About 1-2% of the total light falling upon the leaves is

B. Effect of different wavelengths of light

Sr. No.	Time taken for 5 bubbles in			
	Sun light	Red light	Blue light	Green light
1.	20.1 sec	15.0 sec	18.0 sec	No bubbles
2.	28.0 sec	23.0 sec	19.0 sec	No bubbles
3.	25 0 sec	16.0 sec	20.0 sec	No bubbles
4.	39.0 sec	32.0 sec	36.3 sec	No bubbles
5.	33.0 sec	28.0 sec	32.0 sec	No bubbles
Average	29.2 sec	22.8 sec	25.4 sec	No bubbles

Thus maximum rate of photosynthesis is attained at intensities much below those of the full sunlight.

However, low light intensity may become a limiting factor and photosynthesis is lesser in plants exposed to weak light than in plants exposed to strong light. Thus, the rate of photosynthesis is lesser in shade than in sun light.

If alternate light and dark periods (intermittent) are given, the efficiency of photosynthesis is higher. One of the reasons being, accumulation of CO_2 in the leaves and translocation or conversion of photosynthate into soluble form. This helps to increase the rate of photosynthesis during light periods.

B. Effect of different wavelengths of light. Under sunlight, plants continue to show a definite rate of photosynthesis beacause the pigments active in photosynthesis absorb respective wavelengths. These wavelengths being most effective, the rate of photosynthesis is almost maximum if other factors are also favourable.

If red wavelengths (647-660 mμ) are available, the rate of photosynthesis is the maximum. The wavelength (near 670 mμ) is known to be maximally effective.

Similarly, net higher peak of absorption in the blue wavelengths is 422-492 mμ. It is absorbed in larger quantities than other wavelengths. These wavelengths near 440 mμ are second most effective. The rate of photosynthesis, in this case, would be lesser as compared to red wavelengths.

The rate of photosynthesis is practically zero in green wavelengths. This is beacause the chlorophylls reflect green wavelengths.

Respiration

Exercise 1

***Object* : To demonstrate anaerobic respiration.**

Requirements

A test tube, petri dish, stand, KOH crystals, mercury, germinating seeds, forceps, etc.

Procedure

1. A petri dish is filled half with mercury.
2. A test tube is completely filled with mercury and inverted over this petri dish.
3. Germinating seeds (soaked gram seeds) are introduced into the tube by forceps through its lower open end. The seeds rise to the top of the tube.
4. The apparatus is allowed to remain as such for a few hours.

Result

The level of mercury falls down. Now KOH crystal is inserted into the tube. The mercury again begins to rise.

Conclusion

At the beginning of the experiment, test tube has no air. Germinating seeds respire at the tip of the tube in the absence of air. It results in formation of alcohol and CO_2.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

The CO_2 produced pushes down the mercury column. On introduction of KOH crystal this CO_2 is absorbed. Thus mercury rises once again.

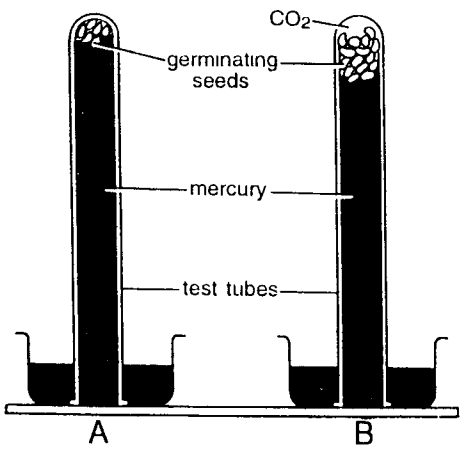


Fig 1 Apparatus to demonstrate anaerobic respiration **A.** Mercury filled tube at the beginning of the experiment. **B.** The tube at the end of experiment showing presence of carbon dioxide at the tip

Exercise 2

***Object* : To demonstrate the process of fermentation.**

Requirements

Kuhne's fermentation vessel, beaker, glucose, water, baker's yeast, etc.

Procedure

1. Prepare 10% solution of glucose in a beaker.
2. Add a small quantity of baker's yeast to the beaker.
3. Pour the mixture into Kuhne's fermentation vessel. The complete upright tube and half of the bent bulb is filled.
4. The open end of the bent bulb is plugged with cotton or cork.
5. The apparatus is allowed to stand for some hours.

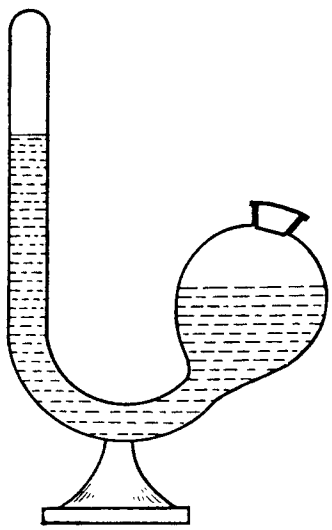


Fig 2. Kuhne's fermentation vessel to demonstrate the process of fermentation

Result

The gas begins to collect in the upright arm of the vessel with a consequent fall in the level of solution.

Conclusion

Gas collecting in the tube is CO_2 which can be tested by introducing caustic potash in the vessel. The smell of alcohol can also be detected from the remaining solution.

The process that occurs in the tube is fermentation. In this case sugar is fermented by the addition of zymase (enzyme from baker's yeast) resulting in the production of carbon dioxide and alcohol. Pure zymase is not known to bring about fermentation. It requires presence of phosphate which acts as a coenzyme. Thus, the fermentation reactions can be summarised as follows —

(i) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_5OH + 2H_2O + C_6H_{10}O_4\ (R_2PO_4)_2$
(Hexose diphosphate)

(ii) $C_6H_{12}O_4\ (R_2PO_4)_2 + 2H_2O =$
(Hexose diphosphate) $2R_2HPO_4 + C_6H_{12}O_6$

Final equation, therefore,

$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH$

The fermentation of sugar stops when concentration of ethyl alcohol reaches 10-15% which kills all the yeast cells.

Exercise 3

***Object* : To demonstrate aerobic respiration.**

Requirements

A bottle, two holed cork, bent tube, reservoir with tube and stop cock, beakers, stand, water, lime water, germinating seeds, etc.

Procedure

1. Germinating seeds are placed in a bottle.
2. It is closed by a cork through which a glass tube bent twice at right angles is inserted. The free lower end is allowed to dip in water.
3. Through another hole is inserted a tube holding a water reservoir with a stop cock.
4. The water seal (water in beaker) and stop cock of the reservoir are closed. The seeds are allowed to respire for some time.
5. Water seal is then replaced by beaker containing lime water. Stop cock of the reservoir is opened and air inside the bottle is driven out.

Result

The air bubbles come out through the bent tube into the beaker containing lime water which turns milky.

Conclusion

The turning milky of lime water indicates the presence of carbon dioxide. This gas is apparently released during germination of soaked seeds.

During the process of aerobic respiration respiratory substrate is broken down by oxidation that uses oxygen and carbon dioxide is released.

(*This process can also be demonstrated by other apparatus*)

Materials (for apparatus shown in fig. 3B)

A conical flask, cork, bent glass tube, a small test tube, thread, water, KOH, germinating seeds, etc.

Procedure

1. Germinating seeds are placed in flask.
2. A bent tube is introduced through the cork.
3. The free end of the tube is allowed to dip into a beaker containing water.
4. A small test tube containing KOH crystals is hung inside the flask.
5. The apparatus is made airtight and respiration is allowed to continue (fig. 3B).

Result

Water in beaker rises in glass tube.

Conclusions

Seeds take oxygen and liberate an equal amount of carbon dioxide. Thus, there should not be any change in the volume of air. However, KOH crystals in the test tube absorb carbon dioxide creating a

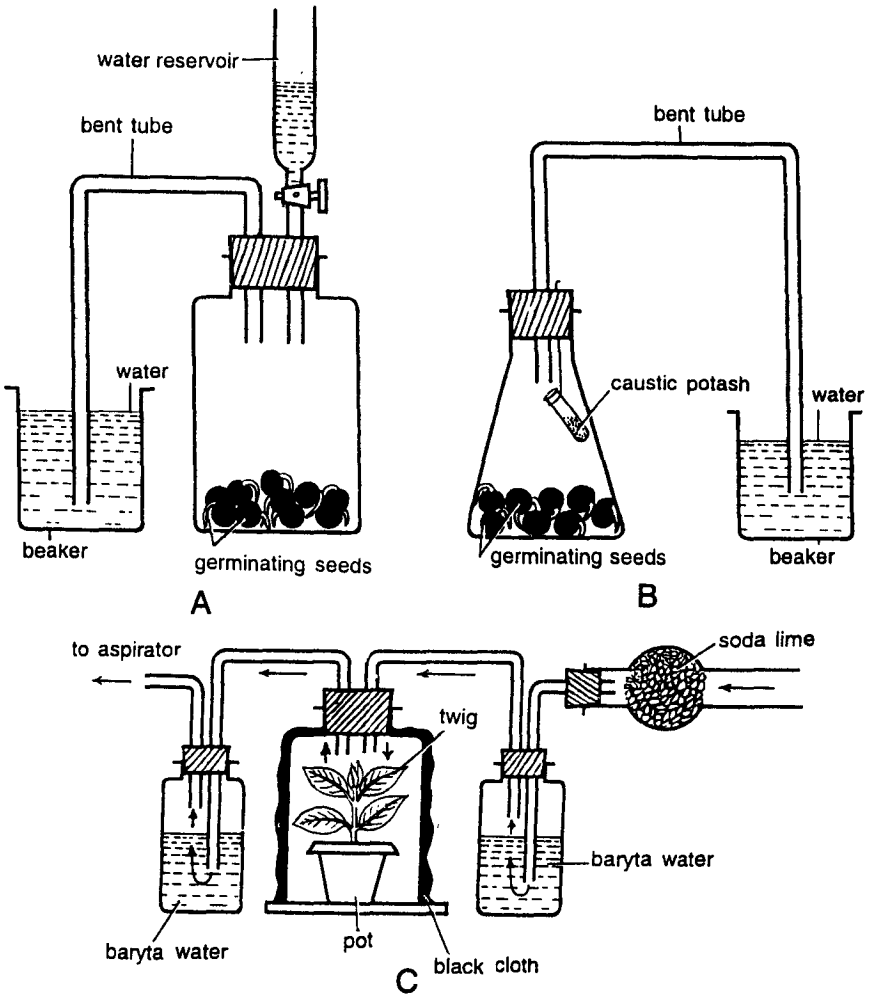


Fig 3. To demonstrate aerobic respiration. **A. B.** and **C.** Different set ups to show aerobic respiration and evolution of carbon dioxide

vacuum in the flask. This results in the rise of water in the glass tube.

Materials (for apparatus shown in fig. 3C).

A bell jar, two bottles, bent tubes, a bulb, soda lime, water, aspirator, connecting rubber tubes, corks, grease, cloth, a potted plant, etc. (Fig. 3C).

Procedure

1. A potted plant is covered by a bell jar (if germinating seeds are to be used, bell jar is replaced by a bottle).
2. The bell jar is connected by connecting tubes to the bottles on both the sides. The bottles contain lime water or baryta water.
3. Bottle at one end is connected with a bulb containing soda lime which is in contact with air.
4. The bottle at other end is connected with an aspirator. The apparatus is made airtight.
5. The bell jar is covered with black cloth to prevent photosynthesis.
6. To begin the experiment, aspirator is allowed to run. (fig. 5).

Results

The lime water in a bottle close to aspirator turns milky while that placed in a bottle at another end does not.

Conclusions

The air enters the glass bulb filled with KOH. This removes carbon dioxide from the air. The air now enters the bottle containing lime water where carbon dioxide if any is also absorbed. The air completely free from carbon dioxide now reaches the bell jar.

The plant inside the bell jar cannot undergo photosynthesis because light is not available. However, plant can respire since carbon dioxide free air still supplies oxygen needed for this process. The respiration releases carbon dioxide which enters the bottle with lime water turning it milky.

This experiment shows that, if a plant is given air free of carbon dioxide, even then it turns the lime water milky. This demonstrates that carbon dioxide is released during respiration of the plant in a bell jar.

Exercise 4

***Object* : To demonstrate that energy is released in the form of heat during respiration.**

Requirements

Two thermos flasks, germinating seeds, mercuric chloride, two thermometers, rubber corks, etc.

Procedure

1. Take two thermos flasks.
2. Introduce seeds which are boiled or dried and treated with mercuric chloride in A. (The mercuric chloride treatment also eliminates fungi and/or bacteria from the seeds and consequently their respiration is prevented.)
3. In flask B germinating seeds are placed.
4. Both the flasks are closed by rubber stoppers. Thermometers are inserted through each of these blanks. The thermometers should be buried in the seeds.
5. The temperatures at the beginning of the experiment are noted.

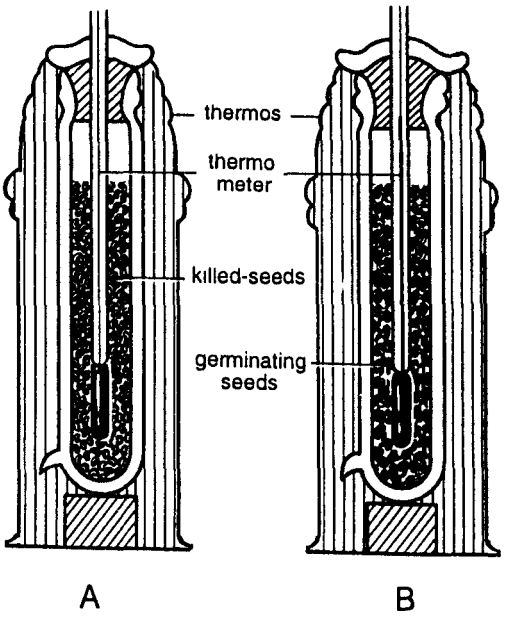


Fig. 4. Thermos flasks used to demonstrate release of energy in the form of heat during respiration.

6. The apparatus is allowed to stand for some time and then the temperatures are recorded once again.

Results

The temperature of the flask A with killed seeds remains unchanged while there is a rise in temperature of the flask B where germinating seeds were placed.

Conclusions

Respiration breaks down respiratory substrate and release about 673 Kcal of energy. Of this, most of the energy is converted and stored (as ATP) before being actually used in other metabolic activities of the cells.

However, some amount of energy escapes as free energy (heat) resulting in the increase in temperature.

In flask A the seeds are dead and hence do not respire, therefore, the temperature remains unchanged. However, the germinating seeds in flask B continue to respire. The energy escaping as heat during this process; consequently causes rise in temperature.

Exercise 5

***Object* : To determine the value of RQ of different respiratory substrates.**

Requirements

Ganong's respirometer, respiratory substrates, caustic potash, saline water, filter paper, stand, etc.

Procedure

1. Open the bulb of the respirometer and pour a few drops of water.
2. Place a filter paper at its base.
3. Place a few germinating seeds in the bulb.
4. Saline water is filled through the other end of tube (called levelling arm). Saline water is used because carbon dioxide is insoluble in this solution.
5. Now place the stopper of the bulb. It is first adjusted in a way that a hole in the bulb and that in the stopper come to lie opposite each other. Air enters through them into the bulb.
6. The solution in the arm is brought to the same level by raising or lowering the levelling arm.
7. The stopper is then rotated, so that the contact between the two holes is cut off.
8. The initial level of the solution is noted again.
9. The experiment is allowed to proceed for a few hours. The level is noted once again.
10. A KOH crystal is introduced in the tube and change in the level is noted.

Results and conclusions

1. If the respiratory substrate is carbohydrate. Germinating grains of cereals e.g. wheat, rice, maize, etc. are rich in carbohydrates. During their respiration, the amount of carbon dioxide released shall be equal to the amount of oxygen absorbed. Thus, there shall neither be rise nor fall in the level. The RQ is, therefore, equal to unity. Similarly, RQ for the leaves of many species of plants and flowers usually have a respiratory quotient of approximately one.

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

$$\therefore \quad RQ = \frac{6CO_2 \text{ released}}{6O_2 \text{ released}} = 1$$

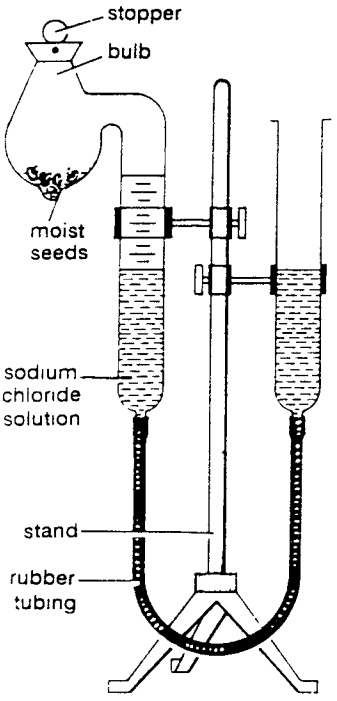


Fig 5. Ganong's respirometer for determining the value of RQ of different respiratory substrates

2. If the respiratory substrate is fat. In case of fats (e.g. castor seeds, mustard seeds, ground nut seeds, etc.), the amount of carbon dioxide released is less than the amount of oxygen absorbed, e.g.

$$2C_{51}H_{98}O_6 + 145O_2 \rightarrow 102CO_2 + 98H_2O$$

Therefore, there shall be initial rise in the level of saline water denoted by V_1. When KOH crystal is added, there is further rise in the level which is denoted by V_2.

V_1 = excess of oxygen (43) i.e. 145 CO_2 – 102 CO_2

V_2 = amount of carbon dioxide released (102)

$V_1 + V_2$ = amount of oxygen absorbed (102 + 43 = 145)

$$RQ = \frac{CO_2 \text{ released}}{6O_2 \text{ absorbed}} = \frac{V_2}{V_1 + V_2}$$

$$= \frac{102}{102+43} = \frac{102}{145} = 0.7$$

3. If the substrate is organic acid. When organic acids are respiratory substrates, more amount of carbon dioxide is released than the amount of oxygen absorbed; e.g. in succulents.

$$C_4H_6O_5 + 3O_2 \rightarrow 4CO_2 + 3H_2O$$

Therefore, the level of solution will fall. This fall would be equivalent to the excess amount of CO_2 — denoted by V_1. On addition of KOH, CO_2, is absorbed and the level rises further — denoted by V_2.

V_1 = excess of CO_2 released (1) i.e. $4CO_2 - 3O_2$.

V_2 = total amount of CO_2 produced (4)

$V_2 - V_1$ = amount of O_2 absorbed (4 – 1 = 3)

$$RQ = \frac{CO_2 \text{ released}}{O_2 \text{ absorbed}} = \frac{V_2}{V_2 - V_1}$$

$$= \frac{4}{(4-1=3)} = \frac{4}{3} = 1.3$$

This type of respiration occurs in succulents during daytime. (Succulents show incomplete oxidation of carbohydrates during night producing organic acids. These are later oxidised during daytime. Thus, if the bulb is covered with black cloth, succulents would show incomplete oxidation of carbohydrates and the value of RQ would be zero or less than one).

RQ can also be calculated.

Rise in the level in tube A is denoted by V_1.

V_1 = excess of oxygen used

Rise in the level in tube B is denoted by V_2.

V_2 = total amount of carbon dioxide released.

$\therefore$ $V_1 + V_2$ = total amount of oxygen used.

$$RQ = \frac{\text{amount of } CO_2 \text{ released}}{\text{amount of } 6O_2 \text{ absorbed}} = \frac{V_2}{V_1 + V_2}$$

Exercise 6

***Object* : Comparison of the rate of respiration of various plant parts.**

Requirements

Flask with a graduated bent tube, germinating seeds, floral parts, (use only non-green parts or else photosynthesis would occur giving wrong results), cotton, KOH pellets, mercury (or oil), stand with clamps, beaker, graph paper, pencil, etc.

Procedure

1. Place measured quantity of germinating seeds or petals in the flask.

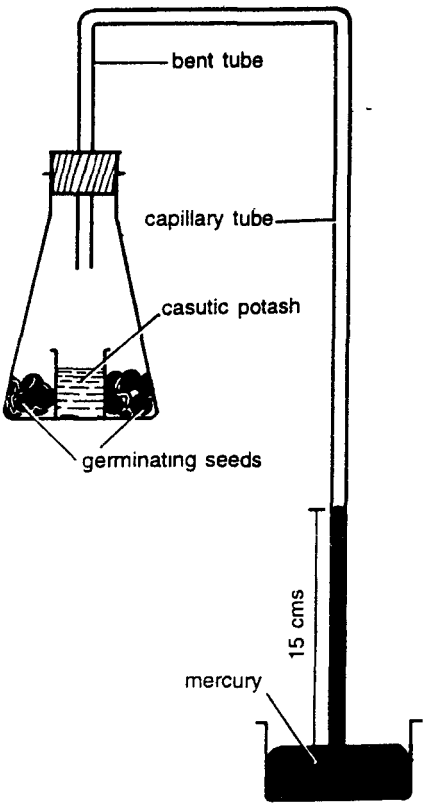


Fig. 6. Apparatus for comparison of the rate of respiration of various plant parts

2. Insert cotton plug in the bent tube followed by pelletes of KOH and another cotton plug.
3. Ensure that cotton plugs and KOH pellets are securely placed.
4. Fill a small beaker or a trough with mercury.
5. Invert the bent tube of the flask so as to immerse it in mercury in the beaker. Leave a small gap between the free end of the tube and bottom of the beaker.
6. Adjust the height of the flask by stand and the clamp.
7. Allow the experiment to remain undisturbed for 1-2 hours.
8. Measure the height of mercury column in the glass tube.

Observations

There is rise of mercury in the glass tube.

Results

The amount of oxygen consumed by respiring material per hour is calculated by the following formula.

Rate of respiration

$$= \frac{\text{reading of mercury column at the end} - \text{reading of mercury column in the begining}}{\text{amount of respiring material (in mgm)}}$$

Conclusion

During respiration, carbon dioxide is released while oxygen is consumed at the same time. Carbon dioxide is absorbed by KOH pellets and a partial vacuum is produced due to use of oxygen. Therefore, mercury rises up in the tube. This rise is equal to the oxygen consumed.

Auxins

Exercise 1

***Object* : Demonstration of *Avena* straight growth test.**

Requirements

Avena (oat) fruits (caryopsis), filter paper, aluminium foil, petri dishes, test tubes, pipettes, measuring cylinder, coleoptile cutter (or sharp blade), hair brush, pencil, graph paper, distilled water, ethanol, IAA, 2% sucrose solution, etc.

Procedure

The following are the major steps —

1. Seed germination. About 50 or more *Avena* seeds are germinated by placing soaked seeds in water and then placed on a damp filter paper in petri dish containing a small amount of water. Petri dish is covered with aluminium foil and placed in the dark at 25 ± 1°C for 2 to 5 days.

2. Preparation of sections. After germination when coleoptiles are about 2-3 cm long, the apical tip, about 4 mm, of each coleoptile is removed. This is done in order to prevent natural auxins

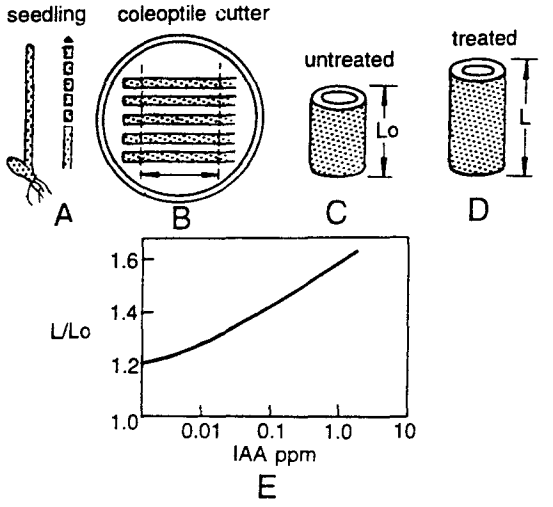


Fig. 1 Diagrammatic representation of *Avena* straight growth test. **A.** *Avena* seedling, **B.** Cutting of coleoptile sections, **C.** Freshly cut segment (**LO** = length), **D.** Treated segments (**L** = length of treated segment), **E.** Graph showing relationship between dose and response.

produced by the coleoptile from affecting the growth. Cut sections of 10 mm length of the remaining part of the coleoptile with the help of coleoptile cutter or a very sharp blade.

3. Preparation of IAA concentrations. Dissolve 100 mg of IAA in 2 ml of ethanol (IAA is not soluble in water, hence it is first dissolved in ethanol). Dilute to 900 ml with distiled water. Warm the solution to 80°C and keep at this temperature for 5 minutes. Make up to one litre with distilled water. This stock solution gives 100 ppm concentration of IAA.

Take six test tubes marked A to F. Add 10 ml of 2% sucrose solution to each test tube. Now do as follows to obtain desired concentrations.

Tube A. Add 2 ml of IAA stock solution to 18 ml sucrose solution already present. Mix the contents thoroughly. This gives 10 ppm IAA solution.

Tube B. Add 2 ml solution from tube A to 18 ml sucrose solution already present. This gives 1 ppm IAA solution.

Tube C. Add 2 ml solution from tube B to 18 ml of sucrose solution already present. This gives 0.1 ppm IAA solution.

Tube D. Add 2 ml solution from tube C to 18ml of sucrose solution already present. This gives 0.01 ppm IAA solution.

Tube E. Add 2 ml sucrose solution to 18 ml sucrose solution in the test tube. There is no IAA in the tube.

Tube F. Add 2 ml of distilled water to 18 ml of sucrose solution already present.

The tubes E and F act as control.

4. The treatment. Transfer solution from tubes A to F to similarly marked petri dishes.

Transfer four or more 10 mm coleoptile sections to each petri dish.

Cover the petri dishes with the lids.

Allow the petri dishes to remain in the dark at 25 ± 1°C for three days.

5. The measurement. The lengths of the sections floated in various test solutions are measured and noted as L.

6. Drawing a graph. Mark L/Lo (Lo= original length, i.e. 10 mm in this case) on Y axis of the graph and IAA concentrations in ppm on X axis. Join the points to complete the graph.

Conclusion

The response of the section is found to be directly proportional to the logarithm of concentration of IAA used.

Exercise 2

***Object* : Cress root inhibition test for Indole auxins.**

Requirements

Cress seeds (of family Cruciferae), petri dishes, distilled water, IAA, ethanol, sharp blade, filter paper, pipettes, measuring cylinder, measuring scale, aluminium foil, sucrose solution, etc.

Procedure

The following are the major procedural steps—

1. Seed germination. About 50 sterilized cress seeds are germinated by placing soaked seeds on a damp filter paper in a petri dish containing some water. The petri dish is covered with aluminium foil and placed in the dark at 25 ± 1°C for two to five days, till the roots of the seedlings are about 2-5 cm long. The seedings are then placed in various test solutions.

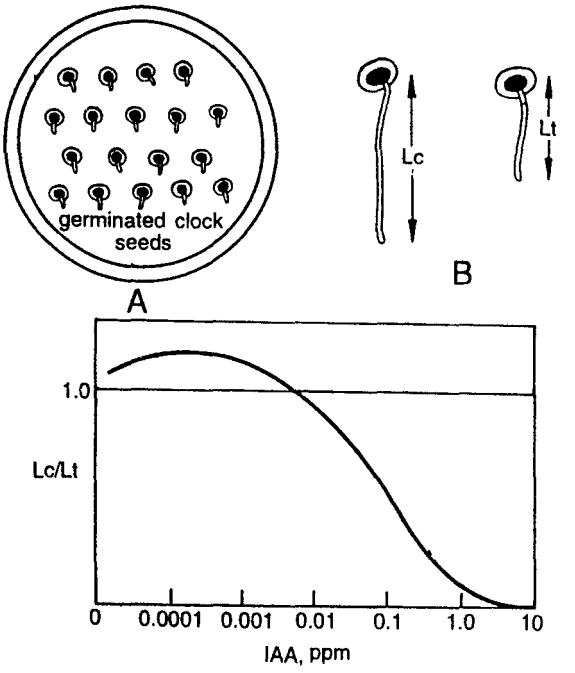


Fig. 2. Diagrammatic representation of cress root inhibition test for Indole auxins. **A.** Germinating cress seeds, **B.** Controlled and treated seedlings (**Lc** = length of controlled root seedling, **Lt** = length of treated root of seedlings), C. Graph showing relationship between dose and response.

2. Preparation of IAA solutions. Dissolve 10 mg of IAA in 2 ml of ethanol (IAA is not soluble in water, hence it is first dissolved in ethanol). Dilute to 900 ml with distilled water. Warm the solution to 80°C and keep at this temperature for 5 minutes. Make up to one litre with distilled water. This stock solution gives 10 ppm concentration of IAA.

Take five test tubes, marked A to E. Add 18 ml of sucrose solution (2%) to each of the five test tubes. Now proceed as follows to prepare different IAA concentrations.

Tube A. Add 2 ml of IAA stock solution to obtain 1 ppm IAA concentration.

Tube B. Add 2 ml of solution from tube A and obtain 0.1 ppm IAA concentration.

Tube C. Add 2 ml of solution from tube B and obtain 0.01 ppm IAA concentration.

Tube D. Add 2 ml of solution from tube C and obtain 0.001 ppm IAA concentration.

Tube E. Add 2 ml of solution from tube D and obtain 0.0001 ppm IAA concentration.

3. The treatment. Transfer the solutions from test tubes A to E to similarly marked petri dishes.

Transfer a few germinated seeds to each petri dish after measuring their root length (Lt).

Cover the petri dishes with lids.

Allow the petri dishes to remain in the dark at 25 ± 1°C for 48 hours.

4. Measurement. Measure lengths of roots of seedlings placed in various test solutions, after the test period.

5. Drawing a graph. Mark Lt/Lc (Lt = length of treated seedling root at the end of test period; Lc = length of control seedling root at the end of test period) on Y axis of the graph and IAA concentration on X axis.

Conclusion

The roots are much more sensitive to auxin than the stem and are, in fact, inhibited by concentration of auxins that normally stimulate stem growth. However, at very low concentration of auxin, root growth may be stimulated. The value of root test, therefore, is that the effect of extremely low concentration of auxin, such as found in plant extracts, may be measured. It is capable of detecting concentrations of IAA as low as 1/100,000 mg. The response is roughly proportional to the logarithm of auxin concentration.

Exercise 3

***Object* : To study the effect of gibberellic acid on plant growth.**

Requirements

Pea (*Pisum sativum*), bean (*Phaseolus vulgaris*) plants, gibberellic acid (GA_3), ethanol, distilled water, a tray with sand, plastic covering for plants, etc.

Procedure

1. Sow pea seeds (preferably dwarf variety) or bean seeds in a tray containing sand. Maintain the seeds in moist condition by occasionally watering the tray.

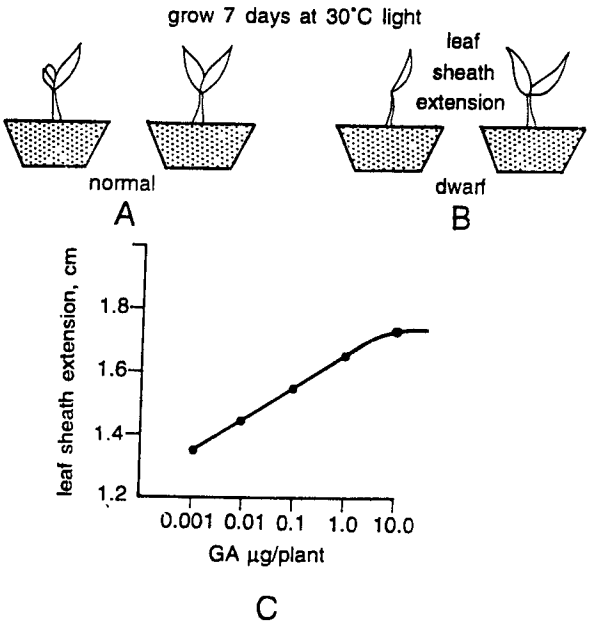


Fig. 3. Diagrammatic representation of effect of gibberellic acid on plant growth. **A.** Response of normal plants to treatments, **B.** Response of dwarf plants to treatments, **C.** Graph showing relationship between dose and response.

2. Cover some of the two-week old pea plants by plastic covers. Spray the rest with gibberellic acid solution (100 mg/litre; dissolve GA_3 in 1 to 2 ml of 95% ethanol and dilute to 1 litre with water). Remove the cover and spray the uncovered plants with amount of ethanol used for GA_3 preparation. (This serves as control.) Allow the plants to grow and measure the following periodically :
   (a) height of plants (from soil level),
   (b) length of internodes,
   (c) length of leaves and
   (d) blade width.

   Measure the above values for both — treated and control plants.
3. Select 2 week old bean plants of approximately the same height. Apply following solutions of GA_3 to the growing tip of two plants each :
   (a) Distilled water (control),
   (b) GA_3 10^{-1} M,
   (c) GA_3 10^{-2} M,
   (d) GA_3 10^{-3} M and
   (e) GA_3 10^{-4} M.

Allow the plants to grow for 2-3 weeks and measure the characters given above.

Tabulate the observations.

Result and conclusion

Growth of Pea plants shows response to gibberellic acid treatment. The bean plants would exhibit effect of GA_3 on stem tip.

1. Genetic dwarfism. The GA_3 eliminates genetic dwarfism in certain plants. The pea plants (dwarf) elongate and acquire height of a normal plant. The normal plants, however, show no effect of GA_3 on height.

2. Effect on stem tip. GA_3 treatment to growing tip of bean seedling shortens the time to flowering and hastens maturity.

