

Based on New Syllabus of C.H.S.E., Odisha

MODERN'S
abc
OF

PRACTICAL BOTANY

ପ୍ରଥମ ଓ ଦ୍ଵିତୀୟ ବର୍ଷ

For +2 1st Year & 2nd Year

BOOKS

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SYLLABUS

+2 PRACTICAL BOTANY

PRACTICAL FOR 1ST YEAR

1. Study of parts of Dissecting & Compound Microscope.
2. Study of typical angiospermic plant

Major Experiments

3. Study of mitosis in onion root tip.
4. Study of meiosis in onion flower bud.
5. Study and description of flowers belonging to the families Malvaceae, Fabaceae, Solanaceae, Brassicaceae, Asteraceae, Liliaceae.
6. Preparation and study of transverse section of dicot and monocot root, stem & leaf.

Minor Experiments

7. Study of cell- Onion scale leaf, Rhoen discolor epidermal peel.
8. Study of cell inclusions : Starch grains & raphides.
9. Qualitative test for catalase activity by leaf disc method.
10. Study of modification of roots, stems & leaves.
11. Study and identification of different types of inflorescences
12. Study of flower and its parts.
13. Study of Parenchyma, Collenchyma, Sclerenchyma, Xylem and Phloem through permanent slides.

Spotting

14. Identifications with reasons of the following specimens / permanent slides of
A. Spirogyra (vegetative filament, scalariform and lateral conjugation) Saccharomyces (cell and budding)
B. Funaria, Dryopteris, Cycas
15. Botanical name, family, habit and parts of the plant used for economic purposes of rice, green gram, ground nut, banana, jute, cotton, Tulsi and neem.
16. Identification with reasons of the following permanent slides :
A. T.S. of a typical angiospermic anther
B. I.S. of ovules : Anotropous / Orthotropous / Campylotropous
C. Monocot and dicot embryo

PRACTICAL EXAMINATION

Full Marks - 15 **Time - 2 Hours**

Major Experiment (One) 7 Marks
(Theory & Procedure = 3, experiments results = 4)

(Sl. No. 3 to 6)

Minor Experiment (One) 3 Marks

(Sl. No. 7 to 13)

Spotting : Three : 3 × 1 (Three minutes each) 3 Marks

(Sl. No. 14 to 16)

Class Record 2 Marks

1. All the above experiments should be conducted by individual students.
2. Questions for major and minor experiments are to be set by drawing lots.

PRACTICAL FOR 2ND YEAR

Major Experiments

1. Study of effect of temperature and chemicals (Ethanol, acetone, formaldehyde) on leaching of pigments in beet root.
2. Study of plant pigments by paper chromatography.
3. Study of the effect of different wavelength of light on photosynthesis by Wilmott's bubbler.
4. Study of effect of dissolved carbon dioxide on photosynthesis by Wilmott's bubbler.
5. Study of transpiration by Ganong's or Farmer's potometer.
6. Study of relation between transpiration and absorption by T/A apparatus.
7. Comparative study of rate of transpiration from upper and lower surface of a dicot leaf.
8. Study of plasmolysis in cells of epidermal peels of Rhoen discolor and to find out the concentration of isotonic condition.

Minor Experiments

9. Quantitative test for the presence of carbohydrates (Glucose, starch, cellulose) / proteins and fats in seeds of rice / wheat / gram or potato tuber.
10. Study of osmosis by potato osmometer.
11. Study of distribution of stomata on upper and lower surface of a dicot and a monocot leaf.
12. Analysis of samples for verification of Mendelian ratio using pea seeds or colour beads.

Spotting

13. Identification with morphological adaptations of the following specimens :
A. Hydrophytes : Hydrilla, Pistia, & Eichhornia
B. Xerophytes : Opuntia & Casuarina
14. Experimental set up showing conditions necessary for germination (air and water)
15. Experimental set up showing types of germination : epigeal / hypogeal.
16. Experimental set up showing phototropism or geotropism.

PRACTICAL EXAMINATION

Full Marks - 15 **Time - 2 Hours**

Major Experiment (One) 7 Marks

(Sl. No. 1 to 8)

Minor Experiment (One) 3 Marks

(Sl. No. 9 to 12)

Spotting : Three : 3 × 1

(Three minutes each) 3 Marks

(Sl. No. 13 to 16)

Class Record 2 Marks

1. All the above experiments should be conducted by individual students.
2. Questions for major and minor experiments are to be set by drawing lots.
3. For each major and minor experiment, candidates have to write the requirements as per their questions which may be verified and signed by the external examiner only.
4. One observation for major experiment may be verified and signed by the external examiner only.