Besides stem elongation and promoting flowering in long-day plants, gibberellins can break dormancy and also produce parthenocarpic fruits.

Exercise 4

***Object* : Demonstration of gibberellin activity by bioassay.**

Requirements

Grains (caryopsis) of dwarf and normal varieties of maize, gibberellic acid (GA_3), distilled water, vermiculite, enamel trays, measuring cylinder, etc.

Procedure

1. Seeds of dwarf and normal varieties of maize are soaked in water. These are separately sown in different enamel trays filled with damp vermiculite.
2. The seeds are allowed to grow till the first leaf emerges.
3. Prepare the test solutions as follows. Dissolve 100 mg of GA_3 in one litre distilled water. (or proportionate amount as required). This gives 100 ppm concentration of GA_3. This can be used as stock solution.
4. Now for preparing lower concentrations, take 90 ml of distilled water and add 10 ml of stock solution to obtain 10 ppm concentration.
5. Take 10 ml of 10 ppm GA_3 solution as prepared above and to it add 90 ml of distilled water in another beaker. This gives 1 ppm concentration. Repeat the procedure to obtain 0.1, 0.01 ppm concentrations of GA_3.
6. Add test material as prepared above to the cup formed by the emerging first leaf. Allow the plants to grow for 7 days at 30°C in light to observe the response.

Observations

Note the observations as given below.

GA concentration (in ppm)	Leaf sheath extension (in cm)
0.001	1.36
0.01	1.45
0.1	1.52
1.0	1.66
10.0	1.73

Draw a graph showing leaf sheath extension on Y axis and GA concentrations used on X axis.

Conclusion

The dwarf habit in many plants is due to single recessive gene. In gibberellin assays only single-gene dwarfs are used. It is specific for gibberellins and the growth response is linear in the range of 0.001-10 ppm (or μg) of GA_3 per plant.

Exercise 5

***Object* : Demonstration of cytokinin activity by bioassay.**

Requirements

Seeds of radish, cytokinin, distilled water, petri dishes, measuring cylinder, beakers, etc.

Procedure

1. . Radish seeds are soaked in water. These are allowed to germinate for 30 hours in complete darkness on moistened filter paper, at 26 ± 1°C.

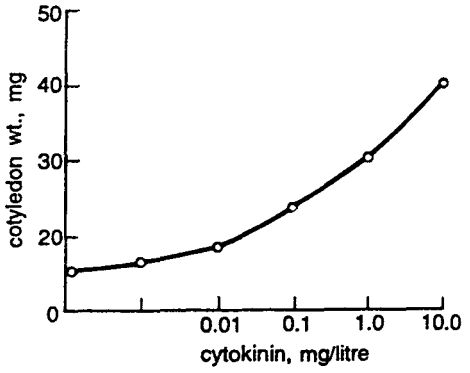


Fig. 4. Diagrammatic representation of radish cotyledon test of cytokinin activity by bioassay. Graph showing relationship between dose and response.

2. Prepare the test solutions as follows. Dissolve 100 mg of cytokinin in one litre of water (or proportionate amount as desired). This gives 100 ppm concentration of cytokinin and serves as a stock solution.
3. Now for preparing lower concentrations, take 45 ml of distilled water in a test tube and add 5 ml of stock solution. This gives 10 ppm concentration of cytokinin.
4. To prepare 1 ppm concentration, take 45 ml of distilled water in a test tube and add 5 ml of 10 ppm cytokinin solution prepared as given above.
5. To prepare 0.1 ppm concentration, take 45 ml of distilled water in a test tube and add 5 ml of 1 ppm cytokinin solution prepared as given above.
6. To prepare 0.01 ppm concentration, take 45 ml of distilled water in a test tube and add 5 ml of 0.1 ppm cytokinin solution prepared as above.
7. To prepare 0.001 ppm concentration, take 45 ml of distilled water in a test tube and add 5 ml of 0.01 ppm cytokinin solution as prepared above.
8. Thus solutions with 10, 1, 0.1, 0.01 and 0.001 ppm concentration of cytokinin are prepared. Filter paper discs of appropriate sizes are cut and placed in different petri dishes. Cytokinin solutions of different concentrations are poured in separate petri dishes to moisten filter paper discs placed in them.
9. Smaller of the cotyledons from each radish seedling is removed and weighed. These are placed on filter discs containing test solutions.
10. Petri dishes are covered and placed under continuous fluorescent illumination for 3 days at 25 ± 1°C. The cotyledons are reweighed.

Observations

The observations are tabulated in the following form.

Cytokinin concentration (in ppm)	Weight (in mg)		Increase
	Before treatment	After treatment	
0.001	30.2	45.3	15.1
0.01	22.6	40.6	18.0
0.1	26.3	48.7	22 4
1.0	25 4	55.6	30.2
10.0	28.5	67.3	30.8

Draw a graph showing increase in the weight of cotyledons on Y axis and concentration on X axis.

Conclusion

The increase in weight is related to the concentrations of the applied cytokinin. Concentrations of cytokinins between 0.001 and 10 mg per litre (or ppm) can be assayed by this procedure.

3

Biochemistry

Exercise 1

***Object* : Separation of plant pigments by chromatographic technique (paper and thin-layer chromatography).**

Requirements

Spinach leaves, chromatography paper Whatman No. 1, microscope slides, mortar, pestle, separatory funnel, beakers, capillary tube, split corks, tubes, chromotographic chambers, pencil, drier, split corks, tubes, sand, silica gel, cellulose, distilled water, benzene, acetone, calcium carbonate, ethyl ether, methyl alcohol, KOH, n- butanol, acetic acid, carbon tetrachloride, anhydrous sodium sulphate, petroleum ether, etc.

Procedure

The following are major steps —

1. **Preparation of paper.** (1) Cut chromatography paper Whatman No.1 into square sheets to a size which would fit in easily in the chromatography jar (or a specimen tube).

(2) Draw a pencil line 1/2 inch above the bottom of each sheet.

2. **Preparation of plates.** (1) Prepare a slurry of silica gel by blending cellulose with silica gel in distilled water (10 g cellulose : 4 g silica gel: 80 ml distilled water).

(2) Another method to prepare slurry is to suspend 2 g of kieselgel in 10 ml of distilled water.

(3) The homogeneous slurry is spread uniformly over the clean microscope slides. Allow the gel to set and to oven dry them overnight at 40°C.

3. **Preparation of extract.** Cut fine strips of spinach leaves, place in a clean mortar and reduce it to pulp with pestle. There are two possible methods. The first (*a)* is simple for the use of under graduate classes.

(a) Extraction of leaf pigment. Add 50 ml of precooled acetone to leaf pulp, stir well and filter through fine linen cloth.

(b) Separation of chlorophylls and carotenoid extracts : (1) Add 50 ml of procooled 80% acetone to leaf pulp to which small quantities of acid-washed sand and a small amount of $CaCO_3$ is added.

(2) Transfer the clear supernatant green coloured liquid to 10 ml of ethyl ether in a separatory funnel.

(3) Add 60 ml of distilled water gradually while rotating the funnel slowly without shaking.

(4) Two layers now separate—1. Lower acetone—water layer, and 2. upper ether layer.

(5) Run off the lower acetone layer and discard it.

(6) Continue to add water till two layers are formed.

(7) Run off the lower layer at least three times thus discarding all the acetone.

(8) Add about 30 ml of methyl alcholic KOH.

(9) Shake the separating funnel and allow it to stand for about 15 minutes.

(10) Add once again some amount of water (20 ml or more) and ether (5ml).

(11) Rotate and shake.

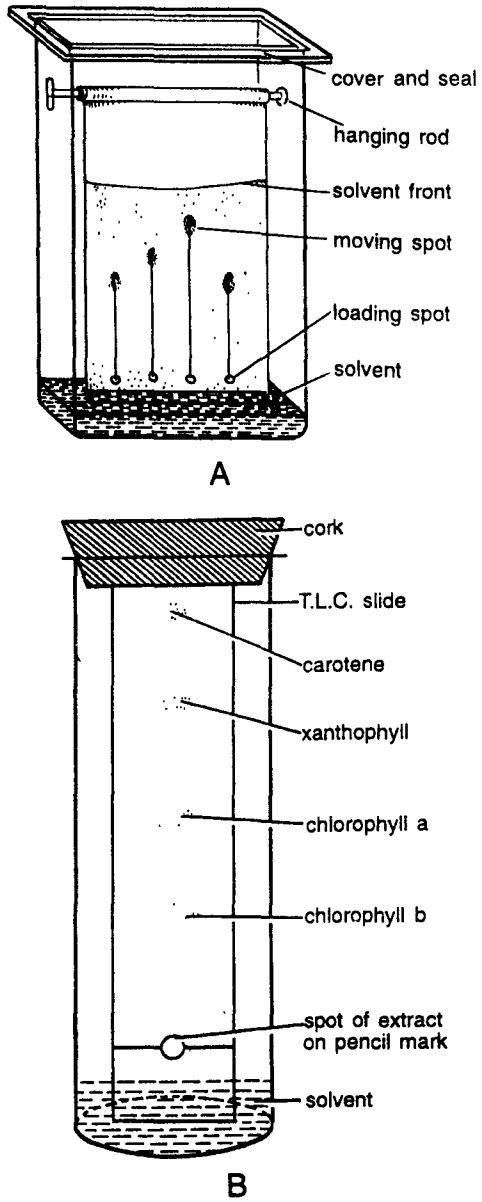


Fig. 1. Chromatographic technique for separation of plant pigments. **A.** Paper chromatography chamber, **B.** Thin layer chromatography.

(12) Two layers appear, upper contains chlorophylls while lower contains carotenoids.

(13) Preserve them in separate containers.

4. Application. ***(a) Paper chromatography.*** (1) Put a spot of the extract on chromatography paper with a capillary tube, 1/2 inch form the left hand margin on the pencil line.

(2) Allow the pigment spot to dry.

(3) Fix the paper strip with clips to the cork of tube or roll it around a glass rod at the top of the jar, so that its bottom just touches the solvent.

(b) For thin layer chromatography. Apply a spot of the extract carefully about 1/2 inch from the bottom and 1/2 inch from the left hand margin of the plate.

5. Development

(1) Develop the paper or plates in any one of the following solvents—

(i) n-butanol, acetic acid, water solvent, 5:1:4;

(ii) carbon tetrachloride and anhydrous sodium sulphate;

(iii) petroleum ether - 95%, acetone, 100:12:

(iv) benzene - acetone, 85:15. (this solvent has been found to be very successful in the practical laboratory).

(2) Pour sufficient solvent (either i, ii, iii or iv) into chromatography jar (or tube) filling about an inch from the bottom.

(3) Place the spotted slide or paper vertically, so that spot is just above the solvent level.

(4) Cover the jar and close the lid tightly.

(5) Allow 1-2 hours for development.

(6) The solvents shall move up the paper or plate (slide).

(7) Remove the chromatogram when the solvent reaches the top and allow it to dry.

(8) Since the colours of the pigments fade, examine the paper or plate when fresh.

Results

The sequence of pigments from top to bottom shall be—

Carotenes: orange yellow;
Xanthophylls : one or more yellow bands;
Chlorophyll a : blue green :
Chlorophyll b : yellow green.
Determine the Rf values by following formula—

$$Rf = \frac{\text{distance from origin travelled by compound}}{\text{distance of solvent front from origin}}$$

Identify the components by comparing results with standardised values.

Table. **Bands of different colours and Rf values.**

	Colour of the band	Rf value	Pigment present
1.	Orange-yellow	0.95	Carotene
2.	Yellow	0.71	Xanthophyll
3.	Blue-green	0.65	Chlorophyll a
4.	Yellow-green	0.45	Chlorophyll b

Exercise 2

Object **: To separate amino acids by chromatography.**

Requirements

Microscope slides, test tubes or specimen tubes with corks, beaker, pipette, oven, glass rod, atomiser, petri dish, glycine, aspartic acid, phenol, ninhydrin, n-butanol, kiesel gel, Whatman chromatography paper, distilled water, etc.

Procedure

[I] Paper chromatography

1. Cut strips of about 14 cm × 1 cm size of Whatman no. 1 chromatography paper.
2. Mark a light pencil dot in the centre of the paper strip one centimeter above at one of the ends. Prepare such three strips.
3. Place a drop of glycine on this marked dot. Place a drop of aspartic acid on similarly prepared another strip of chromatographic paper. On the third strip place a drop of a mixture of glycine and aspartic acid.
4. Take three large specimen tubes and pour 5 ml phenol (80%) in each of the tubes.
5. Cut the cork stoppers of each tube in two halves. Place the strip of chromatographic paper in each cork in such a way that the end with amino acid drop remains free. Also see that the free end of the paper strip just touches the phenol in the tube and amino acid drop remains above the surface of phenol.
6. Allow the experiment to remain undisturbed. Remove the chromatography paper only after the phenol (solvent) reaches the upper region of the paper strip.
7. Keep all the three paper strips in oven at 100°C for about 3 to 5 minutes.
8. Take the strips out of oven and spray ninhydrin (1%) on them with atomiser.
9. Keep these paper strips in oven once again at 100°C for about 3 to 5 minutes.

[II] Thin layer chromatography

1. Prepare a suspension by mixing 2 g of kiesel gel in 10 ml of distilled water.
2. Spread a thin and uniform layer of suspension on thoroughly cleaned microscope slides as a layer.
3. Allow the slides to remain as such for about 15 minutes so that the suspension settles down.
4. Keep the slides in wooden tray and dry them by keeping the tray in oven at 120°C for about 30 minutes.
5. Place a drop of amino acid at one end of the slide, 1 cm above the edge. This is done by dipping the glass rod in amino acid and then transferring it to the slide.

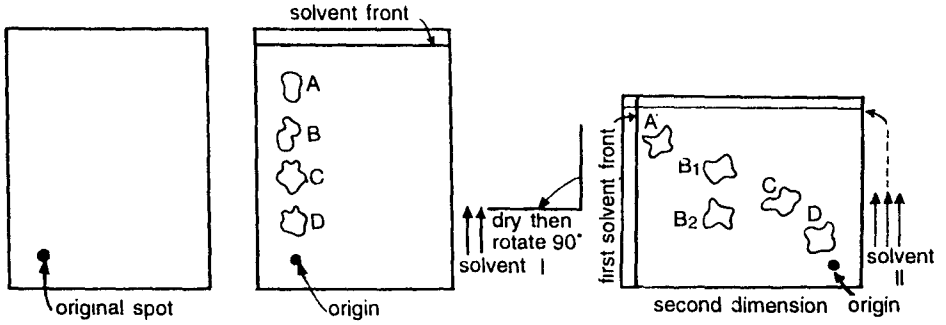


Fig. 2. Technique of two dimensional paper chromatography of amino acids.

6. (i) Place a drop of glycine on one slide.
   (ii) Place a drop of aspartic acid on another slide and
   (iii) Place a drop of a mixture of glycine and aspartic acid on the third slide.
7. Take three large specimen tubes. Pour about 5 ml of phenol (80%) in each one of them.
8. Insert a slide in each specimen tube in such a way that amino acid drop is directed downwards. Though the lower edge of the slide should touch the phenol in the tube, the amino acid drop should remain above the surface of phenol.
9. Close the tube with cork or place a watch glass/petri dish at the open end of the tube.
10. Allow the tubes to remain undisturbed. Chromatogram takes about 15 to 30 minutes to develop. The solvent begins to move upwards during this period.
11. Take out the slide when solvent is seen near the top edge.
12. Keep the slides in oven at 100°C for about 3 to 5 minutes.
13. Spray ninhydrin (1%) on the slides with the help of atomiser.
14. Place the slides in oven once again at 100°C for about 3 to 5 minutes.

Results

1. Purple coloured spots of amino acids are seen on paper/slide.
2. Compare with standard values to find out Rf values and thus identify amino acids.

Exercise 3

***Object* : Separation of chloroplast pigments by solvent method.**

Requirements

Leaves of spinach or *Cucurbita* sps. or *Eichhornia* sps., knife, mortar and pestle, separating funnel, beaker, linen cloth, acetone, alcohol (95%), petroleum ether, KOH, chloroform, test tubes, test tube stand, filter papers, petri dishes, scissors, forceps, etc.

Procedure

1. Cut the leaves into small pieces.
2. Crush them in mortar using a mixture of acetone (80%) and alcohol (95%) in 15 : 85 ml proportion.
3. Filter the extract through a linen cloth to remove debris.
4. Collect the filtered extract in a beaker.
5. Use this extract for pigment separation by solvent extract method.

There are two different methods of separation :

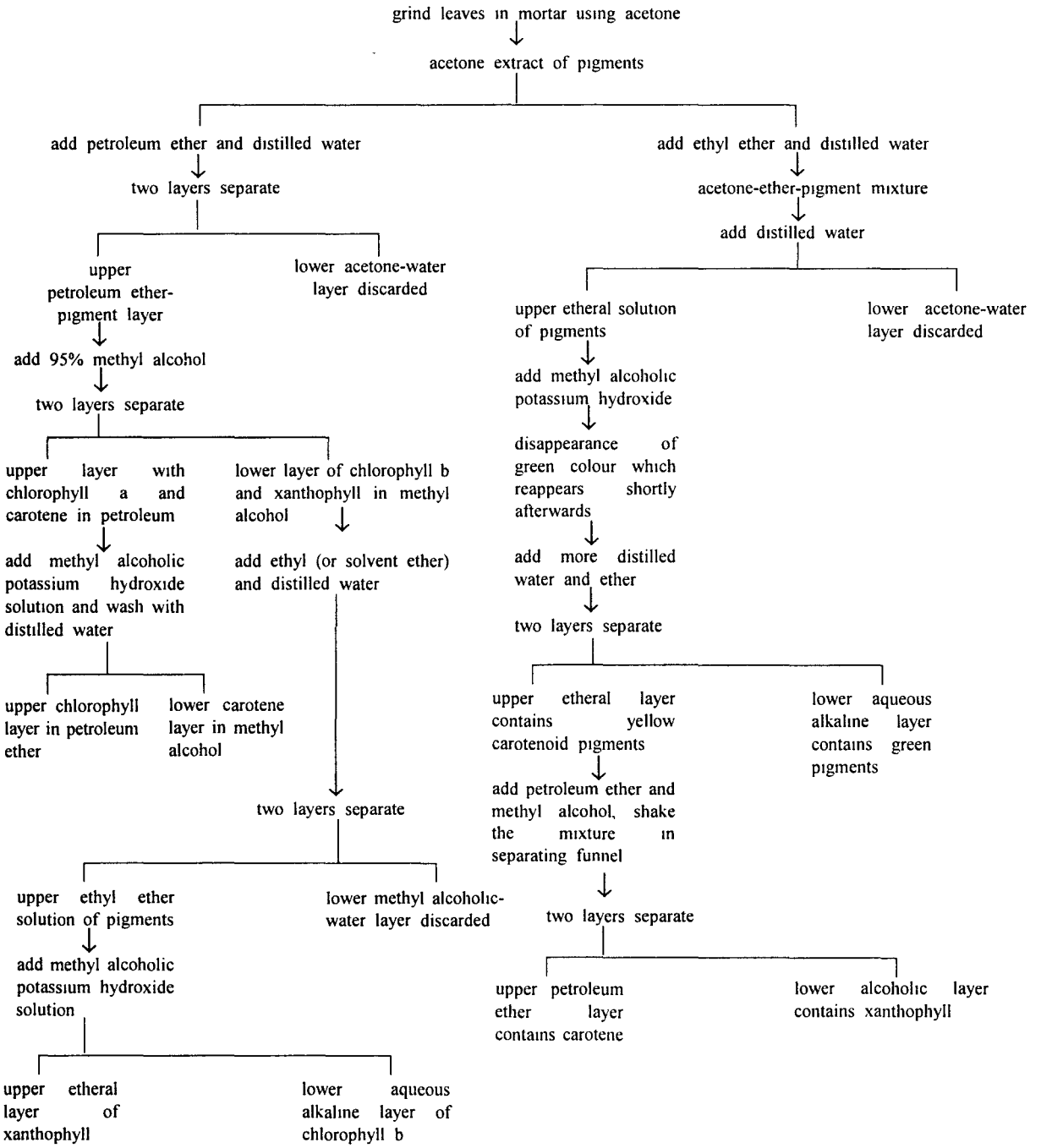
[I] Method 1

1. Take about 100 ml acetone extract in separating funnel (500 ml).
2. Add about 80-100 ml petroleum ether to the funnel.
3. Add a few ml of distilled water to the above mixture and shake thoroughly.
4. Allow the acetone - water layer to settle down.
5. Wash the pigment layer twice by addition of distilled water.
6. Discard lower acetone-water layer.
7. Add 95% methyl alcohol to remaining petroleum ether pigment mixture.
8. Two layers would separate.
   (1) Upper layer with chlorophyll a and carotene in petroleum ether and
   (2) Lower layer with chlorophyll b and xanthophyll.
9. Draw the upper chlorophyll layer and add methyl alcoholic potassium hydroxide solution. Wash with distilled water.
10. Two layers separate
    (1) Upper chlorophyll layer in petroleum ether and
    (2) Lower carotene layer in methyl alcohol.

[II] Method 2

1. Take about 100 ml acetone extract in separating funnel (500 ml).
2. Add about 100 ml of ethyl ether and a few ml of distilled water.
3. Mix the contents and shake thoroughly.
4. Two separate layers appear in separating funnel.
5. Discard the lower water-acetone layer.

SUMMARY OF SOLVENT EXTRACTION METHODS OF CHLOROPHYLL



6. Add methyl alcoholic potassium hydroxide to the remaining etheral solution of pigments.
7. Green colour disappears but reappears shortly afterwards.
8. Add distilled water and ether.
9. Two layers separate
   (1) Upper etheral layer contains yellow carotenoid pigment.
   (2) Lower aqueous alkaline layer contains chlorophylls
10. Discard the esterified lower chlorophyll layer.
11. Add petroleum ether and methyl alcohol to etheral layer containing yellow carotenoid pigments. Shake the mixture in separating funnel.
12. Two layers separate—
    (1) Carotene in upper petroleum ether layer.
    (2) Xanthophyll in lower alcoholic layer.

Exercise 4

***Object* : To study the activity of enzyme catalase.**

Requirements

Potato tuber, knife, petri dishes, test tubes, test tube holder, test tube stand, spirit lamp, hydrogen peroxide (H_2O_2), etc.

Procedure

1. Cut small and thin pieces of potato tuber.
2. Place the pieces in petri dish filled with water.
3. Take a few small pieces and put them in two separate test tubes, marked A and B. Fill both the tubes with appropriate amount of water.
4. Keep the test tube A in the stand. Boil the pieces of potato tuber placed in tube B.
5. Drain out water from both the test tubes. Now add hydrogen peroxide solution (30 parts of water to 1 part hydrogen peroxide), so as to completely immerse the pieces of potato tuber.
6. Note the changes occuring in both the test tubes.

Result

1. Evolution of bubbles is seen in test tube A.
2. There is no evolution of bubbles in test tube B.

Conclusions

Catalase brings about decomposition of hydrogen peroxide into water and oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \uparrow$$

Oxygen evolution, therefore, is an indication of activity of catalase.

The oxygen bubbles are not evolved in test tube B. This is because enzyme is destroyed (denatured) when potato slices are boiled (at high temperature). Hence, there is no enzyme activity.

Exercise 5

***Object* : To study the activity of enzyme peroxidase.**

Requirements

Potato tuber, knife/razor, petri dishes, test tubes, test tube holder, test tube stand, 2% alcoholic solution of gum guaiacum (benzidine), hydrogen peroxide, water, spirit lamp, etc.

Procedure

1. Cut small and thin pieces of potato tuber.
2. Place the pieces in petri dish filled with water.
3. Take a few small pieces and put them in two separate test tubes, marked A and B. Fill appropriate amount of water in each one of them.
4. Leave the test tube A in the stand while boil the pieces of potato tuber placed in tube B.
5. Drain out water from both the test tubes.
6. Add 2% solution of gum guiacum (benzidine) to both the test tubes so that pieces/slices of potato tuber are completely immersed.

Table 1. **Tests for the presence of reducing sugars.**

Experiment	Observations	Conclusions
1. Fehling test Mix 1 ml of Fehling solution A with 1 ml of Fehling solution B. Boil the mixture for about 1 minute. Add a few drops of glucose in Fehling solution and boil.	Solution turns yellow in the beginning Later a brick-red precipitate is obtained.	Presence of reducing sugar in the given sample.
2. Benedict test Take 2-3 ml of given sample in a test tube. Add 2-3 ml of Benedict's reagent and boil for 5 minutes.	The solution turns green, yellow and finally red, depending upon the amount of reducing sugar present in the sample.	Presence of reducing sugar in the given sample.

7. Allow the test tubes to remain undisturbed for about 10-15 minutes.
8. Remove all gum guiacum solution from both the test tubes.
9. Add dilute solution of hydrogen peroxide (3% commercial hydrogen peroxide in 30 parts of water) to both the test tubes.
10. Observe the changes.

Results

1. Potato slices in test tube A change to blue colour very rapidly.
2. There is no change in colour in test tube B.

Conclusion

Absence of blue colour indicates absence of enzyme activity in test tube B, enzymes having been denatured due to high temperature.

Peroxidases are of wide occurrence in the plant tissues and oxidise various substrates (viz. phenols, amines, etc.) in the presence of H_2O_2 as electron acceptos. Hydrogen peroxide with an addition of hydrogen atoms and electrons form water.

$$A.H_2 + H_2O_2 \xrightarrow{\text{peroxidase}} A + 2H_2O$$

Exercise 6

Object **: To perform colour tests for reducing sugar : glucose (grape sugar).**

Requirements

Fehling's solution, Benedict's solution, test tubes, test tube holder, spirit lamp, water, glucose, barley malt, etc.

Procedure

There are two tests to detect the presence of glucose. These are given below.

(a) Fehlings's test. 1. Take about 5 ml of Fehling's solution in a test tube.

2. Add few drops of glucose solution and boil.

(b) Benedict's test. 1. Take about 5 ml of Benedict's solution in a test tube.

2. Add a few drops of glucose solution and boil.

Results

1. The Fehling's solution gives brownish red precipitate confirming the presence of sugar.
2. Benedict's test gives red, yellow or green precipitate cinfirming the presence of sugar.

Exercise 7

Object **: To perform colour test for non-reducing sugars : sucrose.**

Requirements

Sucrose/beet root, Fehling's solution, Benedict's solution, hydrochloric acid, sodium carbonate/sodium bicarbonate, test tubes, test tube holder, spirit lamp, etc.

Procedure

1. Add to sugar equal volume of concentrated hydrochloric acid.
2. Boil the mixture for about five minutes.
3. Neutralise the resulting solution with sodium carbonate or bicarbonate.

Table 2. **Colour tests for the presence of proteins.**

Experiment	Observations	Conclusions
1. Xanthoproteic test		
(a) Take 2-3 ml of aqueous protein solution Add 1 ml of nitric acid to the solution.	White precipitate is obtained	
(b) Heat the white precipitate.	Precipitate turns yellow. It dissolves to yield yellow coloured solution.	
(c) Add sufficient amount of NH_4OH or alkali.	Colour of the solution changes to orange	Presence of protein is confirmed.
2. Biurete test		
(a) Take 2-3 ml of aquous protein solution. Add a few drops (1 ml) of NaOH (4%) solution and		
(b) a few drops of $CuSO_4$ (1%) solution	Violet or red colour is obtained.	Presence of protein in a given solution is confirmed.
3. Millon's test		
(a) Take about 5 ml of aq. protein solution in a test tube. Add a few drops of Millon's reagent A.	Precipitate is formed.	
(b) Boil the mixture.	This gets dissolved on boiling and red coloured solution is obtained.	Presence of protein in a given sample is confirmed.

4. Then subject the solution to the test of reducing sugars by adding Fehling's or/and Benedict's solutions.

Results

1. Fehling's solution given brownish red precipitate to confirm the presence of non-reducing sugars.
2. Benedict's solution gives red, yellow or green precipitate to confirm the presence of non-reducing sugars.

Conclusion

The test reveals the presence of non-reducing sugars.

Sucrose occurs widely in plants. It is formed by condensation of one molecule of glucose with one molecule of fructose. On hydrolysis of sucrose these sugare are formed once again and then give the same results as reducing sugars when subjected to Fehling's and Benedict's solution.

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6} \underset{\text{hydrolysis}}{\overset{\text{condensation}}{\rightleftharpoons}} \underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_2O$$

Exercise 8

Object **: To perform colour tests to demonstrate the presence of proteins.**

Requirements

Gram flour/pea seeds/soya beans, Millon's reagent A and B, nitric acid, ammonium hydroxide, sodium hydroxide (20%), copper sulphate (1%), test tubes, test tube holder, water, spirit lamp, etc.

Procedure

There are two methods to test the presence of proteins.

(a) ***Xanthoproteic test.*** 1. Treat the suspension of tissue in water. Add concentrated nitric acid. A white precipitate is formed.
2. Heat the solution. Yellow colour develops.
3. Now add a few drops of concentrated ammonium hydroxide. Observe the change in colour. The colour deepens to orange.

(b) ***Biurete test.*** 1. Prepare a suspension of material in water.

2. Add 1 ml of sodium hydroxide (20%) and a drop of copper sulphate to the suspension.
3. Observe the developing colour. Add Millon's reagent (*A* or *B*). Mix thoroughly and boil.
4. Note the change in colour in both cases i.e., after adding Millons reagent 'A' and /or 'B'.

Results

(a) ***Xanthoproteic test.*** The yellow colour changes to orange.

(b) ***Biurete test.*** The colour developed is violet. On addition of Millons' reagent 'A' it turns to red and on heating with Millon's reagent 'B' it turns reddish.

Conclusion

The colour changes indicate the presence of protein.

Exercise 9

***Object* : To study the effect of amylase (diastase) on starch.**

Requirements

Starch solution (or extract or slurry of potato pulp), taka diastase or extract from germinating barley seeds, (both are used for amylase), iodine solution, Benedict's solution, test tubes, spirit lamp, etc.

Procedure

1. Take two test tubes.
2. Add a tablet of taka diastase to 100 ml of water in each test tube.
3. Heat and bring to boil the solution in one test tube (A) while keep another (B) as such.
4. Add soluble starch solution to both.
5. Test the solutions in tubes A and B with iodine on porcelain tile or with Benedict's solution.

Results

1. The intensity of the colour in tube A remains unchanged while the colour is intense in tube B which decreases with time.
2. Test the solution in tube B for starch immediately after adding starch solution to the enzyme. Then tests are made for starch after 2, 5, 15 and 20 minutes. Two drops of solution and a drop of iodine on a porcelain tile gives the test (also use Benedict's solution). If blue colour does not appear, dilute stock soln. of amylase with equal volume of $CaCl_2$ solution and repeat the procedure.

The intense colour in the tube A indicates the presence of starch. This intense colour also appears in tube B, thereby indicating presence of starch. However, later, intensity decreases in tube B showing disappearance of the starch.

Conclusions

Amylase (Taka diastase) is an enzyme. It is widely distributed in plant tissues, particularly in germinating seeds. It is specific for starch and breaks it to simple monosaccharides (glucose). It functions to meet the requirement of glucose especially during seed germination.

The tube A when heated does not show reactions because the enzyme is denatured at high temperature. Enzymes are proteins and are most effective in concentrated solutions. Effective temperature and pH range is very narrow and beyond this enzyme is inactivated irreversibly.

Second experiment shows the effect of enzyme concentration on the rate of reaction. The amount of starch (indicated by the comparative intensity of the blue colour) will be highest in a sample where enzyme concentration was lowest. The tube with minimum starch shall have maximum enzyme activity.

If the amount of starch (substrate) is sufficient and does not limit the rate of reaction, then the reaction rate is usually directly proportional to enzyme concentration.

4

Biotechnology

Exercise 1

***Object* : Preparation of different nutrient media for tissue culture.**

Requirements

Weighing balance, ingredients of the nutrient medium, distilled water, plastic/glass bottles, agar, sucrose, culture tubes, culture flasks, non-absorbent cotton, aluminium foil, laboratory facilities like autoclave, deep freeze, water bath, growth chamber, etc.

Procedure

The following procedural steps vary depending upon the specific purpose and the facilities available in the laboratory.

1. Weigh the prescribed quantities of ingredients.
2. Dissolve each one of them separately and mix them before preparation of the medium.
3. A more convenient and commonly used method, however, is to prepare a series of concentrated stock solutions e.g. to prepare MS basal medium, prepare four different stock solutions (Table 8).
   (1) major salts (20 × concentrated)
   (2) minor salts (200 × concentrated)
   (3) iron (200 × concentrated)
   (4) organic nutrients except sucrose (200 × concentrated)
4. Dissolve each component of the stock solutions (1) to (4) separately and then mix with others.
5. Prepare stock solution for each growth regulator by dissolving it in a very minute quantity of appropriate solvent and making up the final volume with distilled water These stock solutions may be prepared at the strength of 1 m mol l^{-1} or m mol l^{-1}.
6. Store all the stock solutions in appropriate sterile plastic or glass bottles under refrigeration.
7. The iron stock solution should be stored in an amber coloured bottle.
8. For storing coconut milk, the water collected from fruits is boiled to deproteinise it, filtered and stored in plastic bottles in a deep freeze at – 20°C. Shake the bottles before using the stock.
9. Required quantities of agar and sucrose are weighed and dissolved in water, about 3/4th the volume of the medium, by heating them in a water bath or an autoclave at low pressure (not required for liquid medium).
10. Add recommended quantities of various stock solutions including growth regulators and other special supplements. Auxins and vitamins are generally added after autoclaving.
11. Make the final volume by adding desired quantity of distilled water.
12. Mix all the ingredients properly. Adjust pH of the medium by using 0.1 N NaOH and 0.1 N HCl.
13. Pour the medium in suitable culture vessels. About 15 ml of the medium is poured in each 25 ml culture tube and about 50 ml in a 150 ml flask.

14. If the medium begins to solidify and becomes gel, during steps (10) to (13), the flask containing medium should be heated in a water bath and poured in culture vessels only when in a uniformly liquid state.
15. Plug the culture vessels (tubes/flasks) with non-absorbent cotton wool, wrapped in cheese cloth or any other suitable material.
16. Cover the cotton plug of the culture vessels with aluminium foil. Transfer them to the baskets of appropriate size.
17. Autoclave the culture vessels at 120°C (1.06 kg/cm^2) for 15 minutes to sterilize the medium.
18. Allow the medium to cool at room temperature and store the culture vessels at 4°C.
19. Make slants by keeping the tubes tilted during cooling when preparing a solid medium.
20. The nutrient medium is ready for inoculation (tables 1, 2 and 3, Pages 51, 52, and 53).

Exercise 2

***Object* : To produce haploid plants by anther culture.**

Requirements

Controlled temperature growth room, dissecting microscope, laminar flow cabinet, autoclave, hydrochloric acid, acetocarmine, glacial acetic acid, ethanol, distilled water, Murashige and Skoog's basal medium, naphthalene acetic acid (NAA), 6-benzyl aminopurine (BAP), kinetin, sodium hydroxide, etc.

Procedure

1. Grow the experimental plant (of which anthers are to be used for culture) under controlled conditions of light, temperature and humidity. Select flower buds with young anthers. In most of the species anthers are best cultured when the pollen is unicellular and uninucleate or is just entering the first pollen meiosis. To determine the suitable stage of anthers for culture, use some external features such as length of bud, emergence of corolla from calyx, length of corolla tube, etc. It is an important factor in anther culture because the androgenic response declines with increasing age.
2. To be sure that the anthers are at the right stage for culture, break open anthers of some of the selected buds on a glass slide and stain the pollens with acetocarmine. Examine the pollens under microscope.
3. Surface sterilize the selected buds. This can be done effectively by rinsing the buds in 70% ethanol for 1 minute. Now immerse the buds in 1% sodium hypochlorite (or 9-10% calcium hypochlorite) for 15 minutes. Rinse the buds 3 times in distilled water. The outer surface of the buds is now contaminant free and the tissue inside the bud are also sterile.
   From this point onwards all procedures should be performed in the laminar flow cabinet or in a perfectly sterile chamber.
4. Carefully cut open the bud, remove the sepals and petals. Now remove the stamens with fine forceps and place them on a sterile petri plate. Separate each anther from its filament. It is necessary to see that filament in not cultured because it is a diploid tissue.
5. Plate the anthers horizontally on MS medium with NAA (1 mg l^{-1}) and Kinetin (1 mg l^{-1}). This serves as induction medium. It contains auxin and cytokinin in equal amounts. Seal the petri plate with paraffin. Incubate the culture plates in alternating light (12-18 hrs. at 28°C) and dark (6-12 hrs. at 22°C) periods.

During this entire procedure take special care to avoid injury to anther wall. Wounds usually stimulate anther wall cells to proliferate and form callus.

Observations

In 3-5 weeks, the wall of the responsive anthers gradually turns brown and bursts. Observe the callus induction under dissecting microscope. Calli developing from pollen grains are usually yellow or white whereas those developing from filaments and anther wall are generally green. Such green calli can be excised and discarded.

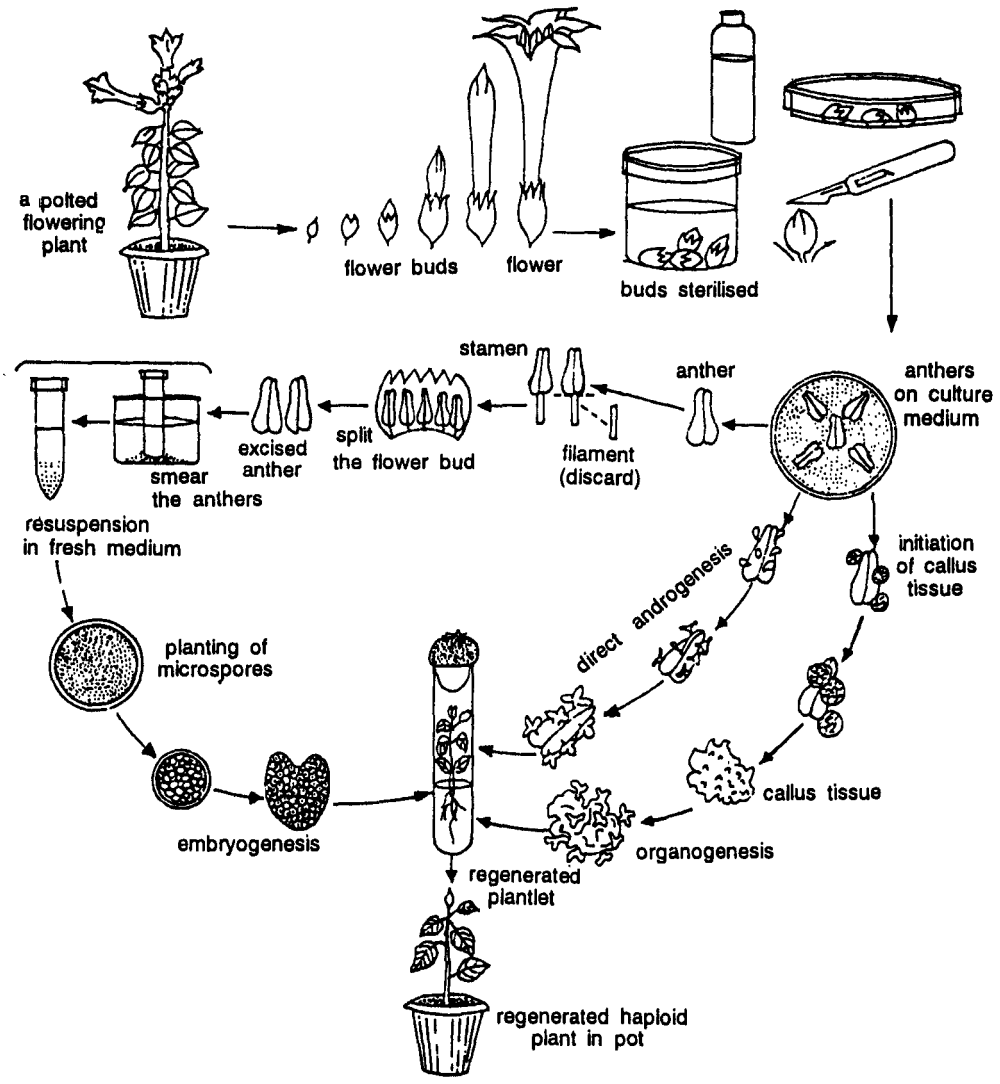


Fig. 1. Production of haploid plants.

[I] Shoot regeneration

The anther calli obtained in the induction medium are transferred to another medium, called shoot regeneration medium. It contains high concentration of cytokinin and very low concentration of auxin. Such a medium induces shoot formation on the anther callus.

(B-52)

[II] Regeneration of roots on shoots

Allow the shoot to grow in shoot regeneration medium till they attain a length of 10 mm. The shoots are now excised with a sharp scalpel from the callus and transferred to a flask containing root inducing medium. It is a solid MS medium which contains no phytohormones. Roots develop within 10-15 days.

Table 1. **Composition of some Plant Tissue Culture Media.**

Constituents	Media (amounts in mg l^{-1})[h]						
	Whites[c]	Hellers[d]	MS[e]	ER[f]	B_5[g]	Nitsch's[h]	NT[i]
Inorganic							
NH_4NO_3	–	–	1650	1200	–	720	825
KNO_3	80	–	1900	1900	25275	950	950
$CaCl_2.2H_2O$	–	75	440	440	150	–	220
$CaCl_2$	–	–	–	–	–	166	–
$MgSO_4.7H_2O$	750	250	370	370	246.5	185	1233
KH_2PO_4	–	–	170	340	–	68	680
$(NH_4)_2SO_4$	–	–	–	–	134	–	–
$Ca(NO_3)_2.4H_2O$	300	–	–	–	–	–	–
$NaNO_3$	–	600	–	–	–	–	–
Na_2SO_4	200	–	–	–	–	–	–
$NaH_2PO_2.H_2O$	19	125	–	–	150	–	–
KCl	65	750	–	–	–	–	–
KI	0.75	0.01	0.83	–	0.75	–	0.83
H_3BO_3	1.5	1	6.2	0.63	3	10	6.2
$MnSO_4.4H_2O$	5	0.1	22.3	2.23	–	25	22.3
$MnSO_4.H_2O$	–	–	–	–	10	–	–
$ZnSO_4.7H_2O$	3	1	8.6	–	2	10	–
$ZnSO_4.4H_2O$	–	–	–	–	–	–	8.6
$Zn.Na_2.EDTA$	–	–	–	15	–	–	–
$Na_2MoO_4.2H_2O$	–	–	0.25	0.025	0.25	0.25	0.25
MoO_3	0.001	–	–	–	–	–	–
$CuSO_4.5H_2O$	0.01	0.03	0.025	0.0025	0.025	0.025	0.025
$CoCl_2.6H_2O$	–	–	0.025	0.0025	0.025	–	–
$CoSO_4.7H_2O$	–	–	–	–	–	–	0.03
$AlCl_3$	–	0.03	–	–	–	–	–
$NiCl_2.6H_2O$	–	0.03	–	–	–	–	–
$FeCl_3.6H_2O$	–	1	–	–	–	–	–
$Fe_2(SO_4)_3$	2.5	–	–	–	–	–	–
$FeSO_4.7H_2O$	–	–	27.8	27.8	–	27.8	27.8
$Na_2.EDTA.2H_2O$	–	–	37.3	37.3	–	37.3	37.3
Sequestrene 330 Fe	–	–	–	–	28	–	–
Organic							
Inositol	–	–	100	–	100	100	100
Nicotinic acid	0.05	–	0.5	0.5	1	5	–
Pyridoxine HCl	0.01	–	0.5	0.5	1	0.5	–
Thiamine HCl	0.01	–	0.1	0.5	10	0.5	1
Glycine	3	–	2	2	–	2	–
Folic acid	–	–	–	–	–	0.5	–
Biotin	–	–	–	–	–	0.05	–
Sucrose	2%	–	3%	4%	2%	2%	1%
D-Mannitol	–	–	–	–	–	–	12.7%

*Growth regulators and complex nutrient mixtures described by various authors are not included here. [b]Concentrations of mannitol and sucrose are expressed in percentage. [c]White (1963). [d]Heller (1953). [e]Murashige and Skoog (1962). [f]Eriksson (1965) [g]Gamborg et al. (1968). [h]Nitsch (1969). [i]Nagata and Takebe (1971).

Table 2. **Data sheet used for media preparation.**

MEDIA NO.	SUMMARY COMPOSITION	VOLUME	PURPOSE	DATE :
				PREPARED BY :
CONSTITUENTS	TYPE	STOCK CONC.	AMOUNT ADDED	
Macro Minerals		20x		
Micro		200x		
Iron		200x		
Organics		200x		
Sugar				
Auxin		1 mmol l^{-1}		
		10 mmol l^{-1}		
Cytokinin		1 mmol l^{-1}		
		10 mmol l^{-1}		
Other				
Agar				
Container	pH required			
	pH original			
	pH adjusted			

Table 3. **Molecular Weights of the Compounds Commonly Used in Tissue Culture Media.**

Compound	Chemical formula	Molecular weight
Macronutrients		
Ammonium nitrate	NH_4NO_3	80.04
Ammonium sulphate	$(NH_4)_2SO_4$	132.15
Calcium chloride	$CaCl_2.2H_2O$	147.02
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	236.16
Magnesium sulphate	$MgSO_4.7H_2O$	246.47
Potassium chloride	KCl	74.55
Potassium nitrate	KNO_3	101.11
Potassium dihydrogen *ortho*-phosphate	KH_2PO_4	136.09
Sodium dihydrogen *ortho*-phosphate	$NaH_2PO_4.2H_2O$	156.01
Micronutrients		
Boric acid	H_3BO_3	61.83
Cobalt chloride	$CoCl_2.6H_2O$	237.93
Cupric sulphate	$CuSO_4.5H_2O$	249.68
Manganous sulphate	$MnSO_4.4H_2O$	223.01
Potassium iodide	KI	166 01
Sodium molybdate	$Na_2MoO_4.2H_2O$	241.95
Zinc sulphate	$ZnSO_4.7H_2O$	287.54
Sodium EDTA	$Na_2.EDTA.2H_2O$ $(C_{10}H_{14}N_2O_8Na_2\ 2H_2O)$	372.25
Ferrous sulphate	$FeSO_4.7H_2O$	278.03
Ferric—sodium EDTA	FeNa.EDTA $(C_{10}H_{12}FeN_2NaO_8)$	367.07
Sugars and sugar alcohols		
Fructose	$C_6H_{12}O_6$	180.15
Glucose	$C_6H_{12}O_6$	180.15

Contd.

Mannitol	$C_6H_{14}O_6$	182.17
Sorbitol	$C_6H_{14}O_6$	182.17
Sucrose	$C_{12}H_{22}O_{11}$	342.31
Vitamins and amino acids		
Ascorbic acid (vitamin C)	$C_6H_8O_6$	176.12
Biotin (vitamin H)	$C_{10}H_{16}N_2O_3S$	244.31
Calcium pantothenate (Ca salt of vitamin B_5)	$(C_9H_{16}NO_5)_2$ Ca	476.53
Cyanocobalamine (vitamin B_{12})	$C_{63}H_{90}CoN_{14}O_{14}P$	1357.64
L-Cysteine HCl	$C_3H_7NO_2S$. HCl	157.63
Folic acid (vitamin B_c, vitamin M)	$C_{19}H_{19}N_7O_6$	441.40
Inositol	$C_6H_{12}O_6$	180.16
Nicotinic acid or Niacin (vitamin B_3)	$C_6H_5NO_2$	123.11
Pyridoxine HCl (vitamin B_6)	$C_8H_{11}NO_3$.HCl	205.64
Thiamine HCl (vitamin B_1)	$C_{12}H_{17}ClN_4OS$.HCl	337.29
Glycine	$C_2H_5NO_2$	75.07
L-Glutamine	$C_5H_{10}N_2O_3$	146.15
Hormones		
Auxins		
$_p$CAA (p-chlorophenoxyacetic acid)	$C_8H_7O_3Cl$	186.59
2, 4-D (2, 4-dichlorophenoxyacetic acid)	$C_8H_6O_3Cl_2$	221.04
IAA (indole-3-acetic acid)	$C_{10}H_9NO_2$	175.18
IBA (3-indolebutyric acid)	$C_{12}H_{13}NO_2$	203.23
NAA (α-naphthalene acetic acid)	$C_{12}H_{10}O_2$	186.20
NOA (β-naphthoxyacetic acid)	$C_{12}H_{10}O_3$	202.20
Cytokinins/purines		
Ad (adenine)	$C_5H_5N_5.3H_2O$	189.13
$AdSO_4$ (adenine sulphate)	$(C_5H_5N_5)_2.H_2SO_4.2H_2O$	404.37
BA or BAP (6-benzyladenine or 6-benzylamino purine)	$C_{12}H_{11}N_5$	225.20
2-ip (6-γ, γ-dimethylallylamino purine or N-isopentenylamino purine)	$C_{10}H_{13}N_5$	203.3
9-[(2 tetrahydroyranyl)-H-purine]	$C_{10}H_9N_5O$	215.21
SD8339 [6-(benzylamino)-9-(2-tetrahydropyranyl)-H-purine]	$C_{17}H_{19}N_5O$	309.40
Zeatin [6-(4-hydroxy-3-methylbut-2-enylamino)-purine]	$C_{10}H_{13}N_5O$	219.20
Gibberellin		
GA_3 (gibberellic acid)	$C_{19}H_{22}O_6$	346 37
Other compounds		
Abscisic acid	$C_{15}H_{20}O_4$	264.31
Colchicine	$C_{22}H_{25}NO_6$	399.43
Phloroglucinol	$C_6H_6O_3$	126.11

Exercise 3

***Object* : Demonstration of micropropagation.**

Requirements

The stock plants, incubator, laminar flow cabinet, growth cabinet or glass house, 70% ethanol, manoxol (or any other surfactant), distilled water, 2% chloros, nutrient media MS or B5 (powdered and prepared), compost (3 : 2, peat : sand), 50 mm and 85 mm diameter sterile petri dishes, 250 ml sterile jars (or conical flasks), propagation trays, seed trays, plastic bags, glass sheets, forceps, blades, scissor, needles, scalpel, hand gloves, laboratory sealing film, pots, etc.

Procedure

The following is a representative schedule for a typical micropropagation. However, the procedure would require modifications and adjustments depending upon the material used and the laboratory facilities available.

1. Cut off the shoot tip with a clean and sharp blade.
2. Put the severed apical meristems (or axillary buds) in distilled water.

3. Conduct the next few steps in laminar flow cabinet or under perfect aseptic conditions.
4. Transfer up to 20 axillary buds in a sterile test tube.
5. Fill the test tube with 70% ethanol. Allow ethanol to remain in the test tube as such for about 2 minutes.
6. Pour alcohol and replace it with 2% chloros solution. Shake the test tube vigorously (about 120 strokes per minute) for about 10 minutes.
7. Decant the chloros solution and fill the test tube with sterile distilled water.
8. Rinse the material for 3 to 4 times. Store it in distilled water (but not for more than two hours).
9. Empty the contents of the tube into a sterile petri dish.
10. Pour MS (MS 1) medium in a sterilized petri dish. Place up to 4 buds (50 mm petri dish) or 10 buds (85 mm petri dish).
11. Take care to ensure that each bud is stuck firmly in the medium but is not buried.
12. Seal the petri dish with laboratory sealing film. Make 2-4 fine holes in the film with sterilised needle.
13. Place the petri dish in growth room or incubator. Allow it to remain for a period of about 1 week to 2 months depending upon the material used.
14. If multiplication is desired, cut internodes and transfer apical cuttings to sterile jars containing medium MS 2 or MS 4.
15. The basal portion of the cutting should be firmly placed in the medium without burying it.

Table 4. **Nutrient Media Useful in Micropropagation by Axillary Bud Culture.** (amount of ingredients $-gl^{-1}$ or mgl^{-1}).

Chemicals	Medium 1 (M&S)	Medium 2 (M&S)	Medium 3 (B5)	Medium 4 (M&S)
NH_4NO_3	1.65g	1.65g	–	1.65g
KNO_3	1.9g	1.9g	2.5g	1.9g
$CaCl_2.2H_2O$	0.44g	0.44g	0.15g	0.44g
$MgSO_4.7H_2O$	0.37g	0.37g	0.25g	0.37g
KH_2PO_4	0.17g	0.17g	–	0.17g
$(NH_4)_2SO_4$	–	–	13.4g	–
$NaH_2PO_4.H_2O$	–	–	0.15g	–
FeNa, EDTA	36.7 mg	36.7 mg	40.0 mg	36.7 mg
H_3BO_3	6.2 mg	6 2 mg	3.0 mg	6.2 mg
$MnSO_4.4H_2O$	22.3 mg	22.3 mg	10.0 mg	22.3 mg
$ZnSO_4.7H_2O$	8.6 mg	8.6 mg	2.0 mg	8.6 mg
KI	0.83 mg	0.83 mg	0.75 mg	0 83 mg
$Na_2MoO_4.2H_2O$	0.25 mg	0.245 mg	0.25 mg	0.025 mg
$CuSO_4.5H_2O$	0.025 mg	0.025 mg	0.025 mg	00.025 mg
$CoCl_2.6H_2O$	0.025 mg	0.025 mg	0.025 mg	0.25 mg
Nicotinic Acid	0.50 mg	0.50 mg	1.0 mg	0.50 mg
Thiamine HCl	0.10 mg	0 10 mg	10.0 mg	0.10 mg
Pyridoxine HCl	0.50 mg	0.50 mg	1.0 mg	0.50 mg
Glycine	2.0 mg	2.0 mg	–	2.0 mg
Sucrose	20.0 g	20 0 g	20.0 g	20.0 g
Inositol	0.1 g	0.1 g	0.1 g	0.1 g
Glutamine	0.1 g	0.1 g	0 1 g	–
BAP (6-benzyl-amino purine)	0.25 mg	0.25 mg	0.25 mg	0 25 mg
GA_3 (gibberellic acid)	0.1 mg	–	–	–
NAA (1-napthalene-acetic acid)	–	–	–	0 05 mg l^{-1}
pH	5.64	5.64	5.64	5.64
Agar (Difco Bacto)	8.0 g	8.0 g	8 0 g	8.0 g

16. If plants are desired to be rooted and transferred to compost, instead of steps 14 and 15 follow the procedure given below.
17. Cut off apex with 2 or 3 nodes (about 5 mm in length).
18. Transfer about 12 plants per 250 ml jar containing culture medium MS 4.
19. Allow them to grow for about 3 to 5 days till approximately 5 mm long roots develop.
20. Remove each plantlet carefully without disturbing the roots. Also try to remove from roots as much agar as possible.
21. Fill polythene bags or small pots with compost. Water the bags or pots as the case may be.
22. Transfer the plantlets to damp compost.
23. Maintain high humid conditions for about 12 to 24 hours.
24. Transfer the polythene bags or pots to glass house. Leave them in shade away from the direct sunlight.
25. Once the plants show signs of maturity (about 70 mm in height), transfer them to larger pots or nursery beds.

Exercise 4

***Object* : Isolation of protoplast.**

Three different tissues are used and therefore, requirements and methods for each one of them are described separately.

[I] Isolation of protoplasts from mesophyll cells of tobacco by simultaneous method

Requirements

Tobacco leaves, 70% ethanol, 0.5% sodium hypochlorite solution, distilled water, 600m mol l^{-1} mannitol – CPW solution, (CPW – cell-protoplast-washing medium contains (mg l^{-1}) : KH_2PO_4 (27.2), KNO_3 (101), $CaCl_2$. $2H_2O$ (1480), $MgSO_4$. $7H_2O$ (246), KI (0.16), $CuSO_4.5H_2O$ (0.025), pH (5.8), enzyme 4% cellulase SS, 0.4% macero-enzyme SS, sucrose, parafilm, forceps, petri dishes, Pasteur pipette, 60-80 mμ mesh, centrifuge tubes, etc.

Procedure

1. Select fully grown leaves from 7-8 week-old plants.
2. Surface-sterilize the leaves by first immersing in 70% ethanol for about 30 seconds followed by rinsing in 0.4-0.5% sodium hypochlorite solution for about 30 minutes.
3. Wash the leaves thoroughly with sterile distilled water to remove all the traces of hypochlorite.
4. Peel off the lower epidermis with fine forceps. Cut the peeled areas into pieces.
5. Place the peeled leaf pieces on a thin layer of 600 m mol l^{-1} mannitol — CPW solution in such a way that the peeled surface is in contact with the solution.

***Table 5.* Stock solutions for Murashige and Skoog's (MS) medium*.**

Constituents	Amount (mg^{-1})
Stock solution I	
NH_4NO_3	33000
KNO_3	38000
$CaCl_2.2H_2O$	8800
$MgSO_4.7H_2O$	7400
KH_2PO_4	3400
Stock solution II	
KI	166
H_3BO_3	1240
$MnSO_4.4H_2O$	4460
$ZnSO_4.7H_2O$	1720
$Na_2MoO_4.2H_2O$	50
$CuSO_4.5H_2O$	5
$CoCl_2.6H_2O$	5
Stock solution III[b]	
$FeSO_4.7H_2O$	5560
Na_2.EDTA.$2H_2O$	7460
Stock solution IV	
Inositol	20000
Nicotinic acid	100
Pyridoxine HCl	100
Thiamine HCl	100
Glycine	400

*To prepare 1 litre of medium take 50 ml of stock I, 5 ml of stock II, 5 ml of stock III and 5 ml of stock IV.

[b]Dissolve $FeSO_4$. $7H_2O$ and Na_2. EDTA. $2H_2O$ separately in 450 ml distilled water by heating and constant stirring. Mix the two solutions, adjust the pH to 5.5 and add distilled water to make up the final volume. to one litre

6. After about 30 minutes replace the mannitol — CPW solution by filter-sterilized solution of enzyme containing 4% cellulase SS, 600 m mol l^{-1} mannitol and CPW salts.
7. Seal the petri dish with parafilm and incubate it in the dark at 24-26°C for 16-18 hours.
8. Gently squeeze the leaf pieces with a Pasteur pipette to liberate the protoplasts.
9. Remove the large debris by filtering through 60-80 µm mesh.
10. Transfer the filtrate to a screw-cap centrifuge tube and sediment the protoplasts at 100 g for 3 minutes.
11. Remove the supernatant and transfer the sediment on the top of 860 m mol l^{-1} sucrose solution (prepared in CPW) in a screw-cap centrifuge tube and centrifuge it at 100 g for 10 minutes.
12. Collect the green protoplast band from the top of the sucrose solution and transfer it to another centrifuge tube.
13. Add the protoplast culture medium to suspend the protoplasts and centrifuge at 100 g for 3 minutes. Repeat these washings for at least three times.
14. After the final washing add sufficient amount of culture medium to achieve protoplast density of 0.5×10^5 to 1×10^5 ml^{-1}.
15. Plate the protoplasts as small (100-150 µl) droplets or a thin layer in petri dishes.

[II] Isolation of protoplasts from mesophyll cells of cereals

Requirements

Wheat/rice seedlings, 0.1% zephirin, 10% ethanol, washing solution (600 m mol l^{-1} sorbitol solution containing 10 m mol^{-1} $CaCl_2$), 0.5% macerozyme, 1% hemicellulase, 2% cellulysin, 600 m mol l^{-1} sorbitol, pH 5.4), nylon mesh (pore size 0.7 mm, 0.05 mm), petri dishes, test tubes, etc.

Procedure

1. Take primary leaves from 5-6 day old seedlings by cutting at the base of the leaf. Discard the apical 0.5 cm region.
2. Surface-sterilize the leaves in 0.1% Zephirin - 10% ethanol solution for 5 minutes.
3. Wash twice with washing solution (600 m mol l^{-1} sorbitol solution containing 10 m mol l^{-1} $CaCl_2$).
4. Cut the leaves into 1-2 mm wide transverse sections and transfer them to the enzyme solution (0.5% maceroenzyme, 1% hemicellulase, 2% cellulysin, 600 m mol l^{-1} sorbitol, pH 5.4) with a ratio of 10 ml enzyme solution for each gram of leaf tissue.
5. Infiltrate the leaves under partial vacuum for 3-5 minutes.
6. Incubate the leaves in the dark, at 23 ± 2°C, on a shaker with 80 strokes per minute.
7. After 2 hours filter the leaf digest through two layers of nylon mesh (first mesh of pore size 0.7 mm and the second of 0.05 mm).
8. Transfer the filtrate to centrifuge tubes and spin at 50 g for 90 seconds.
9. Remove the supernatant and wash the pellet thrice with washing medium.
10. Suspend the protoplast in nutrient medium and culture them.

[III] Isolation of protoplasts from root nodules of *Trifolium* sp.

Requirements

Roots of *Trifolium*, protoplast dilution buffer (PDB), 4% cellulysin, 2% macerase, 1% driselase, 50 µm nylon mesh, Pasteur pipette, 30% sucrose solution,

Procedure

1. Cut the nodules (1.5 mm in length) from aseptically grown plants.
2. Wash thrice with protoplast dilution buffer.
3. Cut the nodules into four pieces and wash again in PDB.
4. Transfer the nodule pieces to the enzyme solution containing 4% cellulysin, 2% macerase, 1% driselase in PDB, pH 5.8.
5. After incubation of about 3-4 hours at 23°C in the dark dissociate the partially digested nodules by passing through the orifice of Pasteur pipette and continue incubation for a further 90 minutes.
6. Sieve through a 50 µm nylon mesh and wash the digested tissue twice in PDB by centrifuging at 200 g for 10 minutes.

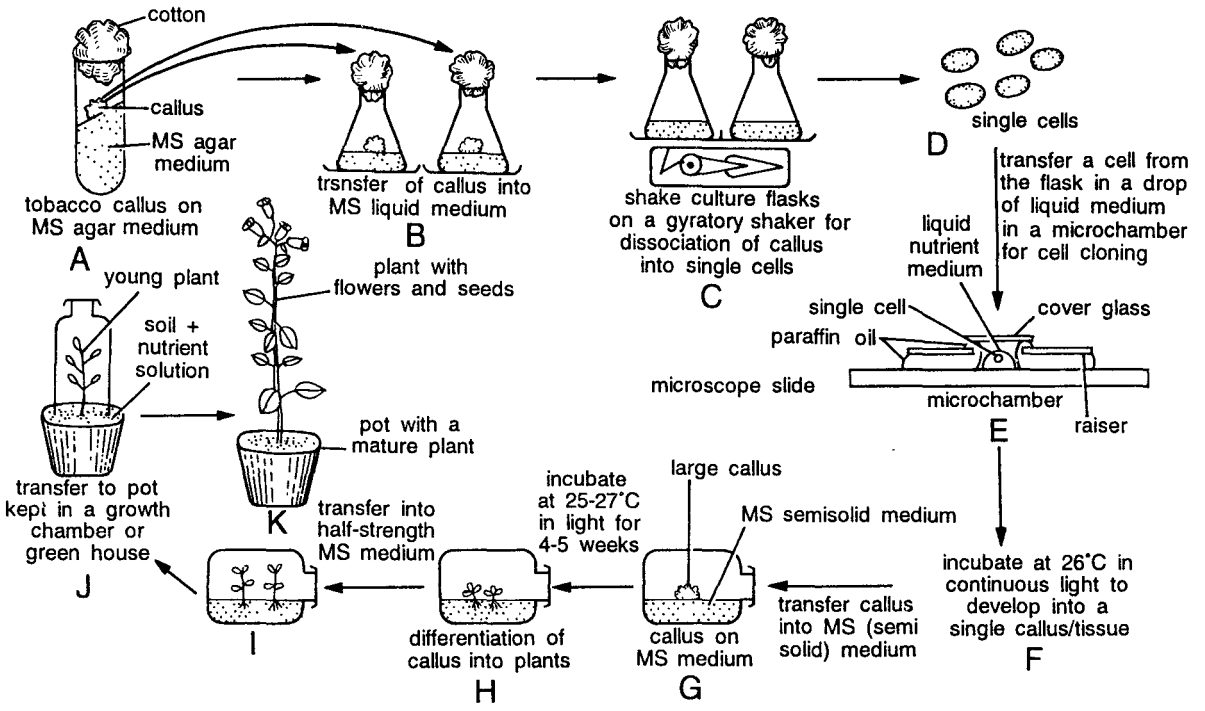


Fig 2 Method root, shoot formation in liquid medium

7. Transfer the suspension onto a 30% sucrose solution and centrifuge at 100 g for 10 minutes.
8. Collect the intact protoplast from the top of the sucrose solution.

Exercise 5

***Object* : Demonstration of root, shoot formation from the apical and basal portion of stem segments in liquid medium containing different hormones.**

Requirements

Controlled temperature growth room, dissecting microscope, laminar flow cabinet, autoclave, hydrochloric acid, Murashige and Skoog's basal medium, naphthalene acetic acid (NAA), 6-benzyl aminopurine (BAP), Kinetin, sodium hydroxide, etc.

Procedure

The following is a tentative schedule for a typical plant tissue culture. The exact procedure would, however, differ depending upon the material to be used and the laboratory facilities available.

1. Cut off the shoot tip or any desired part with a clean and sharp blade.
2. Place the severed part in a petri dish containing distilled water.
3. Conduct the next few steps in laminar flow cabinet or under perfect aseptic conditions.
4. Cut portion (explant) is dipped into 5% teepol (or any other surface steriliser) for 5 to 10 minutes for surface sterilisation. Wash the explant in sterilised distilled water.
5. The explant is further surface sterilised by immersing in 70% ethanol (V/V) for 40 to 60 seconds followed by 5% sodium hypochlorite (V/V) for 20-30 minutes.

6. The explant is finally washed at least three times in sterilised distilled water.
7. A thin solid section of the explant is now cut by a sterilised scalpel and placed in sterilised petri dish. 5% sodium hypochlorite solution is now added to the petri dish. The section is allowed to remain in this solution for about 5-10 minutes.
8. The explant is now repeatedly washed in sterile distilled water.
9. The explant is then placed in another clean and sterilised petri dish.
10. Prepare sterilised conical flasks with nutrient medium and keep them plugged with cotton.
11. The cambium is carefully removed from the sections under total aseptic conditions.
12. The separated cambial tissue is now transferred to the conical flasks containing medium.
13. The flask marked for induction of root should contain low concentration of cytokinin and relatively high concentration of auxins in the medium.
15. The process of incubation involves removal of cotton plug, insertion of explant and replugging under completely aseptic conditions.
16. Allow at least 4-6 weeks for the callus growth to take place.
17. Callus tissue can now be removed for sub-culture.

5

Ecology

Exercise 1

***Object* : To determine the minimum number of quadrats required for reliable estimate of biomass in grasslands.**

Requirements

Quadrat of definite size, graph sheet, herbarium sheet, cellotape, etc.