SECOND YEAR

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INTRODUCTION

GENERAL INSTRUCTION FOR THE STUDENTS IN A BOTANY PRACTICAL LABORATORY

Science is the systematic study based on facts, observation, accuracy and analysis. Scientific observations are the products of curiosity and inquisitiveness of one's mind and scientific results are the outcomes of different laboratories. Laboratory is a place where one can observe an object thoroughly, verify a law and can establish its accuracy and can come out with novel scientific products. Practical observation in the laboratory is, therefore, most important in the study of science.

The students of +2 must learn the basic rules and regulations of a science laboratory which will guide them in future to work in advanced sophisticated laboratories. Some of these are given below.

Discipline in a laboratory

A science student is proud of his laboratory. It is actually the place where the young scientists sprout. A science student can verify his thought and idea here. Therefore the following etiquettes must be followed by a student in a laboratory.

- Laboratory must be maintained neat and clean.
- Laboratory equipments must be kept in proper order and laboratory instruments must be kept in working condition.
- One must come to the laboratory with theoretical preparation and with proper work plan.
- One must not enter the laboratory without permission of the teacher/authority.
- One must maintain absolute silence and keep oneself busy with the work present inside the laboratory. One can consult the teacher, if necessary.
- One must try to complete the assigned work within the specified time.
- Laboratory provisions must be handled with care.
- After the work is over, the working space must be left clean and in order.

Work plan for the practical class in a laboratory

- One must come to the practical class with rough practical record, up-to-date maintained practical record and working tool for practical.
- After entering the room, one must submit the practical record of previous class for verification and then occupy the seat.
- One must ensure that the working space, equipment and glasswares are clean and in proper order before starting the practical work.
- One must listen carefully and try to understand the instructions given by the teacher in the practical class. A thorough understanding brings success in the practical class.
- One must work sincerely in a practical class as per the instructions of the teacher.