Procedure

1. Determine the minimum size of quadrat by species-area curve method.
2. Lay down 25 to 50 quadrats of size thus determined at different sites along the entire grassland.
3. List the different species present in each quadrat.

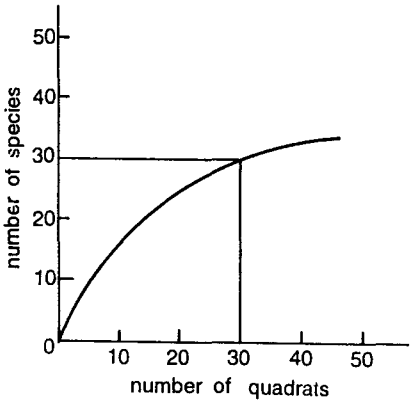


Fig 1. A graph to determine the minimum number of quadrats required

4. Find out accumulating total of the number of species for each quadrat.
5. Plot the number of quadrats on X-axis against accumulating total number of species on Y-axis.

Result

A curve is obtained. The point at which curve starts flattening would give the minimum number of quadrats to be laid down.

Exercise 2

***Object* : To determine the frequency of various species occurring in a given area.**

Requirements

Quadrats of required size, measuring tape, paper, pencil, etc.

Procedure

The following are some of the common methods.

[I] Quadrat

1. Take a quadrat of suitable size, lay it randomly at number of places.
2. Identify the species or distinguish them as **A**, **B**, **C**, etc.
3. Find out the presence or absence of each of the species in each segment (square) of the quadrat and tabulate the data.

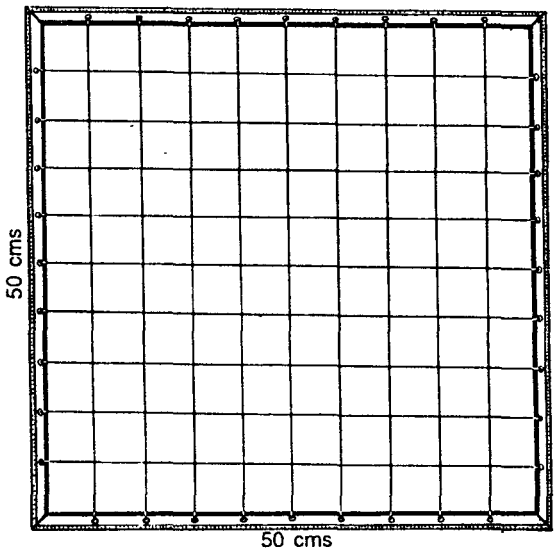


Fig 2. A typical quadrat.

4. If the species are not identified taxonomically in the field, collect them, glue or fix with cellotape to herbarium sheets, use the same identification marks, e.g., **A**, **B**, **C**, etc. as used in the table and properly preserve the sheets.

[II] Line transect

1. In a grassland, line transect can also be used for determining frequency.
2. A measuring tape or a cord marked into one meter segments is used.
3. Take a tape or cord across the grassland in North-South direction.
4. Note the presence or absence of plant species in each one meter segment. Only those plant species are considered which touch the cord or tape.

[III] Belt transect

Similar method is used in belt transects. The plant species occurring in alternate segments or uniform area are recorded.

Results

The record of the observations is kept in the following way (refer to table 2)

Calculations

1. Calculate the percentage frequency as follows :

Percentage frequency =

$$\frac{\text{Total number of quadrats / segments in which species occurred}}{\text{Total number of quadrats / segments studied}} \times 100$$

2. Distribute various species into five frequency classes (Raunkiaer, 1934) as given below —

Write down the frequency class in appropriate column against each species.

3. The distribution of sixteen species in five frequency classes is A = 8, B = 3, C = 2, D = 1 and E = 2. Find out the percentage of these species falling into different frequency

Table 1. **To determine the minimum number of quadrats.**

Species	Quadrats									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	+	–	–	+	–	+	–			
B	+	+	+	–	–	+	–			
C		+	+	+	+	–	+			
D			+	+	–	–	+			
E					+	–	+			
F					+	–	–			
G					+	+	–			
H						+	+			
Accumulating total number of species	2	3	4	4	7	8	8			

Table 2. **To determine the frequency of various species.**

Serial no.	Name of the plant species	No. of quadrats/segments					Total no. of a species	Total no. of quadrats in which species occurred	Total no. of quadrats/studied	Frequency %	Frequency class	Density	Abundance
		1	2	3	4	5							
1.	*Alysicarpus monilifer*	5	5	-	-	-	10	2	5	40	B	2	5
2.	*Convolvulus pluricaulis*	10	-	-	-	-	10	1	5	20	A	2	10
3.	*Cynodon dactylon*	15	10	12	13	15	65	5	5	100	E	13	13
4.	*Cyperus rotundus*	-	6	-	-	-	6	1	5	20	A	1 2	6
5.	*Desmodium triflorum*	-	12	-	-	-	12	1	5	20	A	2.4	12
6.	*Dichanthium annulatum*	12	-	12	10	11	45	4	5	80	D	9	11.25
7.	*Eclipta alba*	5	6	-	-	4	15	3	5	60	C	3	5
8.	*Euphorbia hirta*		-	-	6	-4	10	2	5	40	B	2	5
9.	*Evolvulus nummularius*	-	-	3	-	-	3	1	5	20	A	0 3	6
10.	*Gomphrena globosa*	2	4	3	1	2	12	5	5	100	E	2.4	2.4
11.	*Indigofera linifolia*	-	-	-	6	-	6	1	5	20	A	1.2	6
12.	*Launea nudicaulis*	-	-	-	-	3	3	1	5	20	A	0.6	3
13.	*Phyllanthus niruri*	-	-	-	-	2	2	1	5	20	A	0.4	2
14.	*Rhynchosia minima*	-	-	-	4	3	7	2	5	40	B	1 4	3 5
15.	*Sida cordifolia*	-	-	2	6	4	12	3	5	60	C	2 4	4
16.	*Vernonia cinerea*	-	-	11	-	-	11	1	5	20	A	2.2	11

Number of plants in each frequency class : A = 8, B = 3, C = 2, D = 1, E = 2.

Table 3. **Distribution in frequency classes.**

Frequency %	Frequency class
1-20 %	A
21-40 %	B
41-60 %	C
61-80 %	D
81-100 %	E

classes as follows out of the total number of species recorded.

$$\frac{\text{No. of species falling in frequency class}}{\text{Total number of species recorded}} \times 100$$

for frequency class A = 8/16 × 100 = 50
frequency class B = 3/16 × 100 = 18.75
frequency class C = 2/16 × 100 = 12.5
frequency class D = 1/16 × 100 = 6.25
frequency class E = 2/16 × 100 = 12.5

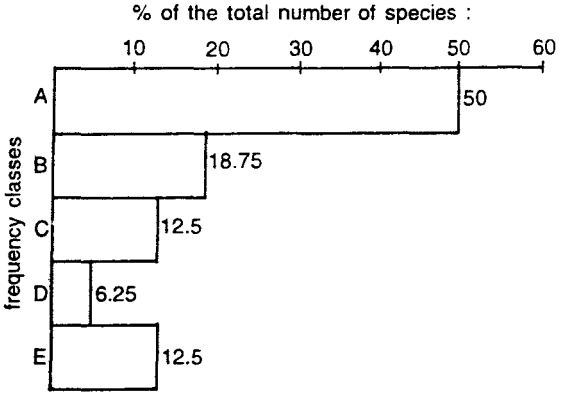


Fig. 3. Frequency diagram of the place studied.

4. Take a graph sheet and show % of the total number of species on y-axis and the frequency classes on x-axis. This is known as frequency diagram.

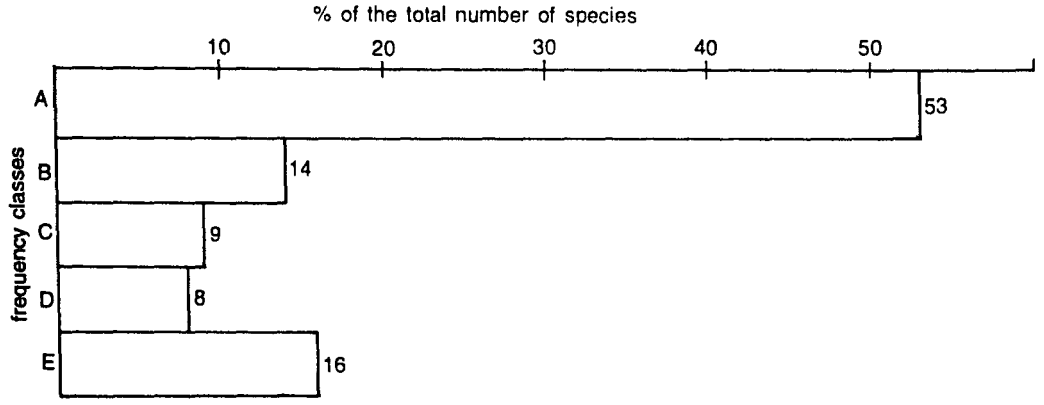


Fig. 4. Raunkiaer's normal frequency diagram.

Conclusions

1. Compare the frequency diagram of the place studied with that of Raunkiaer's normal frequency diagram.
2. When values of frequency classes B, C and D are comparatively higher than their values in normal frequency diagram, the vegetation is said to be **heterogenous**, as is the case in the present study. (Higher values of class E indicate homogeneity of vegetation).
3. Also compare the figures with frequency figures proposed by Raunkiaer as Law of Frequency given below

$$A > B > C \begin{matrix} > \\ < \end{matrix} D < E$$

Exercise 3

***Object* : Determination of local vegetation : frequency and relative frequency, density and relative density and importance value index.**

Requirements

Quadrats, measuring tape, paper, pencil, etc.

Arrange the species in decreasing value of IVI.

Procedure

1. Lay the quadrats, identify the species, count their number in each quadrat and record the observations in a tabular form as done earlier (table 1).

Table 4. **To determine frequency, relative frequency, density relative density and importance value index.**

1	2	3	4	5	6
Serial No.	Name of plant species	Relative Dominance	Relative Density	Relative Frequency	IVI (3+4+5)
1	A				
2	B				
3	C				
4	D				
5	E				

2. Use line transect or belt transect as done earlier (exercise no. 2) and record the observations as per table 2.

Calculations

Importance value index (IVI) is a measure of dominance and ecological success of a species. It takes into consideration relative dominance, relative density and relative frequency. These are calculated as follows —

1. **Relative dominance =**

$$\frac{\text{Total basal area of the species}}{\text{Total basal area of all the species}} \times 100$$

2. **Relative density =**

$$\frac{\text{Number of individuals of the species}}{\text{Number of individuals of all the species}} \times 100$$

3. Relative frequency =

$$\frac{\text{Number of occurrences of the species}}{\text{Number of occurrences of all the species}} \times 100$$

Record the values obtained in the following table

Exercise 4

Object* : Estimation of biomass.

Requirements

Scissors, polythene bags, oven, balance, etc.

Procedure

The following three methods are commonly used.

1. A small sampling unit (e.g. 25 × 25 cm) is chosen and plants are cut close to the ground surface.
2. Select randomly a few plants (e.g., about five) from a sampling unit, the density for which has already been calculated.)
3. In a pond, lower a container (known as dredge) of known volume which opens by special mechanism only after lowering it under water surface at desired depth and then close down.

In all the cases, each sample is packed in suitable plastic bags, dried in the laboratory (blotting papers are used for aquatic plants), weighed and cut into in smaller and suitable pieces.

Take 1000 g of each sample and dry it in an oven at 70°C for 12-24 hours. Note down the dry weight.

Calculate the biomass (dry weight) per meter square area.

(a) Take into consideration the fresh weight and the dry weight of 1000 g from the sample.
(b) Calculate the dry weight for this fresh weight of a sample.
(c) Now determine the area from which the sample is collected and calculate the value for one square meter.

Calculations

1. Fresh weight ... ... Xg
2. Weight after heating (at 74°C for 24 hrs)... ... Yg

∴ Dry weight ... . Yg

Find out the biomass per unit area e.g. the size of the quadrat used was 25 cm × 25 cm = 625 cm^2 and Yg was the dry weight

∴ biomass for 1 m × 1 m (100 cm × 100 cm) would be

$$\frac{Yg \times 100 \times 100}{625} = Z\,g/m^2$$

As such biomass would be Z g/m^2.

Exercise 5

***Object* : To determine the vegetational cover in a given area.**

Requirements

Measuring tape, Vernier callipers, scissors, paper, pencil, etc.

Procedure

The following are the two common methods.

[I] Line intercept method A

Take a measuring tape across the grassland. Measure and note the length of the tape, intercepted by individual plants.

[II] Line intercept method B

Cut a few stems of individual species at the ground surface. Measure the diameter of the cut end by Vernier callipers. Alternatively measure the diameter of the plant at a fixed height above the ground level.

[III] Point frame method

The apparatus has 10 pins. Place the apparatus at random at 5 places across the field of study. Note

* Biomass and standing crop, both are synonyms. Biomass can be expressed in terms of number and measured as fresh weight (living weight), dry weight, ash-free dry weight, energy or any conventional unit which is found useful for the purpose of comparison. Thus, the simplest way is to determine fresh weight of the samples and calculate it for a square meter area.

down the number of species hit by one or more times whenever apparatus is used. Measure either the length or diameter of these plants and calculate vegetational cover by any one of the methods described under observations and results.

Observations and results

1. List the observations in the following way —

***Table 5.* To determine vegetational cover.**

Serial no.	Name of plant species	Length of Individuals					Total %
		1	2	3	4	5	
1	A						
2	B						
3	C						
4	D						
5	E						
Total no. of plant sps		Total no of individuals studied					Total length of all the individuals

Calculate (a) total length of transect covered by all the species and (b) percentage of total length of transect covered by different species. This gives percentage cover.

Relative cover percentage =

$$\frac{\text{length of one type}}{\text{total length of all the individuals}} \times 100$$

2. Tabulate in the following form —

***Table 6.* To calculate vegetational cover.**

Serial no.	Name of plant species	Diameter of Individuals					Total	Average
		1	2	3	4	5		
1	A							
2	B							
3	C							
4	D							
5	E							

Total basal area of all species

Calculate by using Average basal area = πr^2

Where r = radius = $\frac{\text{diameter (Average)}}{2}$

If multiplied by the value of density (D) —

D × average basal area = ... sq cm/sq meter.

(B-52)

Exercise 6

***Object* : To study leaf area and leaf area index of a species in a grassland or forest vegetation.**

Requirements

Planimeter, graph papers, white sheets, polythene bags.

Procedure

1. Collect leaves of a few herb species separately in polythene bags.
2. Measure the basal area of species.
3. Trace all the leaves of a species on the graph paper.
4. Count the total area covered by the leaf from the marked outline of the leaf and express it as cm^2/ plant or cm^2/cm^2.
5. Alternatively trace all the leaves on white paper sheet and calculate the area by planimeter. Express the area as cm^2/plant or cm^2/cm^2.
6. Compare the leaf area estimated with first and second method.

Observations and calculations

To establish statistical relationship between leaf area and length and breadth of the leaf, value of Kemp's constant (K) can be calculated as follows.

Take different sizes of leaves of a plant and note their length and breadth.

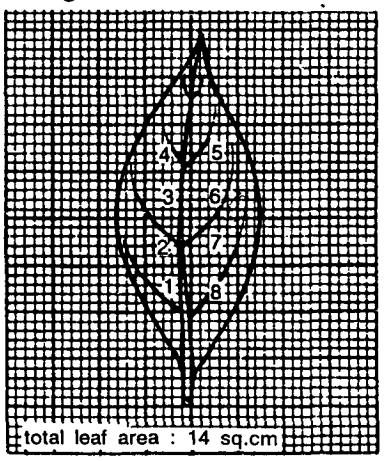


Fig. 5 Measurement of leaf area by using graph paper

Observations and calculations

Leaf area is a very good index of growth and production of a species. Ratio of leaf area and ground area covered by a species is known as leaf area index (LAI)*.

Table 7. **Length and Breadth of Leaves.**
Species .....

Serial no.	Length (L)	Breadth (B)	Leaf Area (L × B)
1.			
2.			
3.			
4			
5			

*Relationship between leaf area and leaf dry weight can also be used

Note : K will be the same for all the leaves of a species If only the length and breadth of the leaves is measured, leaf area can be calculated without using graph paper or planimeter.

Value of constant K can be determined by following formula

$LA = K \times L \times B$

Leaf area index (LAI) can be calculated as

$$LAI = \frac{\text{Leaf area of the species}}{\text{Basal area / ground area covered by the species}}$$

Conclusion

Species A has more leaf area index (more productive) than species B in the vegetation studied.

Exercise 7

Object **: To find out bulk density of a given soil sample.**

Requirements

Soil samples, petri dishes, oven, measuring cylinders, balance, etc.

Procedure

1. Collect soil samples from different places at a depth of 15 cm.
2. Dry the soil in an oven at 105°C till constant weight is attained.
3. Transfer a part of this soil to the measuring cylinder and determine the volume (1).
4. Also determine the weight of soil by first weighing measuring cylinder and the soil (2) and then the weight of measuring cylinder alone (3).

Observations and Calculation

Record the observations as follows.

5. Volume of the soil (1)
6. Weight of measuring cylinder + soil (2)
7. Weight of measuring cylinder (3)
   Calculate by using following formula

$$\textbf{Bulk density} = \frac{\text{Weight of soil (gm)}}{\text{volume of soil}} = \frac{(2)-(3)}{(1)}$$

8. Place the soil in one of the three following classes

	Soil classes	Bulk density (gm/cm^3)
(a)	Medium to fine textured	1.1 – 1.5
(b)	Coarse textured	1.2 – 1.65
(c)	Alkaline saline	1.70 – 1.85

The bulk density is defined as dry weight of unit volume of soil (in gm/cm^3). It is inversely proportional to pore space of the soil.

Exercise 8

Object **: To determine water holding capacity.**

Requirements

Filter papers, brass or tin boxes, balance, soil sample, petri dishes, water, oven, etc.

Procedure

1. Take a soil sample, allow it to dry and crush it.
2. Take a brass or tin box with perforated bottom and weigh the box (1).
3. Take a filter paper and weigh it (2).
4. Now place a filter paper at the bottom of the box. Fill the box gradually with soil by tapping to ensure uniform filling.

5. Place such a soil filled box in a petri dish containing water and allow it to remain overnight. Weigh the container once again (3).
6. Now place this container in an oven at 105°C for about 24 hours, till constant weight is attained. Record the weight (4).
7. Take a few filter papers (similar to one used in container). Dip one in water and find out the average amount of water absorbed by the filter paper (5).

Results

Results are computed in the following way.

[I] Observations

Record the observations as follows.

(1) Weight of the box 40.75 g
(2) Weight of dry filter paper 0.112 g
(3) Weight of wet soil + box + wet filter paper 111.50 g
(4) Weight of dry soil + box + dry filter paper 91.50 g
(5) Weight of wet filter paper 0.634 g

[II] Calculations

Calculate water holding capacity as follows

(6) Weight of wet soil = (weight of wet soil + box + wet filter paper) – (weight of box + weight of wet filter paper).
= (3) – (1 + 5)
= 111.50 g – (40.75 g + 0.634 g)
= 70.12 g

(7) Weight of oven dry soil = (weight of dry soil + box + dry filter paper) – (weight of box + weight of dry filter paper).
= (4) – (1 + 2)
91.50 g – (40.75 g + 0.112 g)
= 50.64 g

(8) Water in soil = weight of wet soil – weight of oven dry soil
= (6) – (7)
= 70.12 g – 50.64 g
= 19.48 g.

Water holding capacity

$$= \frac{\text{amount of water in the soil (8)}}{\text{weight of oven dry soil (7)}} \times 100$$

$$= \frac{19.48}{50.46} \times 100$$

= 38.46%

Exercise 9

Object **: To determine moisture percentage of soil.**

Requirements

Test tubes, box containers, soil sample, balance, oven, water, etc.

Procedure

The following procedure is used —

1. Collect the soil at desired depth and keep in closed test tubes or box.
2. Take an empty box or suitable container and weigh it (1).
3. Now fill the box with soil and weigh it (2).
4. Place this container in an oven at 105°C for about 24 hours till constant weight is attained. Note the weight after drying (3).

Results

Results can be obtained in the following way —

[I] Observations

Record the observations as follows —

(1) Weight of the box 25.0 g
(2) Weight of the box + soil 125.0 g
(3) Weight of the box + oven dry soil 105.0 g

[II] Calculations

Calculate the following values —

(4) Weight of the soil = (weight of box + soil) – weight of box
= (2) – (1) = 125g – 25g
= 100g

(5) Weight of dry soil = (weight of box + oven dry soil) – weight of box
= (3) – (1) = 105g – 25g
= 80g

(6) Amount of moisture in the soil = weight of the soil – weight of dry soil
= (4) – (5) = 100g – 80g
= 20g

Moisture %

$$= \frac{\text{amount of moisture in the soil}}{\text{weight of dry soil}} \times 100$$

$$= \frac{20}{80} \times 100 = 25\%$$

Exercise 10

***Object* : To measure the soil temperature.**

Requirements

Ordinary or soil thermometer.

Procedure

The soil temperature at various depths is measured by any one of the following two methods.

[I] Ordinary thermometer

A hole is dug in the soil up to a desired depth by means of a pointed iron or steel rod. Thermometer is then placed in this hole for about 15 minutes and temperature is recorded.

[II] Soil thermometer

These thermometers have a steel end near the mercury bulb. Thermometer is directly pushed into the soil by steel end to a desired depth and the temperature is recorded.

Exercise 11

***Object* : To determine the soil pH.**

Requirements

Soil sample, distilled water, pH paper/barium sulphate/comparometer/tintometer, pH indicator, etc.

Procedure

The following methods are commonly used.

[I] pH Paper

1. Add a pinch of soil to 5 ml distilled water.
2. Take a broad range pH paper indicator (a small piece) and dip it in the soil-water suspension. The colour of the paper changes.
3. Match the colour with the colour scale given on a booklet. This gives an approximate pH value.
4. For more correct value, narrow range pH paper indicator of the value indicated by broad range paper is now taken (i.e., if the pervious value comes to 8, now use indicator of the scale varying between 7.5 to 8.5).
5. The colour change is compared with the scale given on booklet and approximate pH value is determined.
   (pH papers are indicators of various pH ranges absorbed on them).

[II] Barium sulphate test

1. A spoonful of soil is added to an equal amount of Barium sulphate.
2. About 10-20 ml of distilled water is added to the test tube containing soil suspension.
3. Now sufficient quantity of soil indicator is added to the test tube and contents are thoroughly shaken.
4. Allow the contents to stand.
5. Match the colour developed with the colour chart and note the pH value.
   (For this purpose B.D.H. Barium sulphate soil testing outfit would be very useful).

[III] Comparometers/Tintometers

1. These are boxes with two windows. In one of the windows of the box, test tube containing soil-water suspension is kept, while in another, a tube with standard solution is kept (or in tintometers a rotating colour disc is adjusted) and the comparisons are made.
2. To prepare a soil-water suspension, take a tube supplied with the apparatus.
3. Add a little Barium sulphate and almost twice the amount of soil.
4. Fill the tube up to the mark with distilled water and shake thoroughly. Allow the tube to stand till clear liquid appears.
5. Place the tube in slot of the box and compare the colours to find out the pH value.

Exercise 12

***Object* : To estimate the transparency of water body.**

Requirements

Secchi disc.

Procedure

Secchi disc is a circular disc generally divided into 16 triangular parts. Black and white coloured parts alternate each other.

1. Lower the disc in the water.
2. Note the depth of the water where colour constrast disappears.
3. Repeat the procedure at different depths and/ or different water body.

Exercise 13

***Object* : To determine the chloride content of a water sample to give rough estimate of salinity.**

Requirements

Water sample, 10 cm^3 pipette, burette, distilled water, wash bottle, 3 conical flasks, white tile, potassium chromate indicator, 50 cm^3 silver nitrate solution (2.73 g/100 cc).

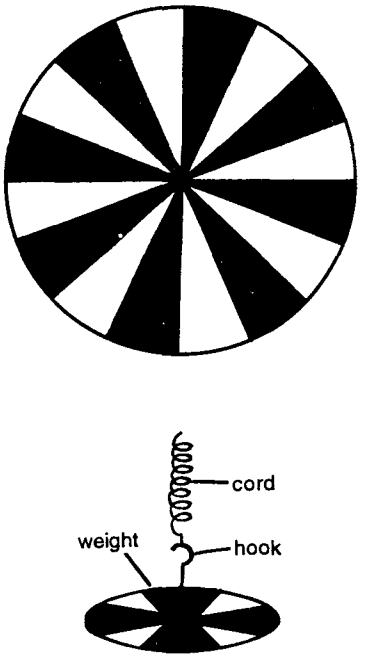


Fig. 6 A Secchi disk to determine water transparency.

Procedure

1. Place 10 cc of water sample into a conical flask and add two drops of potassium chromate indicator solution.
2. Titrate silver nitrate solution from the burette shaking the conical flask constantly.
3. The end point of the titration is given by reddening of the silver chloride precipitate.
4. Repeat the titration on another 10 cc sample of water.
5. Calculate the average volume of silver nitrate used.
6. The volume of silver nitrate solutions used is approximately equal to the chloride content of the water sample (in g dm^{-3}).
7. More is the chloride content more is the salinity. This can be graded as +, ++ and so on.

Exercise 14

***Object* : Comparison of dissolved oxygen content of polluted and non-polluted water by audiometric titration method.**

Requirements

Manganous chloride (40% solution); a mixture of sodium hydroxide (33g) and potassium iodide (10g) dissolved in 100 ml of distilled water; standard sodium thiosulphate solution (N/40, i.e. 0.62 g of sodium thiosulphate dissolved in 100 ml. of distilled water), concentrated sulphuric acid, starch solution, distilled water.

Burette, pipette, beakers, sampling bottles, measuring cylinders, etc.

Procedure

1. Take a narrow mouthed reagent bottle of about 250 ml capacity with a tight fitting ground glass stopper. Fill the water to be sampled in such a way that there is no bubbling. Allow plenty of water to overflow in the sample bottle.
2. Remove carefully the stopper of the sample bottle. Add 0.5 ml of manganous chloride and 0.1 ml of mixture of NaOH and KI for every 70 ml of sample. Use proportionately larger volumes of the two solutions for more quantity of samples.
3. Close the bottles firmly. Care should be taken to see that no air bubbles are included. Shake well and leave to stand for five minutes. A brown precipitate of manganic hydroxide appears and the oxygen in the sample is now fixed. This procedure can be easily carried out in the field and the remaining stages are completed in the laboratory.
4. Add 2 ml of concentrated sulphuric acid to the sample as above. Mix the contents thoroughly by rotation. The precipitate disappears. The liquid becomes clear golden brown in colour due to liberation of iodine.
5. Take about 25 ml of treated sample in a pipette, transfer it to conical flask and immediately titrate against standard sodium thiosulphate solution, using 2 ml of starch solution as indicator.
6. Starch is added until the yellow of iodine has nearly disappeared.
7. The procedure should be repeated at least twice for each sample.

Calculations

1. One ml of the standard sodium thiosulphate is equivalent to 0.1 mg of oxygen.
   Let V_1 be the volume of thiosulphate used
   V_2 be the volume of sample

   $$\frac{V_1 \times 0.1 \times 1000}{V_2} = \text{mg oxygen per litre}$$

   Now 1 ml of sodium thiosulphate
   = 0.0001g of oxygen

   Therefore, 1 ml of sodium thiosulphate

   $$= \frac{0.0001 \times 22.400}{32} \text{ mg of } O_2$$

Hence

$$\frac{V_1 \times 0.0001 \times 22.400 \times 1000}{32 \times V_2} = \frac{V_1 \times 70}{V_2}$$

= mg of oxygen per litre

Exercise 15

***Object* : To determine the per cent leaf area injury of different samples collected around polluted sites.**

Requirements

Graph papers (mm), pencil, scale etc.

Procedure

1. Collect mature leaves from polluted areas such as industries releasing smoke.
2. Observe any injury marks such as chlorotic or necrotic area of the leaf.
3. Place such a leaf on the graph paper and draw outlines along the leaf margins by pencil.
4. Calculate the leaf area by counting the squares on the graph inside the outlines drawn by pencil. Express the area in terms of sq. cm.

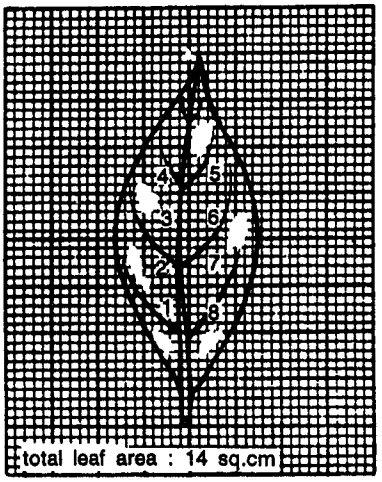


Fig. 7. An injured leaf placed on a graph paper.

5. Now repeat the same procedure for all the necrotic and/pr chlorotic areas of the same leaf.
6. Draw along the margins of each chlorotic and necrotic areas represents the total leaf area injury.
8. Find out the per cent leaf injury by comparing total leaf area injury with total leaf area.

Calculations

1. Tabulate the measurements in the following form.
2. Study at least five leaves of each plant.
3. Calculate the per cent leaf injury. The following formula be applied.
   Percent leaf injury =

$$\frac{\text{Total injured area of the leaf}}{\text{Total leaf area}} \times 100$$

1. **Name of the site studied :**
2. **Industries located.**

Exercise 16

***Object* : To estimate dust holding capacity of different plant species.**

Requirements

Plants of different species from a polluted site, distilled water, physical balance, filter papers, weighing balance, etc.

Procedure

1. Select a few species growing near polluted site.
2. Mark some of the leaves of these plants growing at the height of at least three meters above the ground.
3. Remove the leaves from the plant after a specified period of time.
4. Wash the leaves with distilled water (using wash bottle) and collect the suspension in appropriate container such as petri dish beaker, etc.
5. Filter the distilled water using pre weighed filter paper and collect the dust.
6. Find out the weight of the dust.
7. Also find out the leaf area by placing it on graph paper.
8. Allow the supernatant liquid or liquid after filtering, to evaporate. Find out the weight of insoluble portion after evaporation. Add these weights for calculation of dust.

Observations and calculations

1. Tabulate the observations in the following form.

Name of the plant species	No. of leave studied	Total leaf area	Leaf are of each injured spot					Total area of injured areas	Percent injury
1.	1								
	2								
	3								
	4								
	5								
2.									

Sampling area.....

Name of the plant species	Serial No. of leaf	Weight of the dust	Weight of insoluble fraction	Total weight of dust	Number of days of exposure
1.	1				
	2				
	3				
2.					

2. Calculate the dust holding capacity as follows

$$\text{Dust holding capacity } (gm/m^2/month) = \frac{\text{Total weight of dnst}}{\text{Total leaf area}} \times \frac{30}{\text{No. of days of exposure}}$$

Exercise 17

***Object* : To classify hydrophytes.**

Visit various aquatic habitats and observe the hydrophytes occurring in these places. Study their habits and morphological characters. Classify them into following groups :

The following is one of the most practical classification of hyrophytes.

1. Free floating hydrophytes. These plants float freely on the water surface and are not rooted, e.g. *Eichhomia, Lemna, Limnanthemum, Pistia, Wolffia,* etc.

2. Floating but rooted hydrophytes. These plants float on the surface of water but remain attached to the bottom of water reservoir by their roots, e.g. *Aponogeton, Jussiaea, Nymphaea, Potamogeton, Trapa,* etc.

3. Submerged but not rooted. These plants occur below the water surface and remain free being not rooted, e.g. *Ceratophyllum, Najas,* etc.

4. Submerged but rooted. These plants remain below the water surface but are attached to the reservoir bottom by their roots, e.g. *Hydrilla, Utricularia, Vallisneria,* etc.

List of plants collected
Place of collection.....

Serial No	Group of Hydrophytes	Botanical name
1.	Free floating hydrophytes	1. ..... 2. ..... 3. .....
2.	Floating but rooted	1. ..... 2. ..... 3. .....
3.	Submerged but not rooted	1. ..... 2. ..... 3. .....
4.	Submerged but rooted	1. ..... 2. ..... 3. .....
5.	Amphibious and rooted	1. ..... 2. ..... 3. .....
6.	Emergent but rooted.	1. ..... 2. ..... 3. .....

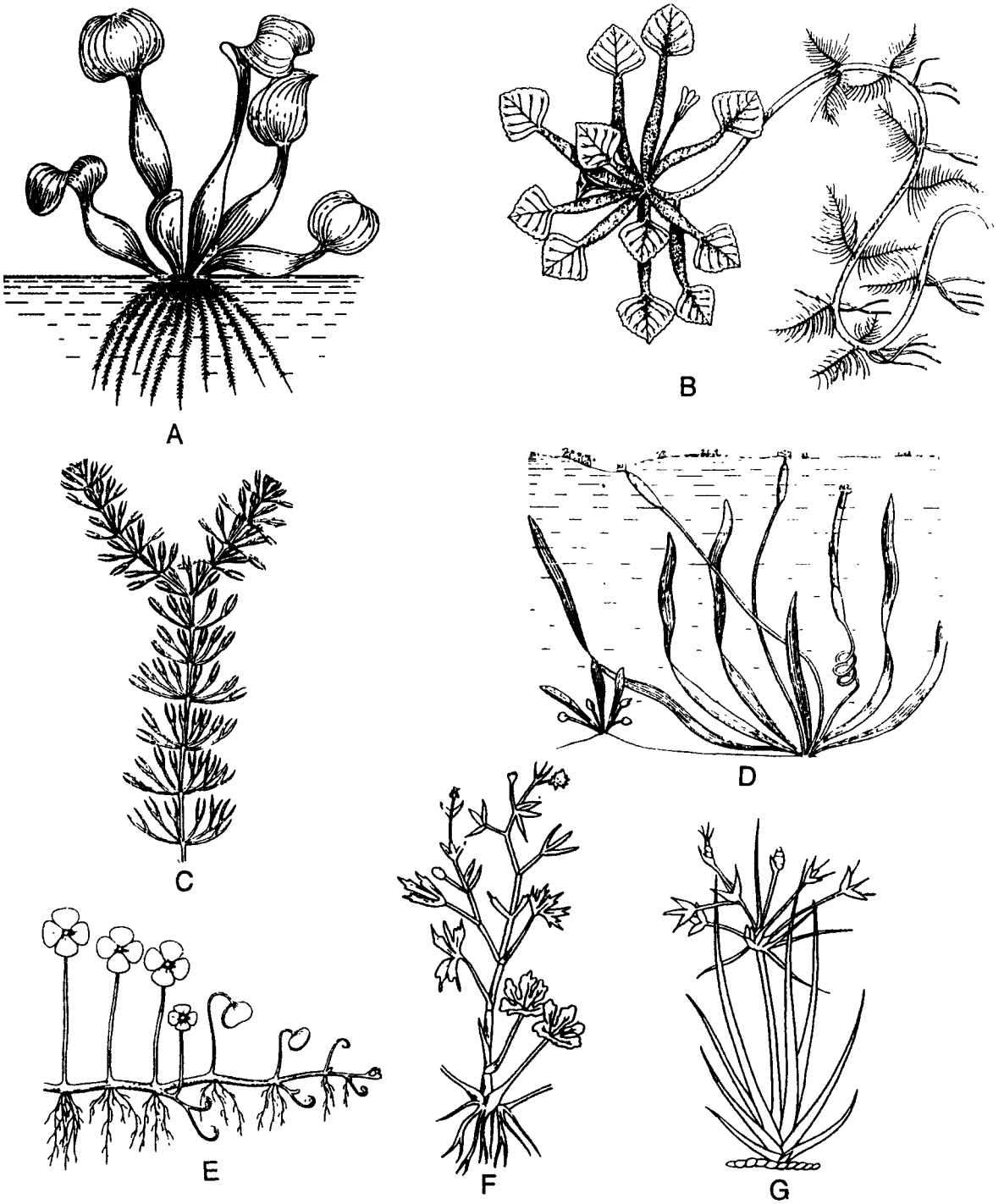


Fig. 8. Different types of hydrophytes. **A** Free floating- *Eichhornia*, **B.** Floating but rooted – *Jussiaea*. **C.** Submerged but not rooted – *Ceratophyllum*, **D.** Submerged and rooted – *Vallisneria*, **E.** Amphibions and rooted – *Marsilea*, **F.** Emergent but rooted – *Ranunculus*, **G.** *Cyperus*.

5. Amphibious and rooted. These plants grow near the water reservoirs in shallow and muddy places, e.g. *Polygonum, Marsilea*, etc.

6. Emergent but rooted. These plants are found in shallow water. They grow half below the water and the half above it, e.g. *Cyperus, Ranunculus, Typha,* etc.

Exercise 18

***Object* : To study the morphological characters of hydrophytes.**

Observe and note down hydrophytic characters given in the list below in various hydrophytes collected by you.

The following are some of the common morphological features shown by hydrophytes.

1. Root. A few major characters are listed below—

1. It is often very poorly developed. The roots may even be absent e.g. *Ceratophyllum, Wolffia,* etc.
2. In some hydrophytes root system is well developed mainly for the purpose of attachment, e.g. *Nymphaea, Cyperus, Typha*, etc.
3. In free floating plants, adventitious roots are developed not for anchorage but for buoyancy e.g. *Eichhomia, Pistia*, etc.
4. Sheath-like root pockets are developed in *Azolla, Lemna, Pistia,* etc. instead of root cap. It helps the plants to float.
5. Spongy roots which are negatively geotropic develop for floating in *Pistia.*

2. Shoot. The stems are spongy, delicate and flexible.

3. Petiole. The following are the hydrophytic characters.

1. Petiole is very long and delicate in plants with roots attached and leaves floating, e.g. *Nymphaea, Sagittaria*, etc.
2. Bulbous petiole of *Eichhomia* helps the plant to float on water surface.

4. Leaves. Leaves of hydrophytes show following characters.

1. The leaves of submerged plants are variously dissected, so that water flows easily without resistance; e.g. *Ceratophyllum, Hydrilla, Vallisneria,* etc.
2. The surfaces of floating leaves possess waxy coating as in *Nymphaea* or leaf hairs as in *Salvinia.*
3. In emergent plants, leaves are heterophyllous. The leaves below the water are narrow, long, segmented and dissected; while the leaves outside the water are broad, small and entire. Such dimorphic leaves are found in *Limnophila heterophylla, Ranunculus scleratus, Sagittaria sagittifolia,* etc.

Exercise 19

Object* : To study hydrophytic characters in T.s. of root of *Eichhornia.

[I] Practical work

Cut a thin transverse section of the root, stain in safranin and fast green and mount in glycerine. Observe the following characters.

[II] Observations

Epiblema 1. This is an outermost layer.
2. It is made of a single layer of cells.
3. The walls are thin and cuticle is absent.

Cortex. 1.It is differentiated into three regions.
2. Outer cortex. The cells of this region are compactly arranged. The cells are parenchymatous.
3. Middle cortex. It is a large region made of many air chambers or lacunae. The partition walls separating are called diaphragms.
4. Inner cortex. It is made of only a few layers of compactly arranged parenchyma.

Endodermis and pericycle. 1. Both these layers are distantly seen.
2. The vascular tissues are surrounded by these layers.

Vascular tissues. 1. Vascular bundles are radial and exarch.
2. These are abut 8-10 groups of xylem. The number of xylem elements in these groups is very less.

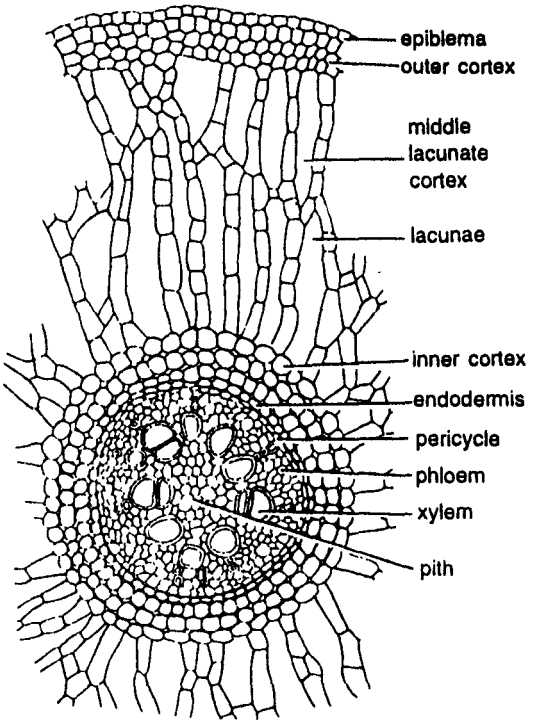


Fig. 9. *Eichhornia*. A part of T. s. root showing hydrophytic characters.

[III] Hydrophytic characters

The following characters help identify the material as hydrophyte.

1. Undifferentiated parenchymatous tissue.
2. Abundance of aerenchyma.
3. Absence of mechanical tissue.
4. Vascular tissue poorly developed.
5. Xylem elements fewer in number.
6. The above characters indicate that the plant is a hydrophyte. Since it shows radial and exarch conditions of vascular bundles it is a root.

Exercise 20

***Object* : To study hydrophytic characters in T.s. stem of *Hydrilla*.**

[I] Practical Work

Cut a thin transverse section of the material; stain in safranin and fast green combination, mount in glycerine and study.

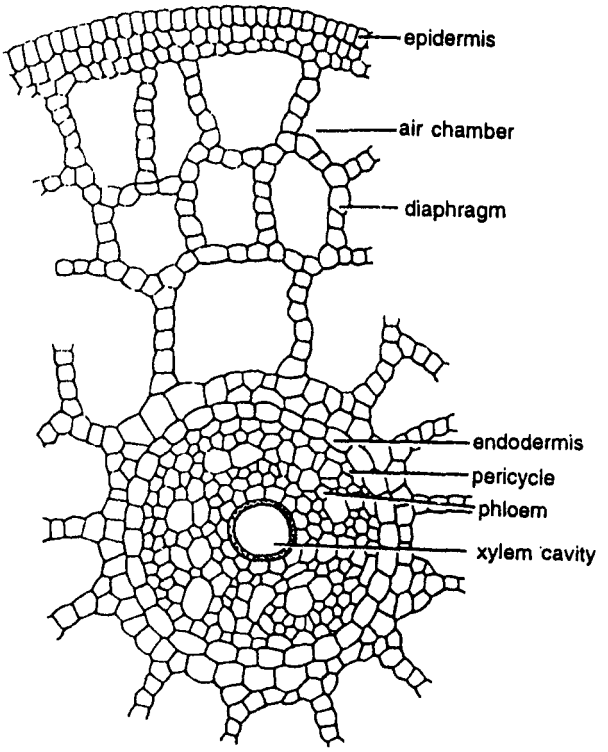


Fig. 10. *Hydrilla*. A part of T. s. stem showing hydrophytic characters.

[II] Observations

The outline of the section is almost circular. It shows following characters

Epidermis

1. This is the outermost single layer of cells.
2. Cutcle is absent.

Cortex

1. It occupies most part of the section.
2. It is made of many, large air chambers.
3. Air chambers are separated from one another by partitions called diaphragms.
4. A few layers just below the epidermis (outer cortex) and a few layers close the endodermis (inner cortex) are compact and parenchymatous.

Endodermis and pericycle

1. Distinct endoermis and pericycle are present.
2. These enclose the underlying vascular tissue.

Vascular tissues

1. It is extremely reduced.
2. Most of the tissue is phloem.
3. Xylem is represented by a single large element situated in the centre.

[III] Hydrophytic characters

1. Epidermis is made of thin walled cells.
2. Cuticle is absent.
3. Absence of mechanical tissue.
4. Aerenchyma and air chambers present.
5. Extremely reduced xylem.
6. Comparatively well developed phloem.

Exercise 21

Object* : To study the hydrophytic characters in T.s. petiole *Eichhornia.

[I] Practical work

Cut a transverse section of petiole of Eichhornia, stain with safranin and fast green, mount in glycerine and study.

[II] Observations

The transverse section is almost circular in outline. It shows following characters.

Epidermis

1. It is the outermost layer made of parenchymatous cells.
2. Cuticle is absent.

Hypodermis

1. It is present just below the epidermis. There are a few layers of parenchyma.
2. The cells are compactly arranged.

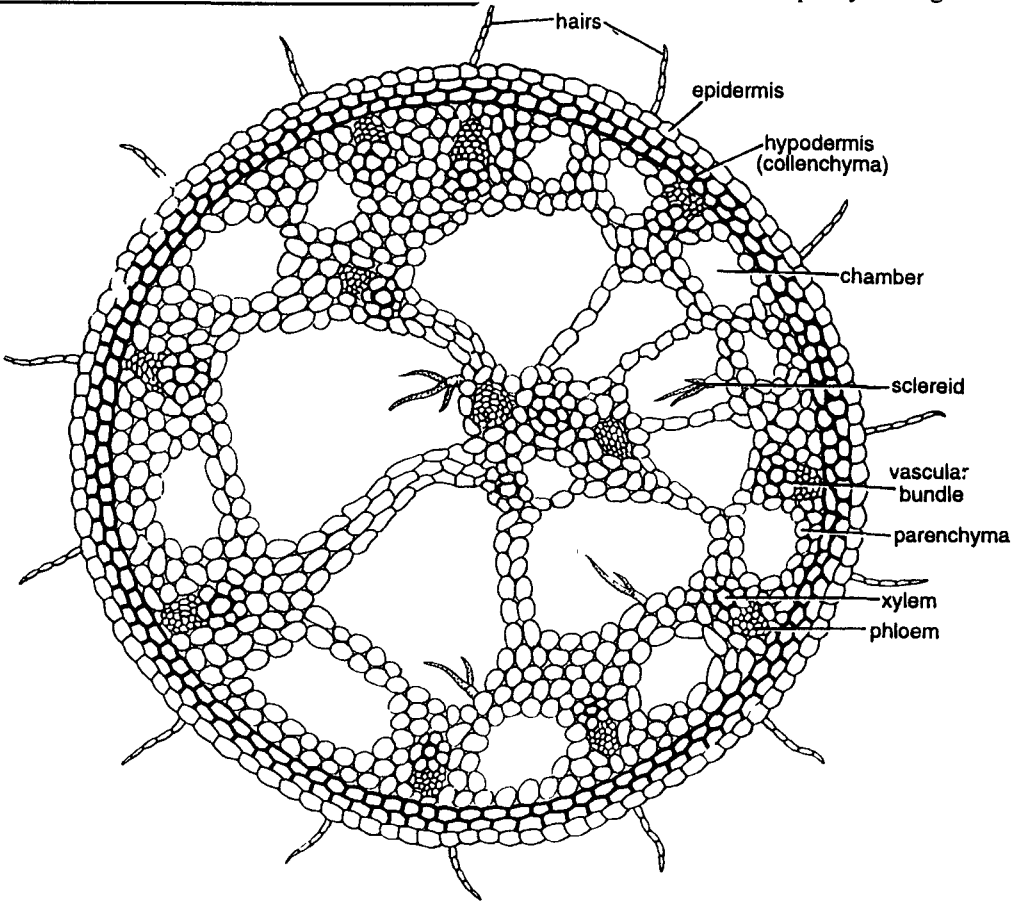


Fig. 11. *Nymphaea.* T. s. petiole showing hydrophytic characters.

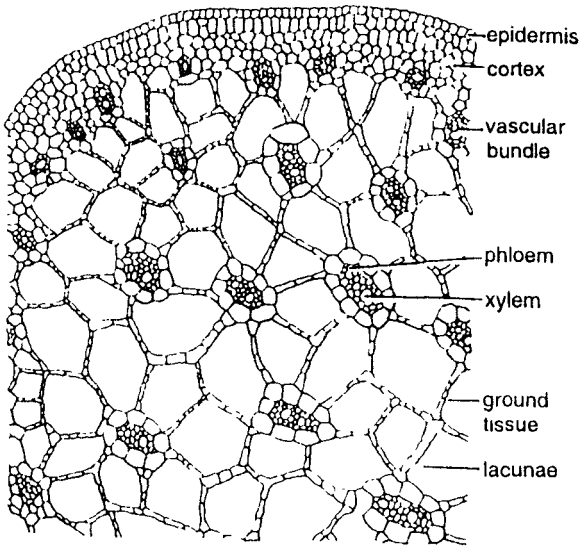


Fig 12. *Eichhornia* A part of T s petiole showing hydrophytic characters.

Ground tissue

1. The remaining part of the section is made of parenchymatous ground tissue.
2. Many large air chambers are distributed throughout this region.
3. Air chambers are separated from one another by diaphragms.

Vascular tissue

1. Vascular bundles are distributed throughout the ground tissue.
2. Vascular bundles lie embedded in parenchyma situated between air chambers.
3. Vascular tissues are poorly developed.
4. Xylem is represented by a single, large element.
5. Phloem is scattered all around the xylem.
6. Vascular bundles are of two types—

(i) large sized vascular bundles lying close to centre. It has two phloem groups, one on each side of xylem element; and

(ii) small sized vascular bundles lying in the outer region of ground tissue. It has only one phloem group situated on its out side.

[III] Hydrophytic characters

The following hydrophytic characters are shown by the section—

1. The cell of the epidermis are thin walled.
2. Cuticle absent.
3. Absence of mechanical tissue.
4. Ground tissue parenchymatous.
5. Presence of aerenchyma.
6. Vascular tissues poorly developed.

Exercise 22

Object* : To study the hydrophytic characters in T.s. petiole of *Nymphaea.

[I] Practical work

Cut a transverse section of petiole of *Nymphaea*, stain in safranin-fast green combination, mount in glycerine and study.

[II] Observations

The transverse section is almost circular in outline. It shows following characters.

Epidermis

1. This is an outermost layer made of parenchymatous cells with chloroplasts.
2. Cuticle is generally absent, if present, it is very thin.
3. A few multicellular, unbranched hairs are present.

Hypodermis

1. It lies below the epidermis and is about 2-3 layered deep.
2. The cells are collenchymatous and compactly arranged.

Ground tissue

1. The remaining part of the section is mostly filled with ground tissue.

2. There are many air chambers scattered throughout this region.
3. A few trichosclereids or internal hairs occur in the air chabers.

Vascular tissue

1. Vascular bundles are distributed throughout the ground tissue.
2. These are situated in the parenchyma between air chambers.
3. Vascular bundles show poorly developed xylem, represented by a single lacuna.
4. Phloem of bundle lies on the outer side and is normally developed.
5. There are two types of bundles—

(i) larger sized towards the centre with two groups of phloem, one on either side of xylem element and

(ii) smaller in size towards periphery with only one (outer) group of phloem.

[III] Hydrophytic characters

1. Thin walled epidermis.
2. Presence of chloroplasts in the epidermis
3. Cuticle absent or very thin.
4. Mechanical tissue reduced.
5. Ground tissue undifferentiated.
6. Presence of large number of air chambers.
7. Vascular tissue reduced.
8. Presence of trichosclereids.

Exercise 23

Object* : To study the hydrophytic characters in T.s. leaf of *Nymphaea.

[I] Practical work

Cut a transverse section of the leaf, stain in safranin-fast green combination, mount in glycerine and study.

[II] Observations

The transverse section shows a bulged and distinct midrib and wings on its both sides. The following characters are seen.

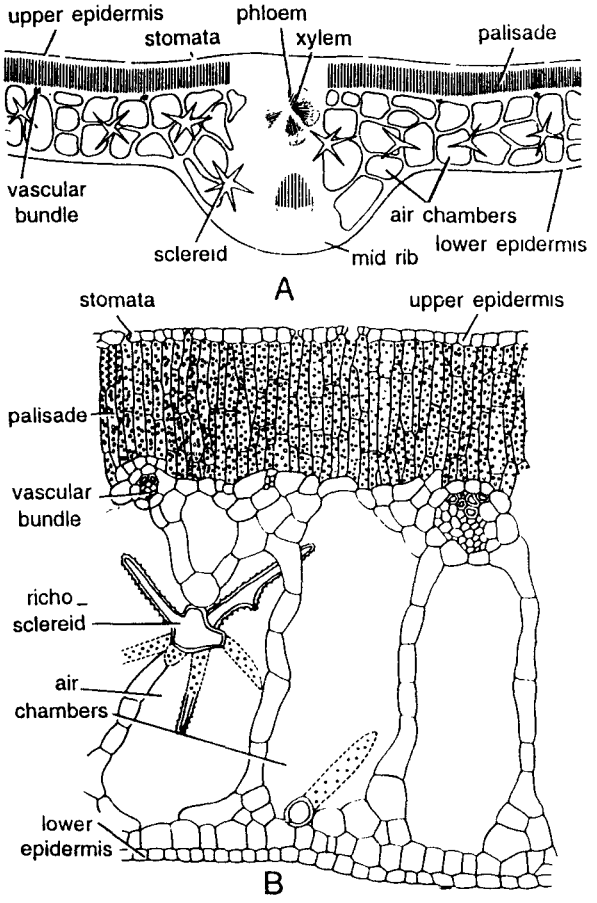


Fig 13 *Nymphaea.* T s. leaf showing hydrophytic characters. **A.** Outlines of section, **B.** Details of part of a section.

Epidermis

1. Both upper and lower epidermal layers are present.
2. Both are made of compactly arranged cells.
3. Upper epidermis has many stomata; which are lacking from lower epidermis.
4. Upper epidermis is covered with waxy cuticle. It is absent from lower epidermis.
5. A few slime glands occur on the lower epidermis.

Mesophyll

1. It is differentiated into upper palisade and lower spongy parenchyma.

2. Upper palisade becomes discontinuopus near epidermis to form sub-stomatal chambers.
3. The lower part of the wings is occupied with large air chambers. Numerous trichosclereids are scattered in this region.

Vascular tissue

1. Vascular bundles occur all along the wings and also in the midrib.
2. There are 3-4 vascular bundles in the midrib. These are similar to those present in the wings.
3. Vascular bundle is surrounded by a parenchymatous bundle sheath.
4. Each vascular bundle is conjoint, collateral and closed.
5. Xylem is poorly developed as compared to phloem.

[III] Hydrophytic characters

1. Presence of waxy cuticle on upper epidermis.
2. Cuticle and stomata present on the upper epidermis and absent from lower epidermis– indicates that the leaf floats on the surface.
3. Large number of air chambers present.
4. Presence of trichosclereids for support.
5. Reduced vascular bundles.
6. Xylem of vascular bundles represented by only a few elements.

Exercise 24

***Object* : To classify Xerophytes.**

Visit various dry and xeric localities nearby. Observe and collect commonly occurring xerophytes. Study their habits and morphologies characters. Classify them into following groups.

The following is one of the useful classifications of xerophytes.

1. Microphyllous. The leaves are small, scaly, reduced, modified or absent, e.g. *Acacia (Australian), Asparagus, Capparis aphylla, Casuarina, Euphorbia, Pinus,* etc.

2. Selerophyllous. The leaves of these plants are thick, coarse and leathery due to excessive lignified and selerified tissues, e.g., *Ficus, Nerium, Spartina, Banksia, Dasilirion,* etc.

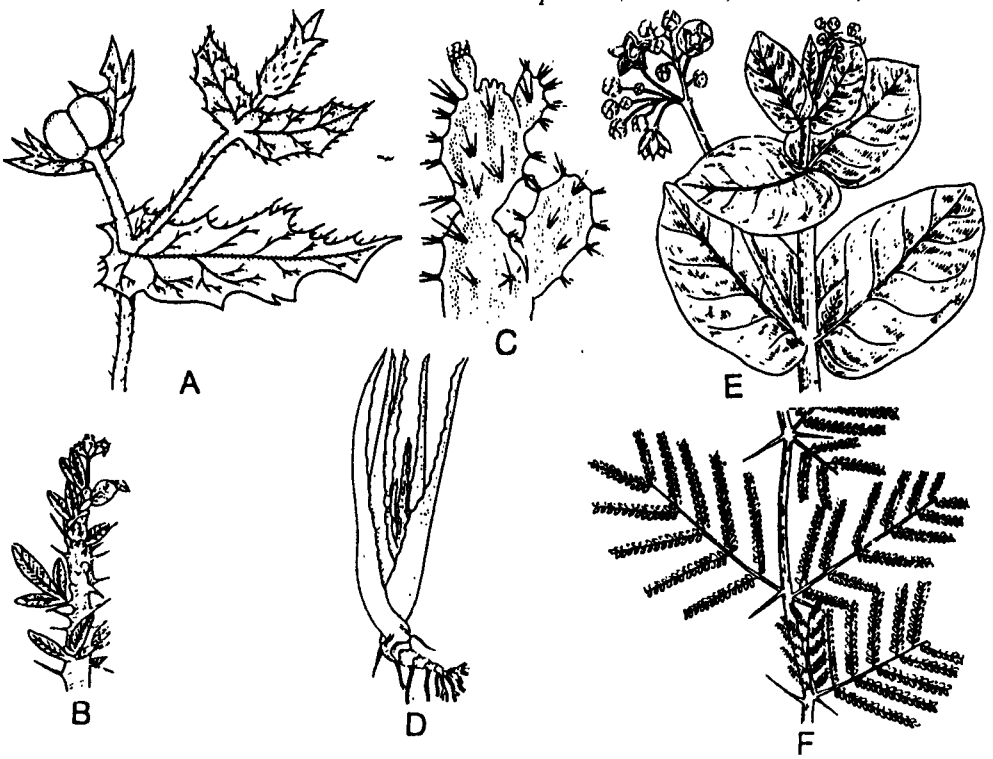


Fig. 14 Different types of xerophytes. **A.** *Argemone,* **B.** *Euphorbia,* **C.** *Opuntia,* **D.** *Aloe,* **E.** *Calotropis,* **F.** *Mimosa.*

3. Triehophyllous. These xerophytes have leaves covered with a thick felt of hairs, e.g., *Calotropis*.

4. Malaeophyllous. The leaves of these plants are fleshy and thick e.g., *Agave, Aloe, Bryophyllum, Begonia, Salicoria,* etc.

Schimper (1903) classified xerophytes on the basis of external morphology of the leaf—

(1) **Selerophylly :** leaves leathery.
(2) **Chylophylly :** (leaf succulence) : leaves fleshy.
(3) **Aphylly :** leaves rudimentary and caducous.
(4) **Sclerocauly :** axes slender, dry and hard.
(5) **Chylocauly :** (stem succulence) axes short, thick and filled with mucilaginous sap.

Prepare a list of plants thus collected.

Exercise 25

***Object* : To study the morphological characters of xerophytes.**

Observe and note down xerophytic characters listed below in various xerophytes collected by you.

The following are some of the common morphological characters shown by xerophytes.

Root. A few major marphological characters of root are listed below.

1. The root system is very well developed and profusely branched.
2. These have a long tap root system that grows deep into the soil and reaches the water table.
3. In some desert plants, roots grow near the soil surface to absorb soil water whenever available.
4. The roots of many xerophytes are perennating.

Shoot. The following are morphological characteristics of the shoot.

1. The stem is generally hard and woody that remains covered with wax, silica, hairs, etc.
2. A few xerophytes possess fleshy and stunted stem. The cells may contain, large quantities of mucilage, thus allowing the stem to store water.
3. In extreme cases stem becomes modified into leaf-like structure to reduce the transpiring surfaces. In Ruscus, the stem becomes leaf-like and is known as phylloclade (also in Opuntia, Muehlenbeckia, etc.). The internodes of Asparagus get modified into leaf-like cladodes, while the leaves are small and scaly.
4. In some xerophytes, the shoot becomes either fleshy, reduced cushion-like or stunted.

Leaves. A few typical morphological characters are given below.

1. In many xerophytes, leaves fall down as soon as they are formed (caducous leaves) e.g., in species of Euphorbia. Capparis aphylla shows complete absence of leaves.
2. In Opuntia and many cacti leaves get reduced to spine-like structures.
3. Rosette arrangement of the leaves is seen in Bromelia which cuts down the light and reduces transpiration.
4. The desert grasses show rolling of the leaves so that stomates located on the upper epidermis stop transpiring e.g., Ammophila, Fesuca, Stipa, etc.

Reproduction. Xerophytes are characterized by following cycles of reproduction. Accordingly three categories can be recognised -

1. Drought escaping (ephemerals). These plants complete their life cycle during the period of available moisture and before the onset of dry season e.g., Artemesia, Astragalus, etc.

2. Drought enduring plants. These xerophytes continue to live during dry period without any injury or damage, though they are at their lowest activities e.g., succulent plants.

3. Drought resistant. These are structurally adapted to resist extreme drought conditions. Besides, most of the xerophytes perennate by roots. These flower only during the season when moisture is available.

Exercise 26

***Object* : To study the xerophytic characters seen in T.s. of *Calotropis* stem.**

[I] Practical work

Cut a transverse section of the material, stain in safranin - fast green combination, mount in glycerine and study.

[II] Observations

The outline of the transverse section is almost circular.

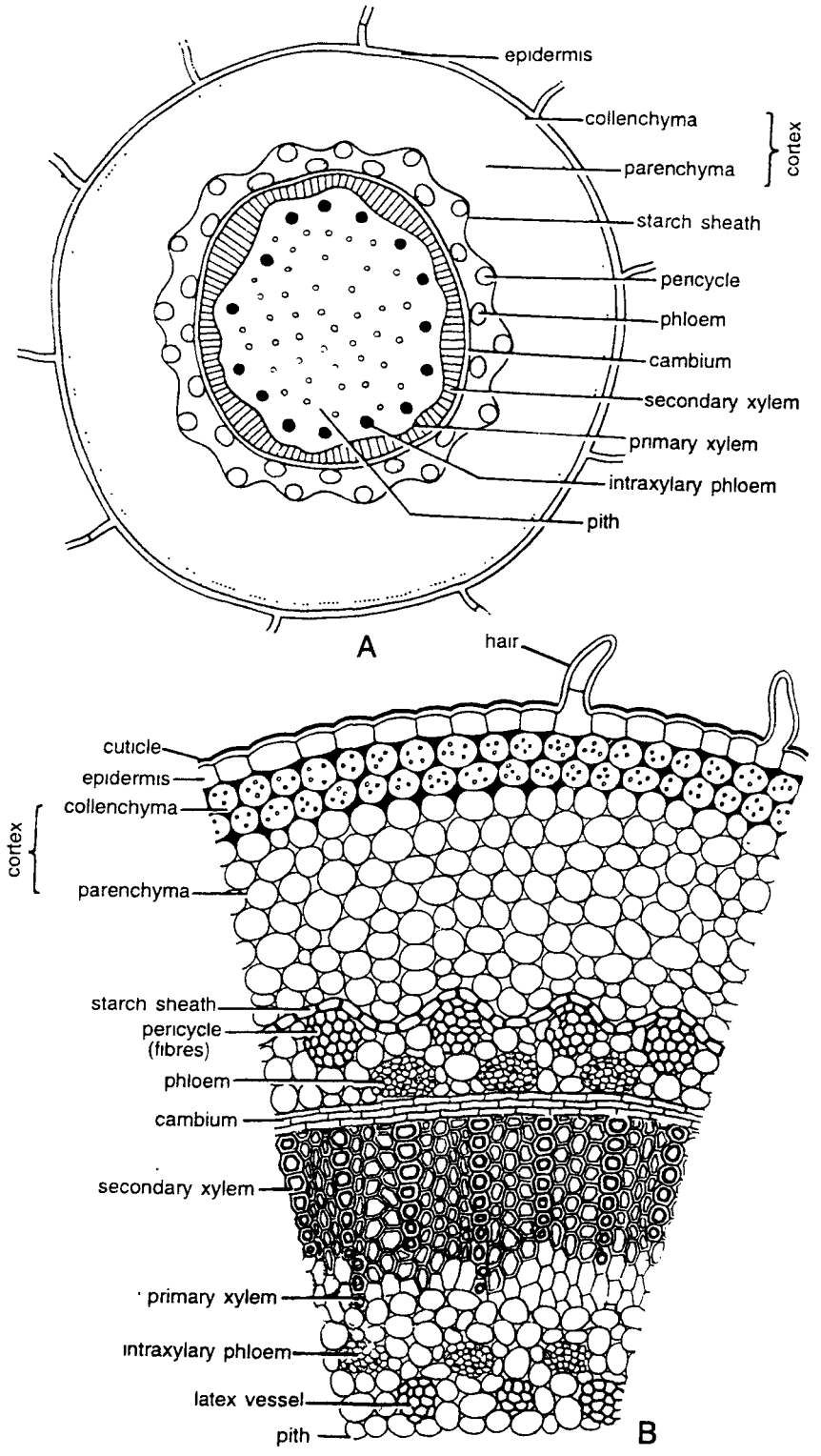


Fig. 15. *Calotropis* T. s stem. **A.** Outlines of the section. **B.** Details of a part of a section

Epidermis

1. This is the outermost single layer of cells.
2. It is thickly cuticularised.
3. Numerous hairs produced by the epidermis form a thick cover.
4. The inner region which forms larger part of the cortex is parenchymatous.

Endodermis

1. This single layer forms a wavy layer around the vascular tissues.
2. The cells lack characteristic casparian strips. The cells are, however, filled with abundant starch.

Pericycle

1. It occurs in the form of small patches of sclerenchymatous fibres.
2. In between sclerenchymatous patches, parenchyma is also present.

Vascular tissue

1. It shows secondary growth.
2. As a result, groups of primary phloem, secondary phloem, cambium, secondary xylem, primary xylem and intraxylary (internal) phloem could be seen.
3. The zone of secondary xylem is the most extensive.

Pith

1. In the centre is a large parenchymatous pith.
2. A few latex vessels are also present close to the groups of intraxylary phloem.

[III] Xerophytic characters

1. A thick envelope of hairs on the epidermis.
2. Presence of thick cuticle.
3. Presence of collenchyma and chloroplasts in the cortex.
4. Sclerenchymatous pericycle.
5. Presence of latex vessels in the pith.

The vascular tissue and the secondary growth indicates that the material is a dicot stem.

Exercise 27

***Object* : To study the xerophytic characters seen in T.s. stem of *Casuarina*.**

[I] Practical work

Cut a transverse section of the material, stain in safranin - fast green combination, mount in glycerine and study.

[II] Observations

The outline of the section shows ridges and grooves. Ridges are almost triangular in shape. The section shows the following characters.

Epidermis

1. This is an outermost single row of cells.
2. The cells are highly cuticularised.
3. Stomata are highly sunken and occur in the grooves.
4. Numerous hairs are present in the grooves and around the stomata.

Cortex

1. It is differentiated into hypodermis, palisade and parenchyma.
2. Hypodermis is present below the epidermis. It is made of sclerenchyma, arranged in T shaped patches.
3. Larger part of the cortex is made of several layers of parenchyma.
4. Ring of vascular bundles called cortical vascular bundles is present in the parenchymatous region. These are situated only below the ridges. Each vascular bundle is conjoint, collateralendarch and open. A sclerenchymatous cap is present above the vascular bundle.

Endodermis

1. This is a single layer of cells which separates cortex from the underlying vascular tissue.

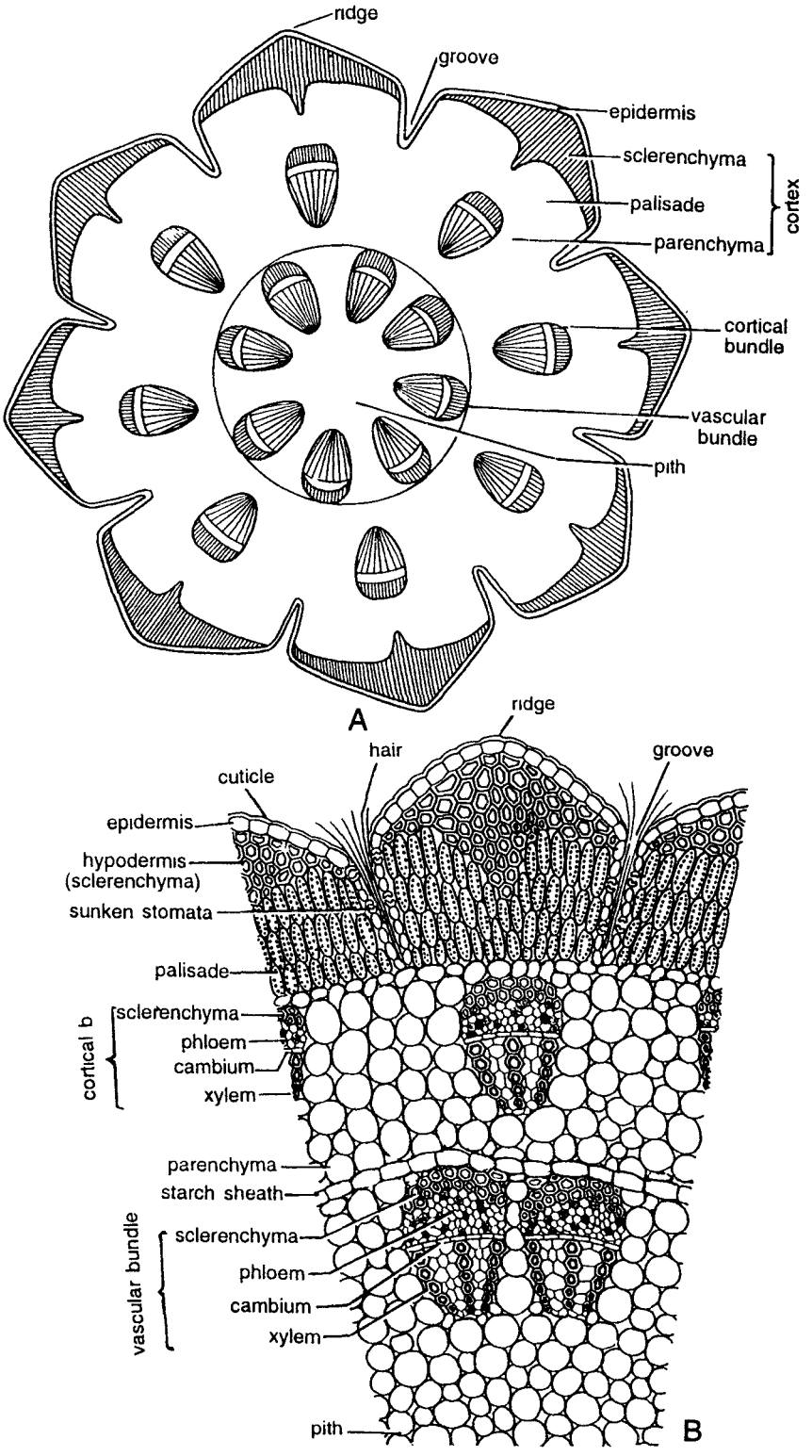


Fig. 16. *Casuarina*. T. s. stem. **A.** Outlines of the section. **B.** Details of a part of a section.

Vascular tissue

1. Vascular bundles are arranged in a ring.
2. Each vascular bundle occurs below the groove.
3. A sclerenchymatous patch, known as bundle cap is present just above the bundle.
4. Each vascular bundle is conjoint, collateral, endarch and open.
5. It shows a small amount of secondary growth.
6. A wide parenchymatous region is present between the two adjacent vascular bundles.

Pith

1. A well developed parenchymatous pith is present in the centre.

[III] Xerophytic characters

The anatomy shows following xerophytic characters.

1. Presence of thick cuticle.
2. Stomata sunken and covered with hairs.
3. Sclerenchymatous hypodermis and bundle cap.
4. Presence of palisade in the cortex.
5. Well developed vascular tissue.

The presence of well developed cortex and the type of vascular bundles indicate that the material is a dicot stem.

Exercise 28

***Object* : To study the xerophytic characters seen in T.s. of *Ficus* leaf.**

[I] Practical work

Cut a transverse section of the material, stain in safranin and fast green combination, mount in glycerine and study.

[II] Observations

Transverse section shows a distinct midrib and the wings on both of its side. The following characters are observed.

Epidermis

1. Both upper and lower epidermis are distinct.
2. Upper epidermis is multiseriate (many layered) and is made of 3-4 layers of cells.
3. Lower epidermis is uniseriate (single layered). Stomata occur in this layer.
4. Both upper and lower epidermis are thickly cuticularised.

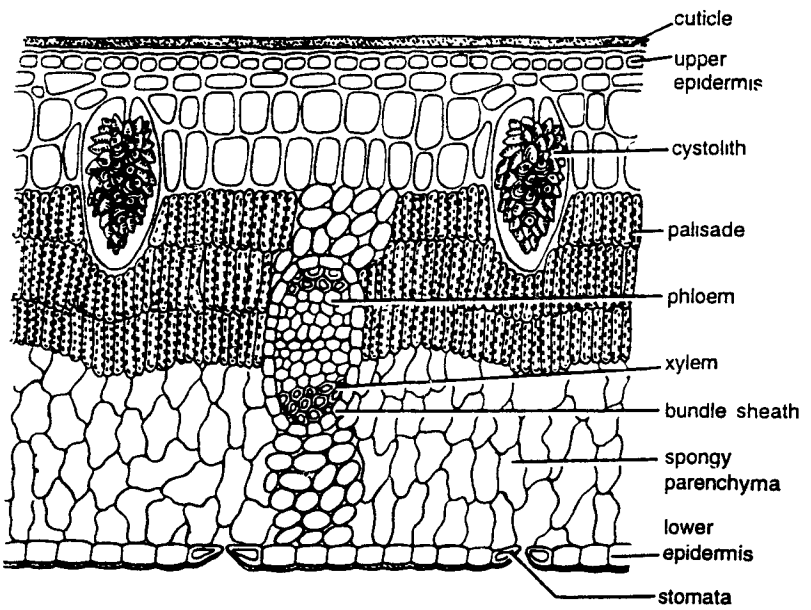


Fig. 17. *Ficus*. A part of T. s. of leaf.

5. Cystolith, grape - like crystalline masses of calcium carbonate are present in one of the lower layers of upper epidermis.

Mesophyll

1. It is differentiated into palisade and spongy parenchyma.
2. Palisade forms 2-3 layers below the epidermis. The cells are rich in chloroplasts.
3. Spongy parenchyma is located near the lower epidermis. The cells are loosely arranged and form large number of air chambers which open into sub-stomatal cavities near the stomata present in the lower epidermis.

Vascular tissue

1. Many vascular bundles are arranged in almost parallel series.
2. A few vascular bundles are slightly bigger than the others.
3. Each vascular bundle is conjoint, collateral and closed.
4. Xylem is situated towards the upper epidermis and the phloem towards the lower epidermis.
5. Parenchymatous bundle sheath surrounds the vascular bundles.

[III] Xerophytic characters

The leaf shows following xerophytic characters.
1. Presence of thick cuticle.
2. Multiple or multilayered epidermis.
3. Presence of cystoliths.
4. Presence of stomata in the lower epidermis only.
5. Sclerenchymatous bundle cap.

Exercise 29

***Object* : To study the xerophytic characters seen in T.s. of *Nerium* leaf.**

[I] Practical work

Cut a transverse section of the material, stain in safranin-fast green combination, mount in glycerine and study.

[II] Observations

The transverse section shows a distinct midrib and wings on either of its sides. Following characters are observed.

Epidermis

1. Both, upper and lower epidermis are distinct.
2. Upper as well as lower epidermis are multiseriate (many layered). Each epidermis is made of about 3-4 layers. The cells are parenchymatous.
3. Both epidermal layers are thickly cuticularised.
4. Stomata occur only in the lower epidermis. These are highly sunken and are present in the infolded parts of lower epidermis.
5. The stomata are covered with hairs.

Mesophyll

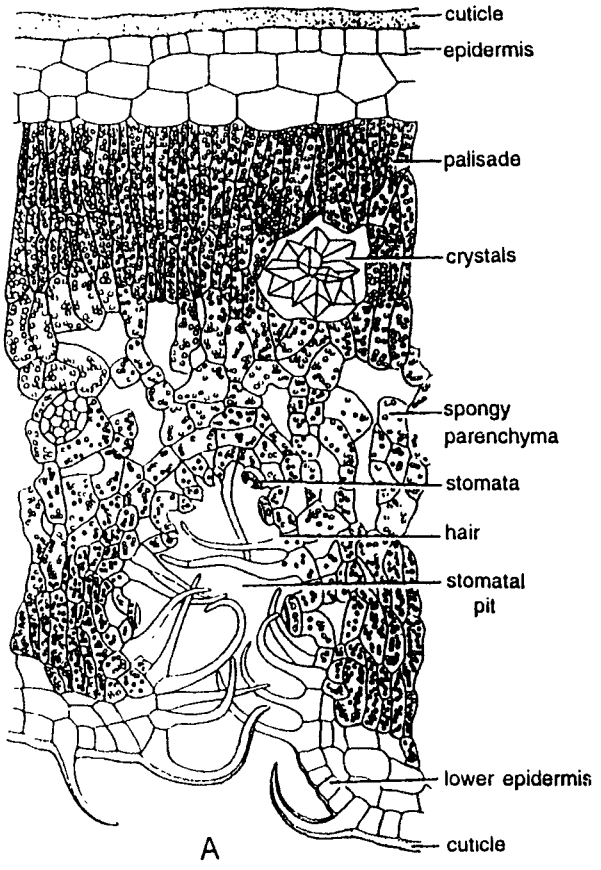
1. It consists of palisade tissue and spongy parenchyma.
2. Palisade lies just below the upper epidermis and is 4-5 cells deep. A few layers of compactly arranged palisade tissue are also present just above the lower epidermis.
3. Spongy parenchyma is present between the palisade of lower and upper epidermis. The cells are loosely arranged and form large air chambers.

Vascular tissue

1. Many vascular bundles occur in the leaf.
2. Vascular bundle in the midrib is larger than the others.
3. Each vascular bundle is conjoint and collateral. The protoxylem is located towards the upper epidermis and the metaxylem towards the lower epidermis.
4. Vascular bundle is surrounded by a parenchymatous bundle sheath.

[III] Xerophytic characters

The leaf shows following xerophytic characters.
1. Presence of thick cuticle.
2. Both epidermal layers are multiseriate.



3. Stomata only in the lower epidermis and highly sunken.
4. Stomata covered with thick envelope of hairs.
5. Presence of palisade near both epidermal layers.
6. Well developed vascular tissues.

Exercise 30

***Object* : To study the xerophytic characters seen in T.s. of grass leaf.**

[I] Practical work

Cut a thin transverse section of the leaf. This would require a sharp razor. Stain in safranin - fast green combination, mount in glycerine and study.

[II] Observations

The section shows following characteristics.

Epidermis

1. Both, upper and lower epidermal layers are present.
2. Each cell of the upper epidermis produces two outgrowths.

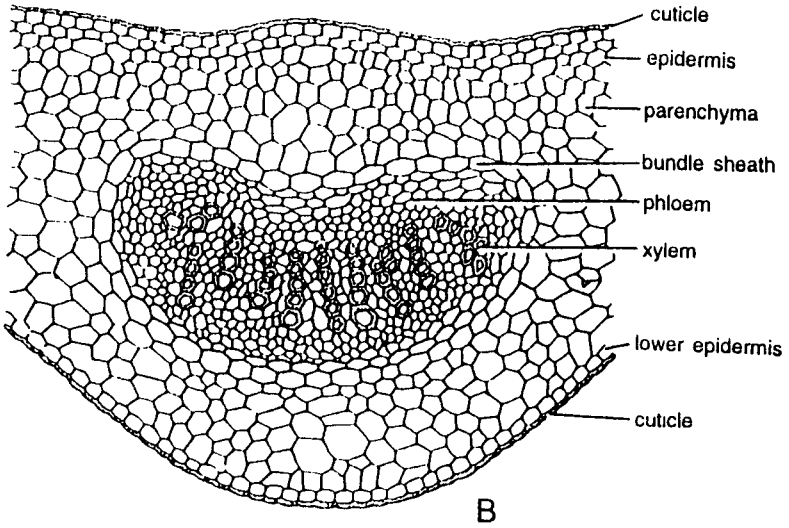


Fig. 18. *Nerium* T s. leaf. **A.** Part of the wing **B.** Details of a part of mid rib.

3. Stomata are present which are generally sunken.
4. A few colourless and large bulliform cells are also present in the upper epidermis. These cells collapse when conditions are dry and, therefore, the upper surface of the leaves roll and cut off the transpiration from stomata in the upper epidermis.
5. The lower epidermis is made of single layer of cells. In the leaves of common grass, stomata are absent (but in many grasses, stomata occur on lower epidermis also).
6. A thick cuticle is present on both epidermal layers.

Mesophyll

1. The mesophyll tissue is situated between upper and lower epidermis.
2. It is undifferentiated and is made of chlorenchyma only.

Vascular tissue

1. Vascular bundles occur in a single row.
2. The size of the vascular bundles differs.
3. Sclerenchymatous bundle sheath envelops the vascular bundle.
4. Each vascular bundle is conjoint and collateral.

[III] Xerophytic characters

Following xerophytic characters are seen.

1. Presence of thick cuticle.
2. Stomata sunken in the epidermis.
3. Stomata present generally on the upper epidermis.
4. Presence of bulliform cells or motor cells.
5. Undifferentiated mesophyll.
6. Rolling of leaves in dry conditions.

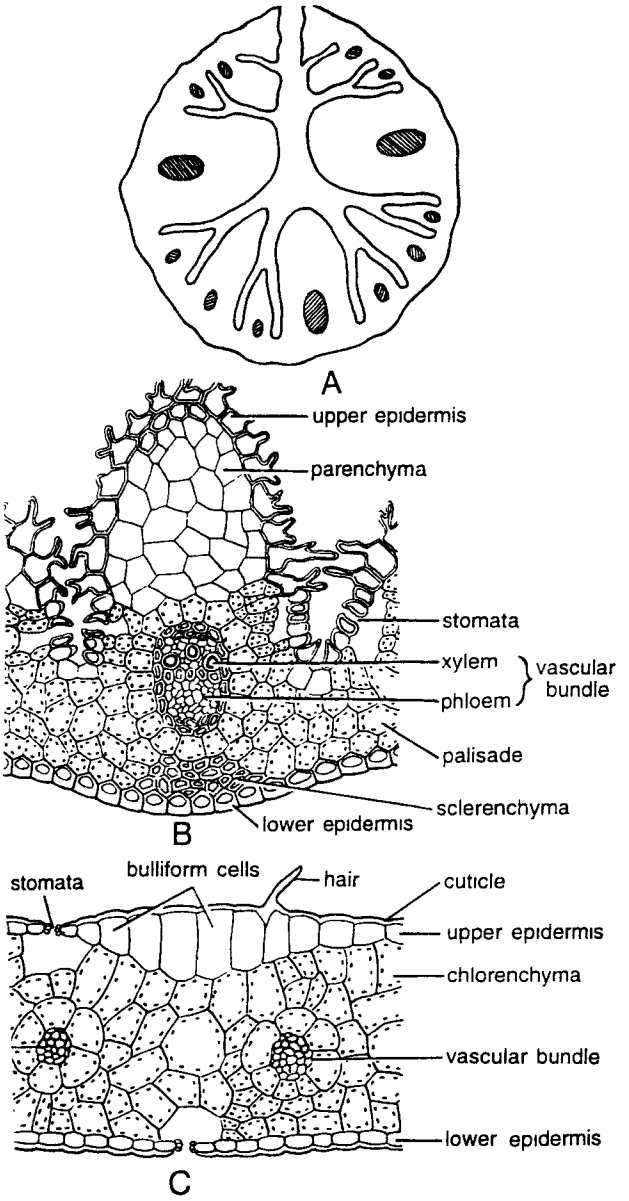


Fig. 19. Grass. T. s leaf **A**. Folded leaf of *Spartina*, **B.** A part of leaf showing details, **C.** An enlarged part of leaf to show bulliform cells.

6

Plant Utilization

It is difficult to visualise the life without plants because plants fulfil three major needs of human life viz. food, clothing and shelter. Most of the useful articles are also plant conversion products. Plants yield fibres, wood, drugs, beverages, oils, cellulose, fats, latex, fumitories, masticatories, spices, tannins, dyes, latex, gums, etc. The daily human life could not have been possible but for green mantle of plants that covers the earth. Some of the important plants and their uses are described in this chapter.

Practical work

The plants of economic importance are generally kept in the laboratory as specimen. A student is expected to study the characters, identify the plant, its useful parts and its importance. A student is also expected to collect information about different uses of plant, its cultivation, production and marketing statistics, etc. In practical examination, common practice is to place a specimen for spotting. Candidate is expected to write comments within a specified time of a few minutes. Therefore, comments written in practical record should be to the point, brief and precise, sufficient enough to be reproduced in the examination within stipulated time. Typical matter for practical record should include the following sequence.

1. Botanical name of the plant.
2. Common English/Hindi/vernacular name.
3. Family
4. Part/parts of the plant used.
5. Morphological characters of the plant.
6. Morphology and anatomy of useful plant part.
7. Cultivation, harvesting and processing.
8. Uses of the plant part/parts.
9. World production.
10. Production in India.

Practical record should also include diagrams of typical plant or plant part which is economically useful.

CEREALS

Exercise 1

Object **: To study the economic Botany of Wheat.**

Comments

Botanical name. ***Triticum aestivum***
Hindi name. Gehoon, Kanak
Family. Gramineae or Poaceae
Edible part is caryopsis which is a fruit or grain.

1. Wheat is an annual herb with 0.6-1.5 m high culms. The inflorescence is a terminal spike made of 15-20 spikelets. The grain is a dry, one seeded indehiscent fruit called caryopsis.
2. The grains are produced in an inflorescence with is a spike of spikelets. A mature grain consists of embryo, starchy endosperm, proteinaceous aleurone layer and husk.
3. Wheat flour is used for breads, cakes, biscuits and other confectionery products. Starch is employed in the preparation of beer, industrial alcohol and other alcoholic beverages, for sizing textiles, etc. Wheat straw is used for

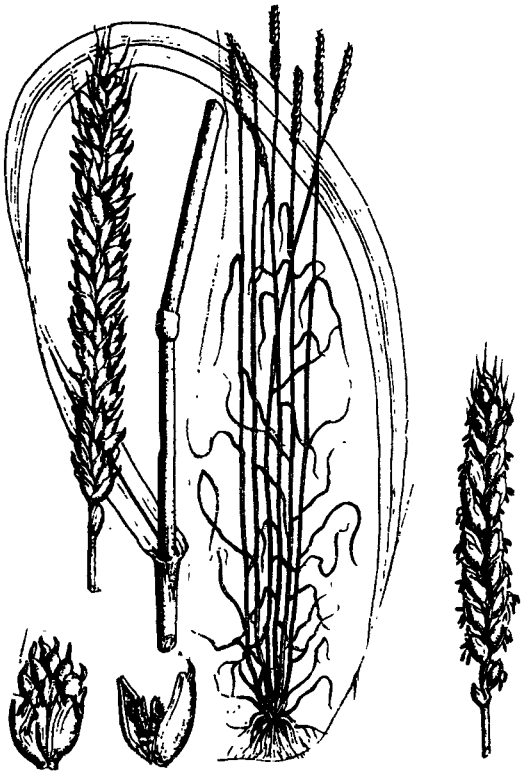


Fig. 1. *Triticum aestium* (wheat), a flowering plant.

weaving chairs, mattresses, stuffing, baskets, packing, cattle feed, etc.

4. Wheat is grown in variety of climates but grows mostly in warm temperate regions of the world.
5. In India it is grown as rabi crop and is sown from October to late November.
6. Largest producer of wheat is U.S.A. Other wheat producing countries are Russia, China, Canada, Australia, India, etc.
7. In India it is a major cereal and covers 12% of the total area under cereals and 76% of that under winter cereals. It is mainly cultivated in U.P., Haryana, Punjab and M.P.
8. Various species used include *T. aestivum*, *T. durum*, *T. dicoccum*, *T. sphaerococum*, etc.
9. Some important Indian wheat varieties are — Lerma Rojo 64, Kalyan Sona, Sharbati Sonora, Moti, Hira, etc.

Exercise 2

Object : To study the economic Botany of Rice.

Comments

Botanical name. ***Oryza sativa***
Hindi name. Chawal, Dhan
Family. Gramineae or Poaceae
Edible part is caryopsis which is a fruit commonly known as grain.

1. Half the world's population, mostly the densely populated regions of the world, use this cereal as a staple food.
2. Plant is a large annual grass. The inflorescence is a terminal panicle, its branches ending into a grain, covered by a husk. The rice grain is a fruit called caryopsis. Grains with husk are popularly known as paddy.
3. The plant grows in hot, most tropics. The area should be flooded with water during early stages. Clay to clay-loam soils are more suitable for paddy cultivation.
4. The grains are used after removal of the husk and are very nutritious. Grain contains considerable amount of proteins, fat and starch. It also forms a raw material for alcoholic beverages. The stems are used as hat fibres and straw is used for mushroom cultivation.
5. China produces about 32% of the worlds rice, India following with 21%. The highest yield in India comes from West Bengal and Bihar.
6. In India, rice is grown at different times in different regions. U.P., A.P., M.P., West Bengal, Tamil Nadu, Gujarat and Kerala are rice growing areas of the country.
7. Some high yielding varieties of rice grown in India include Bala, Jamuna, Krishna, Sabarmati, Gaya, Saket 4, etc.

Exercise 3

***Object* : To study the economic Botany of Maize.**

Comments

Botanical name. ***Zea mays***

Hindi name. Makka, Bhutta

Family. Gramineae or Poaceae

Edible part is caryopsis which is a fruit called grain.

1. The plant of maize (corn) is annual grass. The plants are monoecious and posses both male and female flowers on the same plant.
2. The grain is caryopsis with two types of endosperms (i) hard and yellow and (ii) soft, floury and white. Aleurone layer separates embryo from starchy endosperm.
3. Maize is a sub-tropical crop, grown in alluvial and loam soils. It is grown as Kharif crop.
4. Maize is used as a food for livestock; flour is used in the preparation of corn bread. Other uses include corn flakes, corn starch, syrup, corn oil, dextrins, industrial alcohol, etc. Fibres are also obtained from the main plant for making paper, yarn and as pith. Zein-the maize protein is useful in the manufacture of artificial fibres.
5. U.S.A. produces half the world's output. Other corn producing countries include China, Argentina, Brazil, India, Mexico, etc.
6. In India, maize was introduced by East India company in 12th century. It is now chiefly cultivated in U.P., Bihar, Rajasthan, M.P., Punjab, A.P., etc.
7. Some important maize varieties include Ganga 5, Deccan 101, Sweta, Kanchan, etc.

SUGAR

Exercise 1

***Object* : To study the economic Botany of Sugarcane.**

Comments

Botanical name. ***Saccharum officinarum***

Hindi name. Ganna

Family. Gramineae or Poaceae

Part of the plant used is stem for sugar extraction.

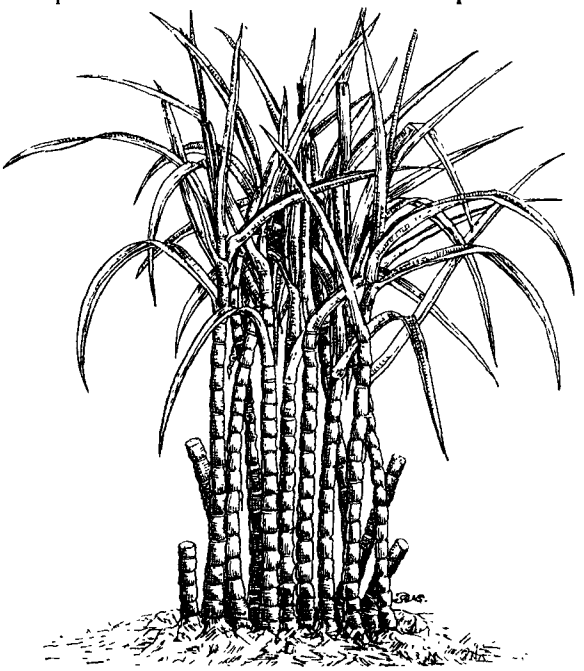


Fig. 2. *Saccharum officinarum* (sugarcane), plants with leaves

1. The perennial grass grows 8 to 12 feet tall and is supported by stilt roots.
2. It grows best in warm humid tropical lowlands. It requires hot and humid climate with high rainfall. It is grown on sandy soils to heavy loams.
3. Sugarcane is propagated by cuttings made from upper joints of old canes. Ratoons are also used for propagation. In India sugarcane is generally grown during January-March and harvested after 10-15 months.
4. The juice extracted from stem by expression is crystallised to manufacture sugar. The bagasse, molasses and filter mud which are by-products of sugar extraction are also used variously.
5. Chief sugarcane producing countries include Brazil, Cuba, India, China, Australia, etc.
6. Eighty per cent sugarcane in India is grown in north India with U.P. leading the list.
7. Most of the varieties in India are developed by Sugarcane Breeding Institute, Coimbtore. These include CO-1148, COS-767, CO-802, etc.

STARCH

Exercise 1

***Object* : to study the economic Botany of Potato.**

Comments

Botanical name. ***Solanum tuberosum***
Hindi name. Aalu
Family. Solanaceae
Part of the palnt used is underground stem tuber.

1. It is rich in starch and forms one of the most commonly used vegetable.
2. Plant, a native of South America, is about a foot tall, spreading annual. The underground branches swell at the tip to form tubers. They bear buds in the axil of scale leaves.
3. Potato is propagated by tubers called 'seeds'. These are used as a whole or after cutting into pieces. It grows over a wide range of soil and climatic conditions.
4. It is essentially a crop of cool, moist regions and grows best in a climate where cool nights alternate with warm days.
5. It is a universal table food and is also used for sizing cotton and paper, production of dextrins, alcohol, adhesives, etc.
6. About 90% production comes from Europe. In India it is largely cultivated in U.P., H.P., Punjab, M.P., etc.
7. Central Potato Research Institute, Shimla engaged in potato research has introduced several improved varieties. Kufri Jyoti, Kufri Badshah are specially developed for tissue culture.

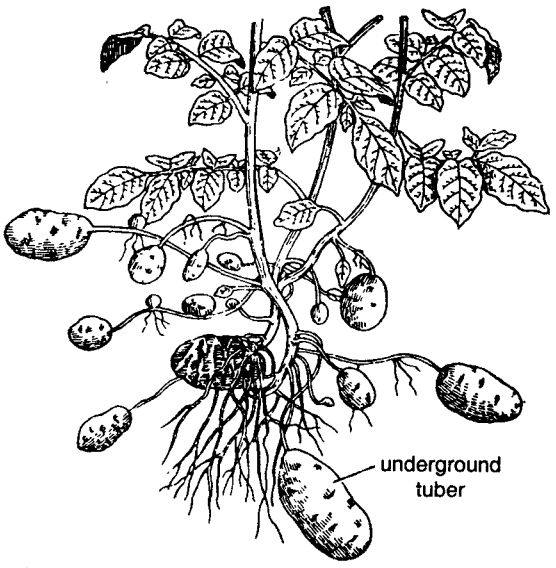


Fig 3 *Solanum tuberosum* (potato), a plant showing underground tubers

FIBRES

Exercise 1

***Object* : To study the economic Botany of Cotton.**

Comments

Botanical names. *NTR* — ***Gossypium arboreum, G. herbaceum***

New World cotton—***G. hirsutum, G. barbadense***
Hindi name. Kapas
Family. Malvaceae

Fig 4. *Gossypium barbadense* (Sea Island cotton), a flowering twig

Parts of the plant used are
(a) seeds for oil extraction and
(b) seed hair as cotton fibres.

1. This plant is an important fibre and oil seed crop. Both oil and fibres are obtained from the seeds. The fibres are epidermal hair, while oil is expressed from the seeds.
2. Plant is a perennial shrub or a small tree. The leaves are simple and palmately lobed. The flowers are solitary axillary, regular and bisexual. The fruit is a loculicidal capsule with 3-5 locules. It is known as cotton boll.
3. Cotton is a tropical crop requiring sunshine, uniform temperature and drier season during flowering. It grows on sandy damp soil of humid regions. Black alluvial soil of the Deccan plateau is considered the best.
4. **Oil** obtained from the seeds is used as salad and cooking oil, in preparation of oleomargarine, oil residue as raw material for soap, washing powders, roofing tar, glycerine, etc. Oil cake is used as food for cattle.
5. Each locule of the fruit contains about nine seeds. The surface of the seeds is covered with long, soft and white or creamy epidermal hairs. **Seed hairs** are used as fibres. The fibres are collected from seeds and after processing, bales are made into varied products. It is an important constituent of cotton fabrics, rubber tyre fabrics, carpets, blankets, cordage, etc. Raw cotton is used for stuffing.
6. Cotton is cultivated in U.S.A., India, Pakistan, China, Egypt and Brazil.
7. In India, it is grown in Maharashtra, Karnataka, Punjab, Assam, Gujarat, Madhya Pradesh, Andhra Pradesh and Uttar Pradesh. Major cotton production in India comes from Gujarat. There are 657 cotton mills in the country and cotton textiles are being exported from India.

Exercise 2

***Object* : To study the economic Botany of Jute.**

Comments

Botanical name. ***Corchorus capsularis***
(also ***C. olitorius***)
Hindi names. Pat, Titapat.
Family. Tiliaceae
Parts of plant used are fibres from phloem (bast fibres) of stem.

1. Of the 40 species of jute, 8 species occur in India, but only two of these are commercially useful.
2. The plant is an annual shrub and is grown from seeds. Under cultivation, the plant remains unbranched. The solitary flowers are hermaphrodite. The fruit is a loculicidal capsule.
3. It is best grown in humid regions with moderate rains and on light, sandy, deltaic loams which receive periodic deposit of fertile silt from flood waters.
4. The fibres are obtained from the secondary phloem by retting the stem. The stems are beaten and fibres are separated.

Fig 5 *Corchorus capsularis* (jute), a flowering branch

5. The fibres occur in long wedge shaped bundles outside the xylem in the stem. Each fibre group consists of 4-50 thin walled cells of phloem.
6. Jute fibre contains on an average 63% cellulose, 22-20% hemicellulose, 10-12% lignin, gums, waxes and minerals. The fibre is used for manufacturing packing cloth, hessian, bags for transport and storage, rugs, curtains, upholstry, linings, ropes, twines, etc.
7. This is the most important cash crop of north-east India, especially valleys of Ganges and Brahamputra in Assam, West Bengal, Bihar and Orissa. About 67% of the products are consumed at home while the rest are exported to U.S.A., U.K., Australia, Canada, Argentina, etc. Other major jute producing country is Bangla Desh.

VEGETABLE OILS

Exercise 1

Object **: To study the economic Botany of Groundnut.**

Comments

Botanical name. ***Arachis hypogaea***
Hindi name. Moongphali
Family. Papilionaceae
Part of the plant used are seeds from which oil is extracted.

1. Ground nut plant is a low growing annual herb. The quadrifoliate leaves are present on grooved petiole. The sessile flowers are borne in the axils of leaves. The fruit is an indehiscent, constricted pod with 1 to 3 or more seeds.
2. Groundnut grows in tropical and subtropical regions. It is cultivated in well drained sandy loams.
3. It is raised as rainfed Kharif crop and is rotated with cereals (wheat, rice) and sometimes with other plants (cotton, potato etc.).
4. Seeds are an important source of vegetable non-drying oil. The oil is expressed by hydraulic presses and expellers.
5. The oil contains fatty acids such as oleic acid, linolenic acid, palmitic acid, etc. It is rich in phosphorus and vitamins (thiamine, riboflavin and niacin).
6. The filtered and refined oil is edible, and is used as salad oil, making margarine, shortening, etc. Poorer grades are used for soap making, as lubricants and illuminants. The residual oil cake is a good cattle feed and is also used as a fertilizer.
7. The major groundnut producing countries are India, China, West Africa, U.S.A., etc.
8. In India groundnut is largely grown in A.P., Gujarat, Karnataka, M.P., U.P., Punjab and Rajasthan.

Exercise 2

Object **: To study the economic Botany of Mustard.**

Comments

Botanical names. ***Brassica campestris*** (also ***B. juncea, B. napus)***
Hindi name. Sarson
Family. Cruciferae or Brassicaceae
Part of the plant used are seeds for extraction of oil.

1. The mustard plants are slender, erect and branched, annual herbs. The small pale yellow coloured flowers are borne in corymbose racemes. The fruit is siliqua. It contains small, yellow brown to black coloured seeds. It yields one of the most important edible oils.
2. Mustard is grown as a rabi crop, either pure or mixed with maize, legumes, sugarcane, etc. Medium or heavy soils are best suited.
3. The oil content varies between 30-48%. This semi-drying oil contains 7% moisture, 36% fats and 25% nitrogenous substances.
4. Erucic acid forms the major part (45-50%) of the total fatty acids. Oleic, linoleic, palmitic, and stearic acid are some other fatty acids present. Pungent character of the oil is due to the presence of allyl-iso-thiocyanate formed by a glycoside-sinigrin present in mustard seeds.
5. The seed and oil are used as condiments in the preparation of pickles and for flavouring curries and vegetables. Oil is also used in lamps, in tempering steel, in oiling wooden goods, in making soaps, etc. The oil cake is used as a cattle feed. The leaves of young plants are used as green vegetable.
6. India is first in the world both with regard to acreage and production. It is chiefly grown in Bihar, M.P., West Bengal, Orissa and U.P. About 60% of the total Indian production comes from Uttar Pradesh alone.

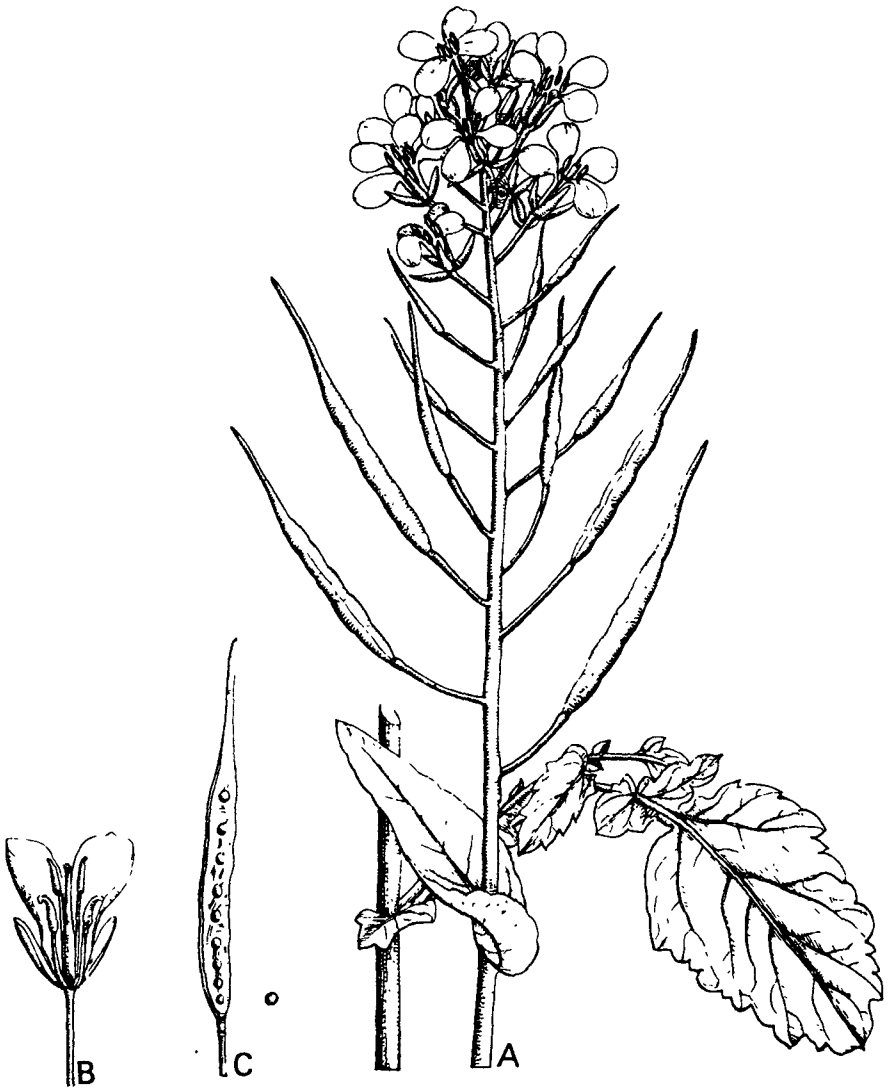


Fig. 6. *Brassica campestris* var *sarson* (mustard), **A.** A branch with flowers and fruits, **B.** A flower, **C.** A fruit (siliqua) with seeds

Exercise 3

***Object* : To study the economic Botany of Coconut.**

Botanical name. ***Cocos nucifera***
Hindi name. Nariyal
Family. Palmae
Parts of the plant used are
(a) mesocarp of the fruit for fibres and
(b) endosperm of the seed for extraction of oil.

1. Coconut palm is a tall, unbranched tree reaching a height of 10-25 m. The stem bears a crown of 20-30 large leaves. The unixexual flowers are arranged in spadix.
2. This tall palm tree bears fruits in bunches on the tree. The fruit is a three sided drupe consisting of a smooth rind or exocarp, a reddish brown fibrous mesocarp and a hard stony endocarp or shell enclosing the seed. The well known coconut meat and milk are actually the endosperm of the seed.

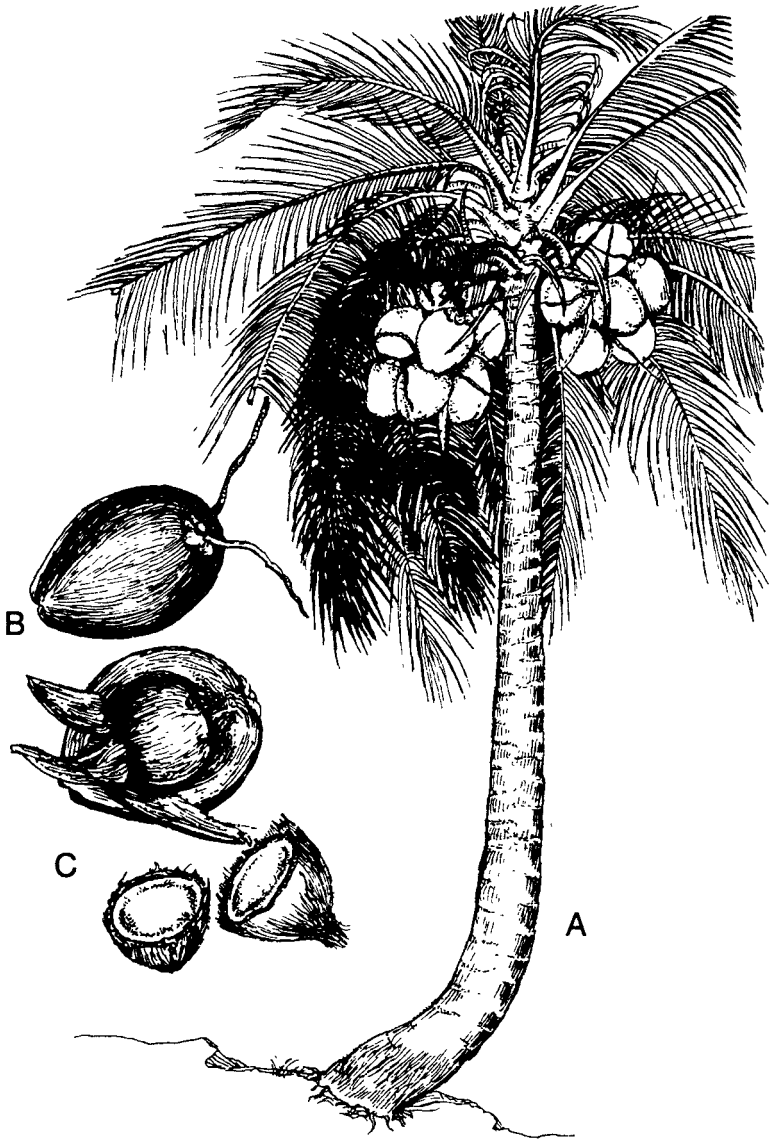


Fig. 7. *Cocos nucifera* (coconut), **A.** A tree bearing fruits at the top, **B.** Fruit with part of husk removed, **C.** Fruit cut open.

3. The palm is widely grown in the coastal and deltaic regions of tropical and sub-tropical countries, mainly Philippines, Indonesia, India, Mexico, Papua and New Guinea and Sri Lanka.
4. The fibrous husk is used for the manufacture of coir which is used for the cordage, mats, foot rugs, brushes, stuffing, etc. The fruits are dehusked and husk is soaked in brackish sea water for 8-10 months. This process of retting is followed by beating husk to separate fibres. The fibres have a thick deposition of lignin and hemicelluloses. Chemically, the coir fibres are made of 26% total water solubles, 14-25% pectins, 8-50% hemicelluloses, 25-29% lignin and 22% cellulose.
5. The shells are used as containers and as fuel.
6. The milk (watery endosperm) is a refreshing drink.

(B-52)

7. The meat (the cellular endosperm) is eaten raw or dried to form copra from which oil is extracted. Coconut oil is used in the manufacture of margarine, vegetable ghee and hard soaps. The oil has the highest saponification value and the lowest iodine value. Amongst many fatty acids of the oil, a few include lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, etc.
8. Unopened inflorescence yields palm sugar.
9. Leaves are used for thatching.
10. In our country, coconut is mainly cultivated in coastal areas of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh.

SPICES

Exercise 1

***Object* : To study the economic Botany of Cardamom.**

Comments

Botanical name. ***Elettaria cardamomum***
Hindi name. Choti elaichi
Family. Zingiberaceae
Parts of the plant used are fruits which are valuable as a spice.

1. The plant is a tall, herbaceous perennial, reaching a height of 2-4 m. Branched rhizome bears many erect leafy shoots. The flowers are borne in long panicles. Fruit is a 3-sided capsule with 10-15 seeds.
2. It grows in tropics. Natural canopy of evergreen forest at an attitude between 600-1500 m is most suitable for its growth.
3. The plant is native of India, indigenous to moist, evergreen forests of South India. It is grown either as a pure plantation crop or as subsidiary to coffee and areca nut. It is also found as a natural undergrowth in some forest tracts.
4. The triangular capsular fruits and seeds have delicate flavour. It is used for flavouring curries, cakes and pickles. The seeds contain 2 to 8% of strongly aromatic volatile oil with a pleasant cooling taste.

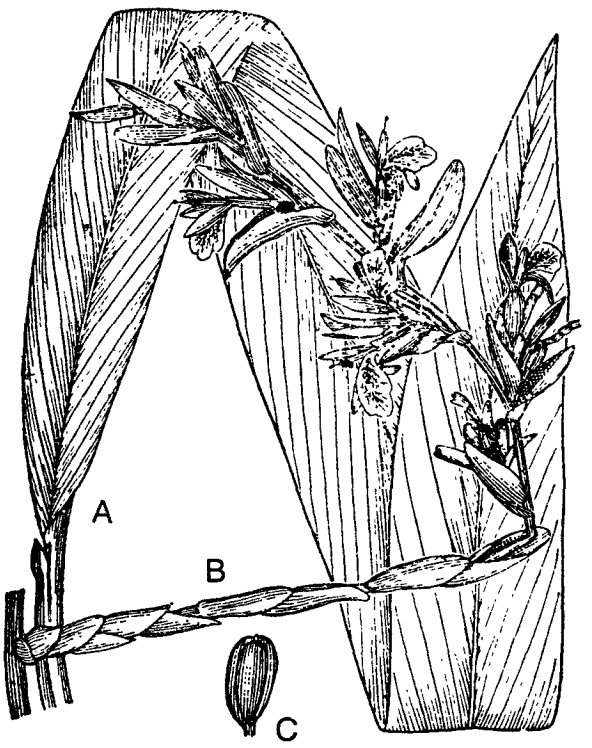


Fig 8. *Elettaria cardamomum*, (cardamom), **A.** A leaf, **B.** Flowering branch, **C.** A seed.

5. The crop is cultivated in hilly forest regions of entire Western Ghats, Mysore, Kerala, Assam and Tamil Nadu.
6. Besides India, cardamom is grown in Sri Lanka, Gautemala, Thailand, Laos, Costa Rica and Tanzania.

Exercise 2

***Object* : To study the economic Botany of Black Pepper.**

Comments

Botanical name. ***Piper nigrum***
Hindi name. Kali mirch
Family. Piperaceae
Part of the plant used are fruits used as a spice.

1. The plant is a weak trailing shrub attaining a height of about 9 m. The swollen nodes bear roots, leaves and axillary buds. The dark green

Fig. 9. *Piper nigrum* (black pepper), **A.** A fruiting branch, **B.** A part of spike with three flowers.

leaves have a pungent taste. The minute white flowers are borne on dense spikes. The mature fruit is bright red which later becomes black and wrinkled.

2. Black pepper grows in humid and warm tropical climate. It requires a fertile well drained loam soil, rich in humus.
3. Black pepper contains moisture, nitrogen, starch, fibre, piperine, pipasin, etc.
4. Seeds yield an oil of aromatic odour. The pungent taste is due to the presence of an oleoresin. It stimulates the flow of saliva and gastric juices and has a cooling effect.
5. It is chiefly cultivated in India, Malayasia and Indonesia. In India, most of the pepper comes from Kerala, other states being Karnataka, Tamil Nadu, Maharashtra and Assam.

Exercise 3

***Object* : To study the economic Botany of Cloves.**

Comments

Botanical name. ***Syzygium aromaticum***
Hindi name. Laung
Family. Myrtaceae
Part of the plant used is dried unopened flower bud used as an important spice.

1. Clove plant is a small, evergreen tree, about 12-15 m in height. The oppositely arranged leaves are dotted with oil glands. The crimson coloured flowers are borne in terminal paniculate cymes. The flowers do not open when cultivated. Fruit is a drupe.
2. Cloves grow best in tropical climate with warm humid conditions. Deep, volcanic loamy soils are best suited. Good deep drainage is essential for proper growth.
3. Unopened flowers are hand picked when these are still dull red in colour. These are dried to brownish-black, crisp and slightly rough, commercial clove.
4. Cloves contain moisture 5.4%, protein 6.3%, volatile oil 13.2%, fats 15.5%, crude fibre 11%,

Fig. 10. *Syzygium aromaticum* (cloves), **A.** A flowering branch, **B.** Unopened flower buds-cloves of commerce.

carbohydrates 57.7%, mineral matter 5.0% and vitamins B_1, B_2, C and A.

5. Cloves are used as a flavouring material and as a spice. Clove oil is useful in toothache and also as a clearing agent during microscope slide preparation.
6. Zanzibar, Pemba islands, Madagascar and Indonesia are the largest clove producing regions of the world.
7. It is also grown to a small extent in Sri Lanka, India, Malaysia and Haiti.
8. In India, it is mainly grown in Nilgiris of Tamil Nadu and Kottayam and Quilon districts of Kerala.

Exercise 4

***Object* : To study the economic Botany of Cinnamon.**

Comments

Botanical names. ***Cinnamomum zeylanicum*** **(= *C. verum*)**
Hindi name. Dalchini
Family. Lauraceae
Part of the plant used is bark.

1. Dried inner bark is one of the oldest spice known to man.
2. The plant is a small evergreen tree attaining a height of 9-15 m. The leathery and dark green leaves are highly aromatic. Axillary and terminal panicles bear yellow flowers. The fruit is a one-seeded fleshy berry.
3. The plants grow best in hot and moist climate of low altitudes. Poor white sands are most suitable.
4. The bark of the tree is peeled off, generally during rainy season. The pieces are allowed to ferment for sometime, cork is removed and then pieces are dried before marketing.
5. The bark of cinnamon contains 9.9% moisture, 4.6% proteins, 2.2% fats, 20.3% carbohydrates, minerals like calcium, phosphorus, iron, sodium, potassium and vitamins such as B_1, B_2, C and A.
6. Cinnamon bark also yields cinnamon oil on steam distillation.

Fig. 11. *Cinnamomum zeylanicum* (Cinnamon), A leafy branch with flowers and fruits.

7. Cinnamon is widely used as spice or condiment. It is astringent, carminative and stimulant.
8. Bark oil is useful in flavouring confectionery, pharmaceuticals and soaps.
9. The plant is a native of Sri Lanka and South India. It is cultivated in Seychelles, China, Malaysia, Indonesia, Kenya, Tanzania, West Indies and South America. Sri Lanka is the largest producer of Cinnamon.
10. In India it is grown in Kerala and Western Ghats.

BEVERAGES

Exercise 1

***Object* : To study the economic Botany of Tea.**

Comments

Botanical name. ***Camellia sinensis*** **(= *Thea sinensis*)**
Hindi name. Chai
Family. Ternstroemiaceae

Parts of plant used are leaves which give a popular beverage called tea.

1. Plant is a perennial shurb reaching a height of 3-4 feet. The leaves posses numerous oil glands giving it a characteristic fragrance and aroma.
2. The tea grows in sub-tropical and tropical mountainous regions at an altitude of about 5000 feet about MSL.
3. The quality of tea depends upon the age of the leaves. The smallest leaf is called orange-pekoe, second leaf as pekoe, third leaf as pekoe-souchong, fourth leaf as souchong and the fifth is known as congou.
4. Tea contains 2.5% theine, 13-18% tannins (polyphenols), volatile oils and a small amount of caffeine.
5. The leaves are plucked and cured and an infusion in boiled water yields most popular of the beverages.
6. India or China or both are native home of tea.
7. India is one of the leading producers and exporters of tea. About 73% of the total output comes from south-east region, especially Assam and West Bengal.

Exercise 1

***Object* : To study the economic Botany of Coffee.**

Comments

Botanical names. ***Coffea arabica*** (also ***C. robusta, C. liberica***)

Hindi name. Kafi

Family. Rubiaceae

Parts of the plant used are seeds which are used for the preparation of a beverage called coffee.

1. The coffee plant is a perennial shrub or a small tree attaining a height of 4.5 to 9 m.
2. The plant grows in hot, moist climate. These are raised from seeds or seedlings and come into bearing in the third year.
3. The fruits are berries and the skin is removed. The seeds are then roasted to develop aroma, flavour and colour. Seeds contain 0.75 to 1.5% caffeine, a volatile oil caffeol, glucose, dextrin, proteins and fatty oils.

Fig. 12 *Camellia sinensis* (tea), a flowering branch.

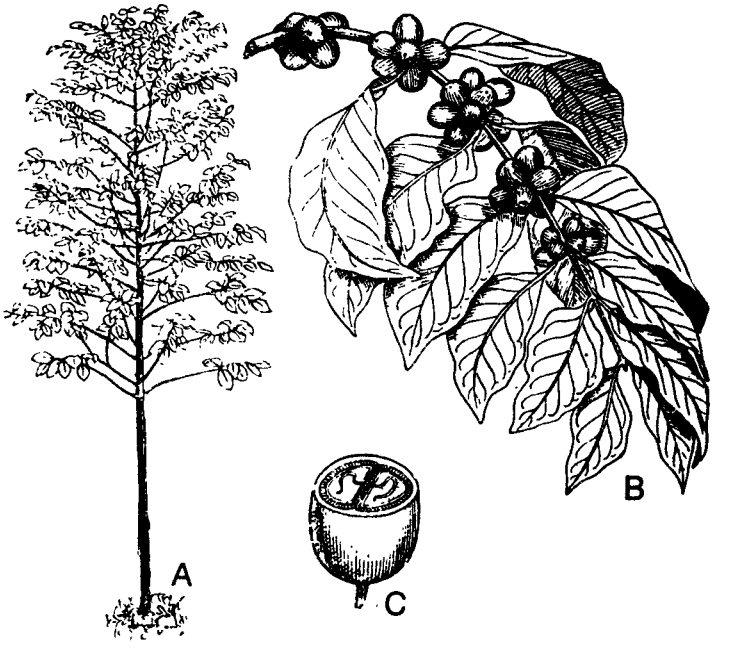


Fig. 13. *Coffea arabica* (coffee), **A.** Trea, **B.** A fruiting branch, **C.** A fruit cut across to show two seeds.

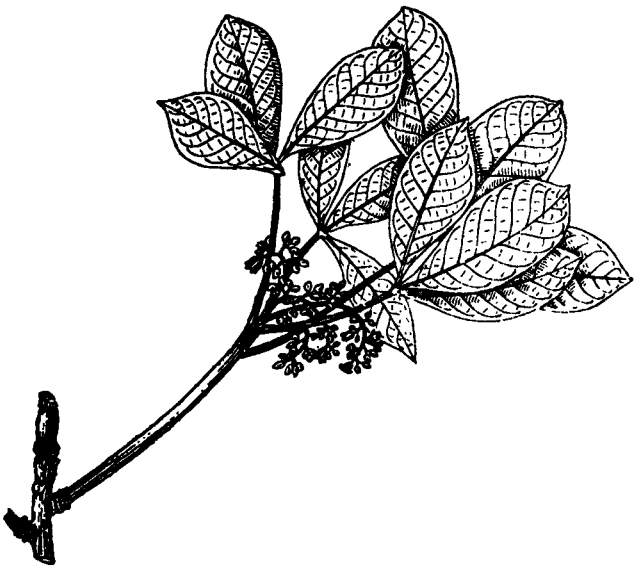


Fig. 14. *Hevea brasiliensis* (rubber plant), a flowering branch with inflorescence.

4. Arabian coffee (*C. arabica*) is a source of 90% of the world supply. It is now grown in Brazil, Guatemala, Angola, India and Sri Lanka. Brazil tops the world production. U.S.A. leads in per capita consumption.
5. Coffee perhaps originated in south-west Abyssinia. Arabian coffee is indigenous to tropical rain forests of Ethiopia.
6. In India, coffee is cultivated in Karnataka, Tamil Nadu and Kerala.

LATEX

Exercise 1

Object **: To study the economic Botany of Rubber.**

Comments

Botanical name. ***Hevea brasilliensis***
Family. Euphorbiaccae
Part of the plant used is the latex from stem.

1. Latex occurs in special cells of inner thin bark, leaves and other soft parts of the tree. The latex vessels are distributed in between phloem. The latex from the lower parts of the tree is usually commercially important.
2. The tree is a native of Amazon. This fast growing tree attains a height of about 60-40 feet. The leaves are trifoliate and the panicle inflorescene bears small unisexual flowers. The fruit is a tripartite capsule which dehisces by explosive mechanism.
3. Rubber plant is well suited for tropical conditions. It requires high humidity and well-drained loamy soil.
4. Rubber is elastic, flexible, air tight, water proof, long lasting and a good insulator of heat and electricity.
5. Latex rubber contains 92 to 94 per cent rubber hydrocarbon, 3 per cent resin, 2 per cent proteins and 0.2 per cent ash.
6. In India rubber is extracted on commercial scale in Kerala, Tamil Nadu, Karnataka, Assam, Andaman and West Bengal.

Common Firewood Plants

	Botanical name	Family	Common names English	Vernacular
1.	*Acacia nilotica*	Mimosaceae	Babul Acacia	Babul, Kikar
2.	*Acacia senegal*	Mimosaceae	Gum Arabic	Kumata
3.	*Adina cordifolia*	Rubiaceae	Yellow teak, Saffron teak	Haldu
4.	*Albizia lebbek*	Mimosaceae	Kokko, East Indian Walnut	Siris
5.	*Anogeissus latifolia*	Combretaceae	Gum ghatti	Dhawa, Dhaura
6.	*Azadirachta indica*	Meliaceae	Margosa	Neem
7.	*Casuarina equisetifolia*	Casuarinaceae	Beefwood, She-oak	Jangli Jhau, Jangli Saru
8.	*Gmelina arborea*	Verbenaceae	Malay bush-beech	Gumhar, Sewan
9.	*Lagerstroemia speciosa*	Lythraceae	Queen Crape Myrtle	Jarul
10.	*Mesua ferraea*	Guttiferae	Iron wood	Nagchampa, Nagkesar
11.	*Prosopis cineraria*	Mimosaceae	-	Jand, Khejri
12.	*Syzygium cumini*	Myrtaceae	Black plum, Java plum	Jamun

Some Common Bamboos*

	Botanical names	Common names	Uses
1.	*Arundinaria racemosa*	River cane	Culms used for cottages, mats and baskets; also as fodder for cattle and ponies.
2.	*Bambusa arundinacea*	Thorny bamboo; **H.** Bans	Used mainly for floating timber and for structural purposes; also for good quality paper pulp; young shoots for pickles; leaves as fodder.
3.	*Bambusa tulda*	**H.** Peka	Used for construction work, mats and basketry; durable and resistant to insects; also source of paper pulp.
4.	*Bambusa vulgaris*	Golden bamboo	Used for scaffolding, roofing, etc.; ornamental; young buds as vegetable; source of paper pulp.
5.	*Chimonobambusa intermedia*	–	Culms used for elegant fishing rods and *hookah*-tubes.
6.	*Dendrocalamus Hamiltonii*	**H.** Kaghgi Bans	Used mostly for paper-making; young culms as vegetable; also suitable for water conduits.
7.	*Dendrocalamus strictus*	Male/Solid bamboo; **H.** Bans-ka-ban	Stems used for rafters, scaffolding, mats, baskets, sticks, furniture, tent poles, water pipes, fishing rods, musical instruments, etc.; pulp for rayon industry; in the preparation of activated carbon; leaves as fodder.
8.	*Melocanna bambusoides*	**A.** Tarai	Prized for house-building, scaffolding, boat making; for preparation of activated charcoal of high adsorption power.
9.	*Ochlandra travancorica*	–	Mature culms used for paper pulp, bamboos for huts and thatching; culms for mats, baskets, umbrella handles and walking sticks; leaves as fodder.
10.	*Phyllostachys bambusoides*	Giant timber bamboo	Used in house construction, bridges, furniture, umbrella handles, walking sticks, etc., young shoots eaten as vegetable; also used for making printing paper; manufacture of rayon.
11.	*Thyrsotachys siamensis*	Umbrella-handle bamboo/ Monastery bamboo	Used for umbrella handles; also for whole-cane fishing rods; employed for paper manufacture.

* Bamboos belong to family Gramineae or Poaceae of Monocots. **A.** Assamese, **H.** Hindi

Some Important Timber Plants

Botanical name	Family	Common name English/Hindi	Uses
1. *Cedrela toona*	Meliaceae (Angiosperms : dicots)	Toon, Red Cedar, **H.** Toon, Mahanim	Wood used for furniture, ceilings, floors, wood red, soft and shining; also used for plywood manufacture.
2. *Cedrus deodara*	Pinaceae (Gymnosperms : dicots)	Deodar	Wood resistant to ants and fungi; straight grained, smooth, used for door and window frames, furniture, railway sleepers, etc.
3. *Dalbergia sissoo*	Papilionaceae/Fabaceae (Angiosperms : dicots)	Sissoo; **H.** Shisham	Wood golden brown — deep brown, grains straight — interlocked-ripple marks distinct; used for high class furniture and cabinet work, railway sleepers and musical instruments.
4. *Mangifera indica*	Anacardiaceae (Angiosperms : dicots)	Mango; **H.** Aam	Wood used for inferior furniture, floor and ceiling boards, beams, rafters, door and window shutters; also for plywood manufacture.
5. *Pinus roxburghii* (= *P. longifolia*)	Pinaceae (Gymnosperms : dicots)	Himalayan long leaved pine /Chir pine	Sapwood white—creamy, heart wood light yellowish brown; odour resinous, moderately hard; used for construction, cheap joinery and furniture, also used for wagons and railway sleepers
6. *Pinus wallichiana* (= *P. excelsa*)	Pinaceae (Gymnosperms : dicots)	Blue pine/ Bhutan pine; **H.** Kail	Wood next to Deodar wood in commercial importance; used in internal fittings of homes, furniture, railway sleepers, match boxes, etc.
7. *Pterocarpus marsupium*	Papilionaceae/Fabaceae (Angiosperms : dicots)	Indian Kino tree/ Malabar Kino tree; **H.** Bijasal, Bija	Wood close grained, yellowish-brown, durable; used chiefly for building purposes such as doors, window frames, beams, etc.
8. *Shorea robusta*	Dipterocarpaceae (Angiosperms : dicots)	**H.** Sal	Sap and heart wood distinct; hard, heavy, tough and brown; resistant to ants and fungi; used for beams, planks in construciton; bridges, furniture, veeners, etc.
9. *Swietenia mahagoni*	Meliaceae (Angiosperms : dicots)	Mahagony tree; **H.** Mahagony	Wood highly prized for cabinet work, panelling and carved wood work; ideal timber for veener and plywood.
10. *Tectona grandis*	Verbenaceae (Angiosperms : dicots)	Teak; **H.** Sagwan	Sap and heart wood distinct; grains straight, texture coarse; heart wood resistant to termites and fungi; used for poles, beams, flooring, panelling, furniture, railway carriages, etc.
11. *Terminalia tomentosa* (= *T. alata*)	Combretaceae (Angiosperms : dicots)	**H.** Asan, Sain	Wood dark brown with darker streaks, hard and strong; used for doors and window frames, electric and telegraph poles.
12. *Xylia xylocarpa*	Minosaceae (Angiosperms : dicots)	Irul; **H.** Jambu, Suria	Most important timber for sleepers; good susbstitute for sal and teak, also useful in house construction.

MICROCHEMICAL TESTS

Exercise 1

***Object* : To detect the presence of cellulose.**

Requirements

Microscope, slides, cover glasses, iodine solution, sulphuric acid 75%, water, paper or cotton fibres, etc.

Procedure

1. Tear paper or cotton in a way so that fibres, are exposed.
2. Place the fibres in a drop of water on a slide.
3. Add a few drops of iodine and allow the fibres to take stain.
4. The fibres turn brown.
5. Add a drop of 75% sulphuric acid and then wash with water.
6. The colour of the fibres changes.

Results

The fibres turn blue.

Conclusion

The change into blue colour indicates the presence of cellulose in the wall thickenings.

This is because cellulose dissolves in cold concentrated sulphuric acid and is precipitated as amyloid on dilution.

Exercise 2

***Object* : To detect the presence of cutin.**

Requirements

Microscope, slides, cover glasses, razor or blade, watch glasses, water, potassium hydroxide (KOH), *Ficus* or *Nerium* leaf, etc.

Procedure

1. Cut the section of the leaf and place in water.
2. Treat the section with KOH solution.
3. Observe the colour of the outermost deposit on epidermis.

Results and conclusion

The yellow colour of the deposition on epidermits indicates that it is composed of cutin — a fat-like substance.

Exercise 3

***Object* : To detect the presence of suberin.**

Requirements

Bottle cork/natural cork, Sudan IV (alcoholic), alcohol 50%, slides, cover glasses, water, glycerine, etc.

Procedure

1. Cut thin slice of the material.
2. Leave the fresh section in Sudan IV to take stain for about 20 minutes.
3. Wash the excess of stain with 50% alcohol.
4. Transfer the section to water and mount in glycerine.
5. Observe the colour under the microscope.

Results and conclusion

The suberised portions become red stained indicating the presence of suberin in the wall.

Exercise 4

***Object* : To detect the presence of lignin.**

Requirements

Match shavings/match sticks/ wood shavings, phloroglucin (1% alcoholic), hydrochloric acid (25%), 1% neutral aqueous potassium permanganate, ammonium hydroxide (sodium bicarbonate), slides, cover glasses, water, etc.

Procedure

Follow any of the two methods given below.

1. Method 1. Prepare thin slices of the material. Place them in 1% alcoholic phloroglucin. Cover the section with coverglass. Allow 25% hydrochloric acid to diffuse along the edge of coverglass.

2. Method 2. Treat the section with 1% aq. neutral potassium permanganate for about 15-20 minutes. Wash with 2% hydrochloric acid followed by repeated washings with water. Add a few drops of either ammonium hydroxide or sodium bicarbonate.

Results and conclusion

1. In the first case red violet colour is taken by lignified walls.
2. In the second method, deep red colour develops in the lignified elements of the deciduous plants.

Exercise 5

***Object* : To detect the presence of mucilage.**

Requirements

Linseed testa, copper sulphate (10%), potassium hydroxide (10%), water, slide, cover glasses, glycerine, etc.

Procedure

1. Cut thin sections of linseed testa.
2. Soak the sections in 10% copper sulphate solution for 20 minutes.
3. Wash the section in water and transfer to 10 % potassium hydroxide.
4. Mount the section in glycerine and observe the colour.

Results and conclusion

The cells with mucilage are stained bright blue indicating that the material possesses mucilage.

Exercise 6

***Object* : To detect the presence of latex.**

Requirements

Latex from *Calotropis*/members of Euphorbiaceae/ Apocynaceae, sucrose, alcohol, conc. sulphuric acid, test tubes, test tube holder, water, etc.

Procedure

1. Prepare an alcoholic extract of latex.
2. Add an equal amount conc. sulphuric acid and sucrose to the latex extract.

Results and conclusion

The colour turns pinkish-purple indicating the presence of latex.

Exercise 7

***Object* : To test for the presence of hemicellulose.**

Requirements

Soyabean seeds, iodine, water, slides, cover glasses, glycerine, etc.

Procedure

1. Cut a thin section of the seed.
2. Observe the section under microsocpe.
3. Treat the section with iodine for a few minutes.
4. Observe the colour of the section.

Results and conclusion

The colour turns blue indicating the presence of hemicelluloses.

Exercise 8

***Object* : To test for the reducing sugar : glucose (grape-sugar).**

Requirements

Fehling's solutions, Benedict's solution, test tubes, test tube holder, spirit lamp, water, glucose (grape-sugar), etc.

Procedure

There are two tests to detect the presence of glucose. These are given below.

Method 1. *Fehling's test.* 1.Take about 5 ml of Fehling solution in a test tube.

2. Add a few drops of glucose solution and boil.

Method 2. *Benedict's test.* 1. Take about 5 ml of Benedict's solution in a test tube.

2. Add a few drops of glucose solution and boil.

Results

1. The Fehling's solution gives brownish red precipitate.
2. Benedict's test gives red-yellow or green precipitate.

Conclusion

The tests reveal the presence of glucose.

Sugar when treated with alkali undergoes enolization to produce enediols. These being highly reactive reducing agents, are capable of reducing oxidising Cu^{+++} ions. Both Fehling's and Benedict's solution contain soluble Cu^{++} ions in soluble form as complexes with citrate or tartrate. On coming in contact with enediols Cu^{++} (cupric) ions are reduced to Cu^{++} (cuprous) ions which later combine to precipitate yellow cuprous hydroxide. Yellow precipitate of cuprous hydroxide on heating, gets converted to reddish cuprous oxide.

$$\text{sugar} \xrightarrow[\text{enediols}]{\text{alkali}} \text{enediols}$$

$$Cu^{++} \longrightarrow Cu^{+}$$

$$Cu^{+} + OH \longrightarrow CuOH \text{ (yellow)}$$

$$2CuOH \longrightarrow Cu_2O \text{ (Red)} + H_2O$$

Overall reaction :

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + 2Cu(OH)_2 \xrightarrow{\text{Boil}} \underset{\substack{\text{Gluconic}\\ \text{acid)}}}{C_6H_{12}O_7} + \underset{\substack{\text{(Cuprous}\\ \text{oxide)}}}{Cu_2O} + H_2O$$

Exercise 9

***Object* : To test for starch/sucrose (non- reducing sugars).**

Requirements

Sucrose/ strach/beet root, Fehling's solution, Benedict's solution, hydrochloric acid, sodium carbonate, sodium bicarbonate, test tubes, test tube holder, spirit lamp, etc.

Procedure

1. Add to sugar equal volume of concentrated hydrochloric acid.
2. Boil the mixture for about five minutes.
3. Neutralise the resulting solution with sodium carbonate or bicarbonate.
4. Then subject the solution to the test of reducing sugars by adding Fehling's or/and Benedict's solutions.

Results

1. Fehling's solution gives brownish red precipitate.
2. Benedict's solution gives yellow red or green precipitate.

Conclusion

The test reveals the presence of non-reducing sugars.

Sucrose occurs widely in plants. It is formed by condensation of one molecule of glucose with one molecule of fructose. On hydrolysis these are formed once again and then give the same results as reducing sugars when subjected to Fehling's and Benedict's solution.

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + \underset{\text{(Fructose)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(Sucrose)}}{C_{12}H_{22}O_{11}} + H_2O$$

Exercise 10

***Object* : To test for the presence of proteins.**

Requiremnts

Gram flour/legumes/soyabean, Millon's reagent A and B, nitric acid, ammonium hydroxide, sodium hydroxide (20%), copper sulphate (1%), test tubes, test tube holder, water, spirit lamp, etc.

Procedure

There are two methods for testing the presence of proteins.

Method 1. ***Xanthoproteic test.*** 1. Treat the suspension of tissue in water. Add concentrated nitric acid. A white precipitate is formed.
2. Heat the solution. Yellow colour is developed.
3. Now add a few drops of concentrated ammonium hydroxide. Observe the change in colour. The colour deepens to orange.

Method 2. ***Biurete test.*** 1. Prepare a suspension of material in water.
2. Add 1 ml of sodium hydroxide (20%) and a drop of copper sulphate to the suspension.
3. Observe the developing colour. Add Millon's reagent (A or B). Mix thoroughly and boil.
4. Note the change in colour in both cases — Millon's reagents 'A' and/or 'B'.

Results

1. ***Xanthoproteic test.*** The yellow colour changes to orange.
2. ***Biurete test.*** The colour developed is violet. On addition of Millon's reagent's 'A' it turns to red on heating and with Millon's reagent 'B' it turns reddish on heating.

Conclusion

The colour changes indicate the presence of protein.

Biurete develops violet colour when treated with dilute $CuSO_4$ solution. This reaction is also shown by compounds containing — $CONH_2$ groups joined directly or by C or N atoms. Proteins also give this reaction because they possess CO — NH — C — CO — NH — peptide bond architecture.

In Xanthoproteic reaction nitration of phenyl rings occurs to yield yellow substitution products which turn orange upon addition of alkali (salt formation).

Exercise 11

***Object* : To test for the presence of fats/oils.**

Requirements

Seeds of almond/soyabean/groundnut, Sudan III (alcoholic), water, osmic acid 1%, test tubes , test tube holders, glycerine, microscope, slides, coverglasses, etc.

Procedure

Any of the two following methods could be followed.

1. **Method 1.** Cut thin sections of the material. Place the sections in Sudan III for about 10 minutes. Wash the sections with 50% alcohol. Mount a section in glycerine after repeated washes with water. Observe the colour under microscope.
2. **Method 2.** Add a few drops of osmic acid to the material in the test tube. Observe the developing colour.

Results

1. The oil drops take red stain.
2. Black colour is developed.

Exercise 12

***Object* : To test the presence of starch.**

Requirements

Starch, test tube, test tube holder, spirit lamp, iodine, HCl, Benedict's solution, NaOH (or Na_2CO_3), etc.

Procedure

Follow any one of the two methods given below.

1. **Method 1.** Take a pinch of starch and add water. Add a few drops of iodine.

Table 1. **Methods to detect food adulteration.**

Food Stuffs	Adulterant	Method of Detection
1. Cereals and pulses	(a) Foreign matter	Take a known quantity (50g) and pick up all foreign matter by hand or forceps Weigh the amount and calculate the percentage (it should not exceed 4%).
	(b) Insect infection	(i) Take a known quantity (50 g). Pick up all the damaged grains Calculate percentage (it should not exceed 1%) (ii) Put the grains in water. Infested grains float on the surface Calculate the percentage.
2. Pulses (arhar, masoor and chana)	Khesari dal	Pick up the Khesari dal (triangular and gray coloured seeds) and calculate percentage as before (it should not exceed 1%).
3. Turmeric (Haldi)	Lead chromate	Weigh 2 g of Haldi powder, reduce it to white ash in crucible (600° C for 4 hours). Cool Add 5 ml of 1 7 dil H_2SO_4 and filter. Add a few drops of 0.2% diphenyl carbazide (alcoholic) Pink colour indicates the presence of lead chromate.
4. Milk	(a) Water added or fat removed	Specific gravity determination of milk by lactometer.
	(b) Starch	Iodine test
5. Tea leaves	Arificial colour	(i) Place the leaves on white paper. The appearance of yellow or reddish colour over the paper will show the presence of artificially added colour. (ii) Spread a little slaked lime on glass plate Sprinkle a little tea dust on the lime. Any colour (e g. red, orange, etc.) other than greenish yellow (due to presence of naturally occurring chlorophyll) indicates the presence of coal tar dye
6. Chillies	Coloured saw dust, brick powder, talcum powder.	Ash a spoonful of chillies. Abundant amount of ash indicates adulteration.
7. Oil	(a) Mineral oil	Take 2 ml of sample Add an equal amount of N/2 alcoholic potash. Heat for 15 minutes in boiling water bath. Cool and add about 10 ml of water Presence of turbidity indicates presence of mineral oil.
	(b) Argemone oil	Add nitric acid. If red colour appears, it indicates the presence of argemone oil
8. Pure ghee	Vanaspati ghee	Bodoudouin test (for the presence of sesame oil) To 5 ml of melted ghee add 0.1 g of sucrose dissolved in 5 ml of dilute HCl Shake well and keep for 15 minutes. A permanent pink colour indicates the presence of sesame oil.
9. Sweets	Metanil yellow	Dissolve a little sample in water, shake and transfer the water extract to another tube. Add dilute HCl A violet red colouration indicates the presence of metanil yellow.

2. **Method 2.** Take a pinch of starch and add water. Boil it by adding HCl. Make this solution alkaline by adding NaOH or Na_2CO_3. Test with Benedict's solution.

Results

1. Black colour appears.
2. Red precipitate is formed.

Conclusion

The tests indicate the presence of starch.

PROJECT REPORT

Compiled Project Report

This of project report is based on data collection. The information that can be collected is available to you in the books.

Such a project work need not be longer than 10 typed or written pages. The following are some important suggestions for compilation of such a report.

1. Select a topic for which sufficient material is available in the books in the library.
2. Collect subject matter from various sources available to you besides library books.
3. Sort out the relevant material that can be included in the project.
4. Arrange the material into following headings.
   (a) Contents
   (b) Introduction
   (c) Subject matter : Timber plants
   (d) List timber plants
   The list should contain —
   Botanical name,
   Common names – English, Hindi
   Family
   Botanical characters
   Uses
   Distribution
   Production
5. Also add a photograph/drawing or visual material about the plant or use of the material, at appropriate place.
6. Acknowledgments. At the end, remember to thank the person/s who helped you to compile the report.
7. List of literature consulted. Also add a list of books, journals, newspapers, etc. from which subject material was drawn.
8. Arrange pages in nice looking file or folder and add a suitable title page.

Field Project Report

One of the important work now introduced as a part of practical is the project work. Since botany is the study of plants and plants occur in nature, field work often forms the subject of projects. The projects provide an opportunity to come to understand the nature itself and hence are valuable. The plant studies would be incomplete if one does not know the plants around and can not tell about their uses. It is, therefore, strongly advised that one keen to pursue Botany in future should undertake frequent visits to areas with thick vegetation. Plants collected could be identified, studied and their uses listed from available books. Such a project would be of great help in knowing about the plants in the neighborhood. It would also offer us information about their uses in various fields.

On plant collection visit one should keep in mind certain things. Some of them are listed below.

1. An area to be visited is first survyed and necessary information such as nature of vegetation, the route, distance, conveyance available, etc. should be obtained.
2. Necessary equipment required for collecting plants should always be carried while on trip.
3. Record of collection should be faithfully maintaned in field record book.
4. At least one plant with leaves and flowers be collected for preparing herbarium sheet.
5. If possible the local names of the plants be also obtained from the residents of the area.

On reaching home or laboratory, the plants should be identified, their botanical names correctly acquired and the information such as its family, morphological characters, uses, distribution, etc. should be collected.

A report should now be prepared. This should contain all the information acquired so far.

The report should contain all the information listed uniformly under definite headings. Wherever possible, drawings of the plant being described or a photograph or a picture be adjusted along with the script at appropriate place.

Useful information on medicinal plants which can be easily collected from your surroundings is given here.

Common Medicinal Plants

Following is the list of some commonly found medicinal plants often used as home remedies. These can be easily collected from the areas surrounding you. It is suggested that a plant be collected and herbarium sheet prepared as a record. The sheet should also have necessary information such as

1. Field book no.
2. Family
3. Botanical name
4. Local name
5. Economic importance
6. Place of collection
7. Locality
8. Notes
9. Date
10. Collected by

A neat and clean diagram of the plant essentially including the plant part used economically should be drawn. Alternatively a figure or a picture be obtained from sources available to you. The matter be systematically arranged and figure or a picture of the plant be suitably included. A list of some very common plants with their required details is given below.

1. *Abrus precatorius* Linn.

Sans. Gunja, **Eng.** Jequirity, Crab's eye.
Common names. Rati, Gunchi.
Family. Papilionaceae/Fabaceae.
Parts useful. Roots, seeds, leaves.
Drug class. Diuretic, tonic, emetic.
Used for the treatment of diseases such as sour throat, cough, chronic inflammation of mucous membrane, etc.

2. *Aconitum ferox Wall ex ser.*

Sans. Visha, **Eng.** Indian aconite.
Common names. Bacchang,bish, mitha zahar.
Family. Ranunculaceae.
Parts useful. Roots.
Drug class. Sedative, diaphoretic.
Used in the treatment of nasal catarrh, tonsillitis, sore throat, gastric disorders, debility and fever of inflammatory origin, also used in neuralgia and rheumatism.

3. *Adhatoda vasica* Nees

Sans. Vasaka
Common name. Arusha
Family. Acanthaceae
Parts useful. Fresh and dried leaves.
Used in the treatment of bronchial troubles, diarrhea, dysentery, glandular tumours, uterotonic, abortifacient for stopping postpartum haemorrhage.

4. *Aegle marmelos* Correa ex Roxb.

Sans. Bilva, **Eng.** Stone apple
Common name. Bel
Family. Rutaceae
Parts useful. Fruit, bark, root, leaf.
Drug class. Antibacterial, anti-inflammatory, laxative, diuretic, cardiac depressant.
Used in the treatment of constipation, diarrhea, peptic ulcers, ear diseases, respiratory diseases.

5. *Aloe vera* Mill.

Sans. Kumari, **Eng.** Indian Aloe
Common name. Ghrit kumari
Family. Liliaceae

Parts useful. Dried juice, leaf
Drug class. Emmenagogue,cathartic, refrigerant.
Used in the treatment of acne, wounds, burns, eczema, cutaneous leishmaniasis, inhibits the growth of *Mycobacterium tuberculosis.*

6. *Atropa belladonna* Linn.

Sans. Suchi, **Eng.** Belladonna,
Common name. Belladonna
Family. Solanaceae
Parts useful. Leaf, roots
Drug class. Narcotic, sedative, mydriatic, antidote of opium and aconite
Used in ophthalmology to dilate pupils.

7. *Azadirachta indica* A-Juss.

Sans. Nimb, **Eng.** Margosa tree,
Common name. Neem
Family. Meliaceae
Parts used. Seed, leaf, bark.
Drug class. Antiseptic, tonic, spermicidal.
Useful in the treatment of skin disorders, hair care, burn, eczema, worms, gingivitis, measles, diabetes, rheumatism, scrofula, teeth care.

8. *Bauhinia variegata* Linn.

Sans. Kachnar, **Eng.** Mountain ebony
Common name. Kachnar
Family. Caesalpiniaceae
Parts used. Bark, roots, leaf.
Drug class. Alternative tonic, astringent, antiulcer, laxative, carminative, anthelmintic.
Used in the treatment of skin disorders, obesity, piles, ulcers.

9. *Butea monosperma* (Lam.) Kuntze

Sans. Palas, **Eng.** Flame of the forest
Common names. Palas, Dhak.
Family. Papilionaceae/Fabaceae
Parts used. Flower, gum, seed, leaf.
Drug class. Astringent, diuretic, depurative, aphrodisiac, d-galactose binding lectins, anti-inflammatory.
Used in the treatment of diarrhoea, skin disorders, diabetes, leucorrhoea, eye diseases, filaria, herpes.

10. *Catharanthus roseus* G. Don.

Sans. Sadabahar, **Eng.** Periwinkle
Common name. Sadabahar
Family. Apocynaceae
Parts used. Roots, leaf.
Drug class. Anticancer, vermifuge, hypotensive, sedative, tranquilizer.
Used in the treatment of diabetes, diahhroea, toothache, menorrhagia.

11. *Datura stramonium* Linn.

Sans. Sada Dhattura, Unmatta, **Eng.** Datura
Common name. Datura
Family. Solanaceae
Parts used. Leaf, seeds.
Drug class. Narcotic, antispasmodic, mydriatic, anodyne, sedative.
Used in the treatment of impotency, asthma, baldness, dandruff, gums.

12. *Glycyrrhiza glabra* Linn.

Sans. Yasti-madhu,madhuka **Eng.** Liquorice.
Common name. Mulhatti, Jethi-madh,
Family. Papilionaceae/Fabaceae
Parts used. Roots
Drug class. Stomachic, demulcent, expectorant, rejuvenation, tonic, laxative.
Used in the treatment of constipation, muscular pain, mouth ulcers, baldness, corns, in irritable conditions of the membranes of urinary organs, sore throat.

13. *Holarrhena antidysenterica* (Linn.) Wall.

Sans. Kutaja, Kalinja, **Eng.** Telicherry bark.
Common names. Kurchi, Kada chhal.
Family. Apocynaceae

Parts used. Bark, seeds, leaf.
Drug class. Stomachic, purgative, liver stimulant, anthelmintic, antipyretic, antidysenteric, tonic.
Used in the treatment of amoebic dysentery and diarrhea, skin diseases.

14. *Hyoscyamus niger* Linn.

Sans. Dipya, Prasikaya, **Eng.** Henbane, black henbane.
Common name. Kurasaniajvayan
Family. Solanaceae
Parts used. Leaf and seeds.
Drug class. Sedative, narcotic, anodyne, mydriatic, antiseptic, anti-inflammatory, analgesic.
Used in the treatment of asthma, whopping cough.

15. *Leucas aspera* Spreng.

Eng. Leucas,
Common name. Chota halkusa
Family. Labiatae/Lamiaceae
Plant part used. Entire plant.
Drug class. Laxative, anthelmintic.
Used in the treatment of fever, scabbies, cough, cold, paralysis, jaundice, psoriasis, chronic skin eruptions and painful swellings.

16. *Madhuca indica* J. F. Gmel.

Sans. Madhuka, **Eng.** Mahua, Illipe, Butter tree.
Common name. Mahua
Family. Sapotaceae
Parts used. Bark, flowers.
Drug class. Galactogogue, astringent, tonic, laxative.
Used in the treatment of bronchitis, rheumatism, diabetes, piles, orchitis, tonsillitis, eczema,

17. *Mentha piperita* Linn. emend. Huds.

Eng. Peppermint,
Common names. Peparmint, gamathi, podina.
Family. Labiatae/Lamiaceae
Part used. Leaves
Drug class. Stimulant, stomachic, carminative.
Used in the treatment of nausea, flatulence, stomach ache, colicky diarrhoea , rheumatism, tooth-ache.

18. *Moringa oleifera* Lam.

Sans. Shigru, **Eng.** Drumstick, horseradish,
Common names. Shobhanjan, Shahajan Sainjana
Family. Moringaceae
Part used. Seeds
Drug class. Antifungal,antiviral,flocculating agent, stimulant, abortifacient, diuretic, antipyretic.
Used in the treatment of hypertension, ulcer, rheumatism, sterility, alopecia, cardiac tonic, paralysis, liver disorders, spleen disorders.

19. *Ocimum basilicum* Linn.

Sans. Visva, Munjariki, Surasa, **Eng.** Sweet basil,
Common names. Krishna tulsi,kali tulsi, babui tulsi
Family. Labiatae/Lamiaceae
Plant part used. Entire plant
Drug class. Carminative, digestive, stomachic, alexipharmic, antipyretic, expectorant, diaphoretic, anthelmintic, diuretic.
Used in the treatment of respiratory diseases, skin diseases, insecticidal, nasal douche, ringworm, habitual constipation, piles and in poultice for sores and sinuses.

20. *Papaver somniferum* Linn.

Sans. Ahifen, chosa, khasa, **Eng.** Opium poppy, white poppy.
Common names. Afim, post, khas-khas
Family. Papaveraceae
Plant part used. Seeds, fruits
Drug class. Strong analgesic, narcotic, sedative, anodyne, antispasmodic, hypnotic, sudorific.
Used in the treatment of insomnia, dysentery, pains, itching.

21. *Phyllanthus niruri* Hook.f.

Sans. Bhumymalaki
Common name. Jangli amla
Family. Euphorbiaceae
Parts of the plant used. Plant, fruits.
Drug class. Liver stimulant, diuretic, astringent, deobstruent, febrifuge.
Used in the treatment of dropsy, viral hepatitis, diarrhoea, dysentery.

22. *Piper nigrum* Linn.

Sans. Maricha, **Eng.** Black pepper,
Common name. Kali mirch
Family. Piperaceae
Parts used. Seed
Drug class. Stimulant, alterative, rubefacient, stomachic.
Used in the treatment of cough, sore throat, piles, cutaneous troubles, weakness following fever.

23. *Psoralea corylifolia* Linn.

Sans. Bakuchi, **Eng.** Psoralea.
Common name. Babchi.
Family. Papilionaceae/Fabaceae
Parts used. Fruit, seed.
Drug class. Anthelmintic, aphrodisiac, stomachic, deobstruent, diuretic, laxative, diaphoretic.
Used in the treatment of leucoderma, psoriasis, leprosy, inflammatory diseases of the skin.

24. *Pterocarpus marsupium* Roxb.

Sans. Pitsala, **Eng.** Malabar kino, Indian kino.
Common name. Bijasal
Family. Papilionaceae/Fabaceae.
Parts used. Gum, wood.
Used in the treatment of diabetes, diarrhoea, dysentery, abdominal pains.

25. *Rauvolfia serpentina* Benth. ex Kurz

Sans. Sarpagandha, **Eng.** Serpentina root
Common names. Sarpagandha, Chota chand.
Family. Apocynaceae
Part used. Root
Drug class. Antihypertensive, hypnotic, sedative.
Used in the treatment of nervous disorders such as anxiety, maniacal behavior, schizophrenia, insanity, increases uterine contractions in labor.

26. *Ricinus communis* Linn.

Sans. Erand, **Eng.** Castor.
Common name. Arand.
Family. Euphorbiaceae
Parts used. Seeds, root.
Drug class. Galactogogue, aphrodisiac, cathartic, purgative.
Used in the treatment of rheumatism, skin diseases, dandruff, constipation, epilepsy.

27. *Sida cordifolia* Linn.

Sans. Bala, **Eng.** Country mallow.
Common names. Bala, Kungyi
Family. Malvaceae
Parts used. Plant, roots.
Drug class. Aphrodisiac, demulcent, febrifuge, cardiac tonic, astringent, diuretic, refrigerant, tonic, stimulating agent.
Used in the treatment of ulcers, rheumatism, urinary troubles, cystitis, sciatica, facial paralysis.

28. *Strychnos nux-vomica* Linn.

Sans. Karaskara, **Eng.** Nux-vomica,
Common name. Kuchla
Family. Loganiaceae
Part used. Seed

Drug class. Aphrodisiac, stomachic, tonic, analgesic, stimulant, febrifuge, emetic.
Used in the treatment of dribbing micturition, bed wetting, nervous disorders, epilepsy.

29. *Taraxacum officinale* Webber ex Wiggers

Eng. Common dandelion,
Common names. Dudal, kanphul
Family. Compositae/Asteraceae
Part used. Leaf, root.
Drug class. Diuretic, tonic for liver diseases, stomachic, laxative, vit A supplement.
Used in the treatment of breast cancer, renal diseases, dyspepsia, hepatic stimulant.

30. *Terminalia arjuna* (Roxb.) Wight & Arn.

Sans. Arjuna, **Eng.** Arjuna,
Common name. Kahu.
Family. Combretaceae
Part of the plant used. Bark
Drug class. Cardiac tonic, antianginal, diuretic, astringent, styptic, febrifuge, antidysenteric.
Used in the treatment of hypertension, cirrhosis of liver, asthma, fractures.

31. *Withania somnifera* Dunnal.

Sans. Ashwagandha, **Eng.** Winter cherry
Common name. Ashwagandha
Family. Solanaceae
Part used. Root
Drug class. Aphrodisiac, general tonic, diuretic, nervine sedative, adaptogenic, immunomodulator, febrifuge.
Used in the treatment of hiccup, cough, dropsy, rheumatism, inflammation, ulcers, scabies.

32. *Ziziphus jujuba* Mill.

Sans. Badar, **Eng.** Ziziphus
Common name. Ber
Family. Rhamnaceae
Part used. Fruit
Drug class. Stomachic, anodyne, tonic.
Used in the treatment of nausea, vomiting, insomnia, anxiety, mental retardation, cold, flu, mouth ulcers, skin diseases, conjunctivitis, hair care.

Appendix

1. Fixing Agents

Fixing Agents and Preservatives

1. Carnoy's fluid

100% ethyl alcohol	—	30 cc
Glacial acetic acid	—	5 cc
Chloroform	—	15 cc

It is used for root tips, anthers, etc. and is preferred for its great penetrating power.

2. Formalin-Aceto-Alcohol

50% or 70% ethyl alcohol	—	90 cc
Glacial acetic acid	—	5 cc
Formalin	—	5 cc

It is popularly known as FAA and is a standard universal fixative. It is the most extensively used fixing and killing agent.

3. Randolph's modified Navashin fluid

Solution A.	Chromic acid	—	5 gm
	Glacial acetic acid	—	50 cc
	Distilled water	—	320 cc
Solution B.	Natural formalin	—	200 cc
	Saponin	—	3 gm
	Distilled water	—	175 cc

At the time of use, mix solutions A and B in equal amounts. Recommended for buds, roots tips and similar objects.

2. Stains

1. Aniline blue
(cotton blue, china blue, water blue)

Aniline blue		1 gm
Alcohol 90% or water	—	100 cc

For better results stain or alcohol should be slightly acidified with hydrochloric acid.

2. Crystal violet (Gentain violet)

Crystal violet	—	1 gm
Distilled water	—	100 cc

3. Erythrosine
The following are two recipes :

(a)	Erythrosine	—	1 gm
	Alcohol 90%	—	100 cc
(b)	Erythrosine	—	1 gm
	Absolute Alcohol	—	5 cc
	Clove oil	—	95 cc

4. Fast green
The following are two recipes :

(a)	Fast green	—	0.5 gm
	Alcohol 90%	—	100 cc
(b)	Fast green	—	0.5 gm
	Absolute alcohol	—	25 cc
	Clove oil	—	75 cc

5. Gram's iodine

Iodine	—	2 gm
Potassium iodide (KI)	—	3 cc
Distilled water	—	300 cc

6. Hematoxylin
It is a chromogen derived from logwood *Haematoxylon campechianum* of Leguminosac. Two types of hematoxylins are commonly employed (a) Heidenhain's and (b) Delafied's hematoxylin.

(a) Heidenhain's hematoxylin.
Half per cent solution of the stain is prepared in warm and distilled water. It is then stored in dark in a closed bottle to ripen for at least four days before use.

(b) Delafield's hematoxylin.
  (i) A saturated aqueous solution (100 cc) of ferric ammonium sulphate is prepared.
  (ii) One gram of stain is dissolved in 6 cc of absolute alcohol.
  (iii) Mixture of solutions 1 and 2 is prepared. Add to this solution 25 cc of glycerine and 25 cc of absolute alcohol are added to this mixture.
  (iv) The solution thus prepared is allowed to remain for sufficient time till the colour becomes dark red.

7. **Safranin**

(a) Safranin	—	1 gm
Alcohol 90%	—	50 cc
Distilled water	—	50 cc
(b) Safranin	—	1 gm
Distilled water	—	100 cc

3. Mounting Media

1. **Glycerine jelly**
Soak some gelatin for 2 to 3 hours in cold water, pour off the excess water and heat until melted. To 1 part of this, add 1 1/2 parts of glycerine and filter while still hot. Add 2 or 3 percent phenol. Still keeping the mixture hot and fluid, add drop by drop a saturated solution of methyl green in 50 percent alcohol, until the glycerine becomes fully as dark as green ink.

2. **Lactophenol**
Mix equal parts of phenol crystals, lactic acid glycerine and distilled water. Cotton blue may be mixed to stain fungi.

4. Laboratory Reagents

Acetone. (a) 80% Acetone AR 800 ml; distilled water to make 1000 ml
(b) 80% Acetone 800 ml; distilled water to make 1000 ml.

Acids

	Mol. wt.	Gm/ litre	Molarity	ml required for 100 ml. N. som.
HCL	36·47	445	12·2	82·0
HNO_3	63·02	989	15·7	63·8
H_2SO_4	98·08	1742	17·8	28·2

Agar-Agar. Bring 1000 ml distilled water to boil, add 30 gm of agar with constant stirring. Pour uniformly in petri dishes after agar has dissolved completely. If desire to store for more than twelve hours, autoclave the containers with agar-agar.

Barium chloride. 20 gm of Barium chloride dissolved in 100 ml water.

Barium hydroxide. N/10.
$Ba(OH)_2$ 12.15 gm.
Boiled and distilled water 500 ml.

Benedict's solution. (a)Dissolve 173 gm of sodium citrate and 100 gm of sodium carbonate (Na_2CO_3) in about 600 ml of distilled water. Warm the solution. Filter if necessary.

(b) Dissolve 17.3 gms of cupric sulphate in about 150 ml of distilled water. Add the latter solution to the former slowly and with constant stirring. Dilute to one litre.

Benzidine solution. Dissolve 4 gm of benzidine (p-diaminodiphenyl) in 100 ml of glacial acetic acid.

Chloral hydrate-iodine. Add about 1 cc iodine to about 10-15 cc of chloral hydrate and shake the mixture.

Cobalt chloride. For 3% : 3 gm of cobalt chloride dissolved in 100 ml of water.

Collodion. A solution of pyroxylin (cellulose nitrates) in ether or acetone, available commercially as reagent.

Copper sulphate. For 1% : 1 gm of $CuSO_4$. $5H_2O$, per 100 ml water. For 10% : 10 gm of $CuSO_4.5H_2O$ per 100 ml of water.

Dichlorophenol indophenol (DCPIP). For 0.1% : 0.1 gm of DCPIP dissolved in 100 ml of water; For 25×10^{-3} M : dissolve 1 gm of DCPIP in 100 ml of water.

Eosin. For 1% : ! gm of eosin dissolved in 100 ml of water.

Fehlings solution. Fehlings A : Dissolve 34.65 gm of $CuSO_4.5H_2O$ in water and make the volume uptp 500 ml.

Fehlings B : Dissolve 173 gm of potassium sodium tartrate and 125 gm of potassium hydroxide in 500 ml of water.

Mix both solutions A and B in equal amount just before use.

Grinding medium.

(a) NaCl (0.25M) add 1000 ml 1.6125 gm or alternatively 250 ml to 3.6531 gm

(b) KH_2PO_4 (0.1M) add 1000 ml water to 13.62 gm or alternatively 100 ml to 1.362 gm

To prepare add (a) 250 ml of NaCl (0.25) to (b) 100 ml of KH_2PO_4 (0.1M)

Gum guiacum. For 2% : 2 gm of gum guiacum dissolved in 100 ml of 95% ethyl alcohol.

Hydrogen peroxide. Dilute 10 ml of 30% H_2O_2 to 100 ml of water.

Iodine. Iodine (I_2) 5 gms, potassium iodide (KI) 10 gm, distilled water to 100 ml. For less intense solution : iodine I gm, potassium iodide 2 gm, distilled water 300 ml.

Millon's reagent. (a) Digest one part by weight of mercury with two parts weight of concentrated nitric acid. Dilute the resulting solution into twice its volume of water or

(b) 10% $HgSO_4$ in 10% sulphuric acid.

Ninhydirn (Triketohydrindine hydrate).

(a) 0.1 gm of ninhydrin dissolved in n-butanol and volume is made upto 100 ml. The reagent is stored in dark bottle and not for more than a week.

(b) To 50 ml of absolute ethyl alcohol add 0.05 gm of ninhydrin, 2 ml of collodine and 0.5 ml of glacial acetic acid.

Phenolphthalein solution. Dissolve I gm of phenolphthalein in 50 ml of 95% ethyl alcohol and then add 50 ml of water.

Phloroglucinol. Dissolve I gm of phenolphthalein in 50 ml of 95% ethyl alcohol and then add 50 ml of water.

Potassium hydroxide. 30 gm of potassium hydroxide per 100 ml of methyl alcohol.

Potassium permanganate. Dissolve I gm of potassium permanganate in 100 ml of water.

Silver nitrate. For 10% : 10 gm of $AgNO_3$ dissolved in 100 ml of water.

Sodium carbonate. For 1 M solution : $Na2CO_3.H_2O$ 124.2 gm, distilled water to 1000 ml.

Sodium hydroxide.

For 0.1N : 4.0 gm dissolved in 1000 ml;

For 20% : 20.0 gm dissolved in 1000 ml.

Sodium hypochlorite. For 1% : 1 gm of sodium hypochlorite dissolved in 100 ml of water.

Solvents (chromatography)

for chlorophylls	
(a) n-butanol	500 ml
glacial acetic acid	100 ml
water	400 ml
(5 : 1: 4)	
(b) petroleum ether	200 ml
acetone	24 ml
(100 : 12)	
(c) benzene	
acetone	85 ml
(85 · 15)	15 mi
for amino acids	
n-butanol	300 ml
glacial acetic acid	100 ml
water	100 ml
(3 : 1 . 1)	
Two dimensional chromatography	
I solvent	
t-butanol	300 ml
glacial acetic acid	100 ml
water	100 ml
(3 : 1 · 1)	
II solvent	
10% acetic acid	10 ml of acetic acid in 1000 ml of water

Sudan III. Add sudan III slowly in 70% alcohol. Warm to dissolve.

Sudan IV. Add sudan IV slowly in 70% alcohol. Warm to dissolve.

Tetrazolium chloride. 0.1 gm of 2, 3, 5 triphenyl tetrazolium chloride per 100 ml of water, keep in brown bottle.

Thin layer plates.

(a) 10 gm cellulose
4 gm silicalgel
80 gm distilled water.

Prepare a homogeneous slurry, spread uniformly, use 1 ml per 19 sq cm or dip slides in slurry for photosynthetic pigments experiment.

(b) Suspend 2 gm of Kiesel gel G or H (E. Merck) in 10 ml of water, spread the suspension over 3 to 5 slides. Allow the slides to set for about 30 minutes at 120°C.