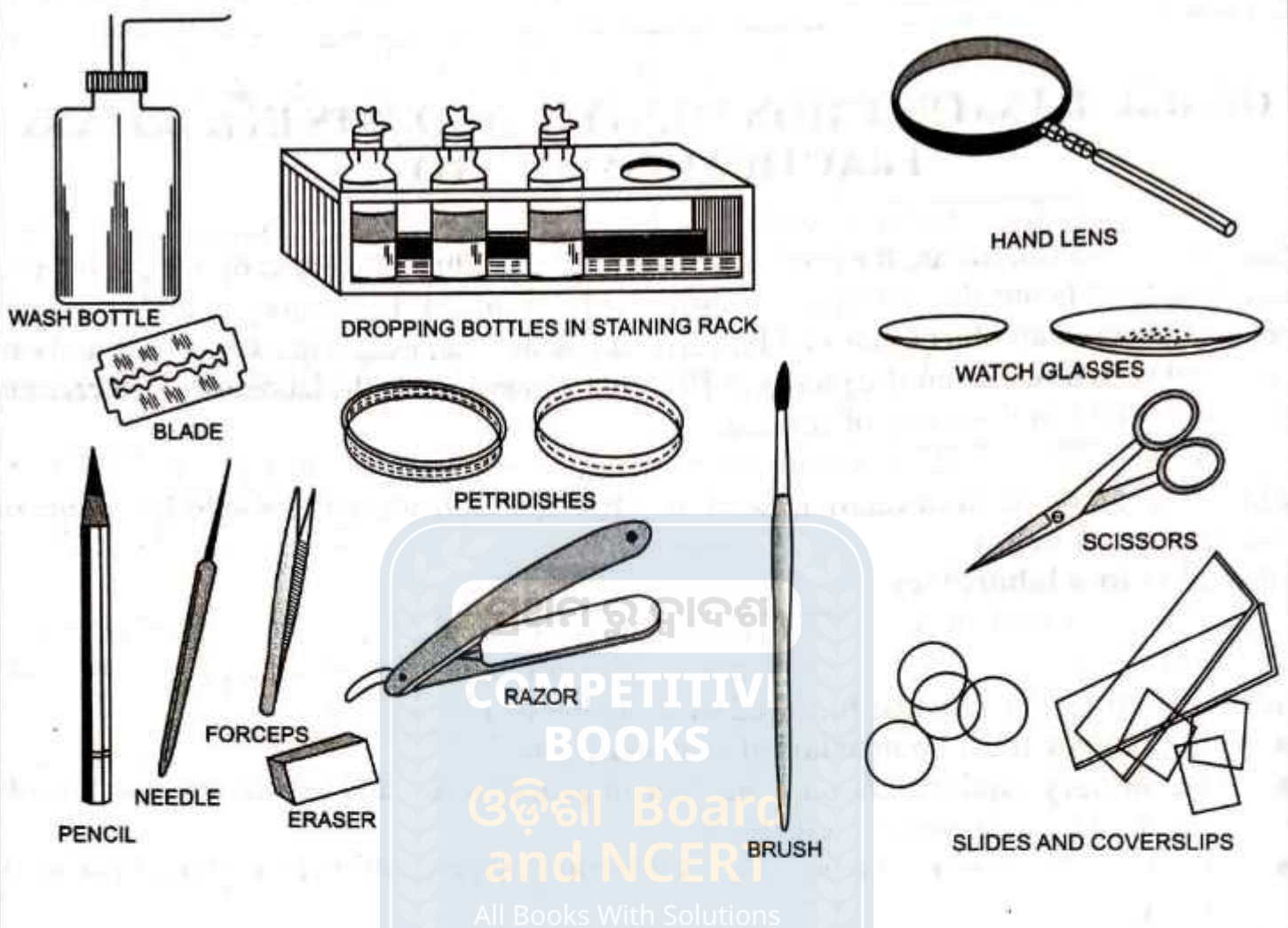


FIG. 1. SOME BASIC LABORATORY PROVISIONS AND INSTRUMENTS.

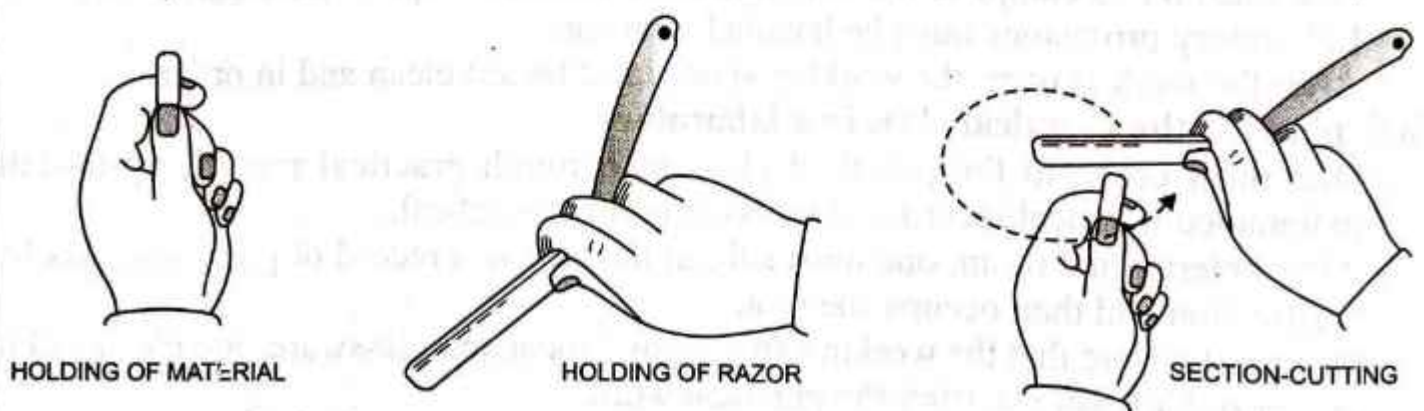


FIG. 2. PROPER HOLDING OF THE MATERIAL AND RAZOR FOR SECTION-CUTTING.

- One must observe the material carefully, prepare the slides, if required, or study the specimen or slide and try to identify them and draw a conclusion.
- Scientific study without conclusion hardly carries any meaning.
- You must draw suitable neat, labelled diagram in practical record / rough practical record (as instructed by the teacher).
- Get your record checked by the teacher in the practical class and make necessary corrections.
- Try to enjoy the practical work in the class. Success in practical work brings maximum pleasure.
- Above all, be regular to a practical class. Once you miss a practical class, you may miss it forever.

Necessary instruments for Botany practical

Each student for a Botany practical class must bring –

- A sharp razor (plano-concave) or a new blade to each class
- A pair of scissors with fine tips
- A pair of forceps
- A pair of needles
- An arrow-head needle
- Two camel hair brushes (No. 1)
- A piece of white muslin cloth / handkerchief
- Pencils of different shades (at least one HB, one B and one 2B)
- Pencil sharpener
- Pencil eraser
- A scale
- Few pieces of blotting paper
- A hand lens
- Rough practical record
- Original Practical record

Basic Laboratory Provisions

Apart from the above instruments required for the practical work in a Botany practical laboratory, certain basic provisions available. These include wash bottle, dropping bottles, staining rack, tray, petridishes, watch glasses, slides, coverslips, etc. Some of the necessary instruments and basic provisions are shown in Fig. 1.

Some Basic Laboratory Techniques

Section cutting : Section of fresh or preserved materials are cut in suitable planes for various anatomical and ecological studies. Razor or blade can be used for the purpose. Proper holding of the material and the razor have been shown in Fig. 2.

Staining : Staining procedure has been described in Chapter 11.

Mounting : Mounting procedure has also been described in Chapter 11.

STUDY OF MICROSCOPE

Expt. No. 1
Date : _____

AIM OF THE EXPERIMENT :

REQUIREMENTS :

PROCEDURE :

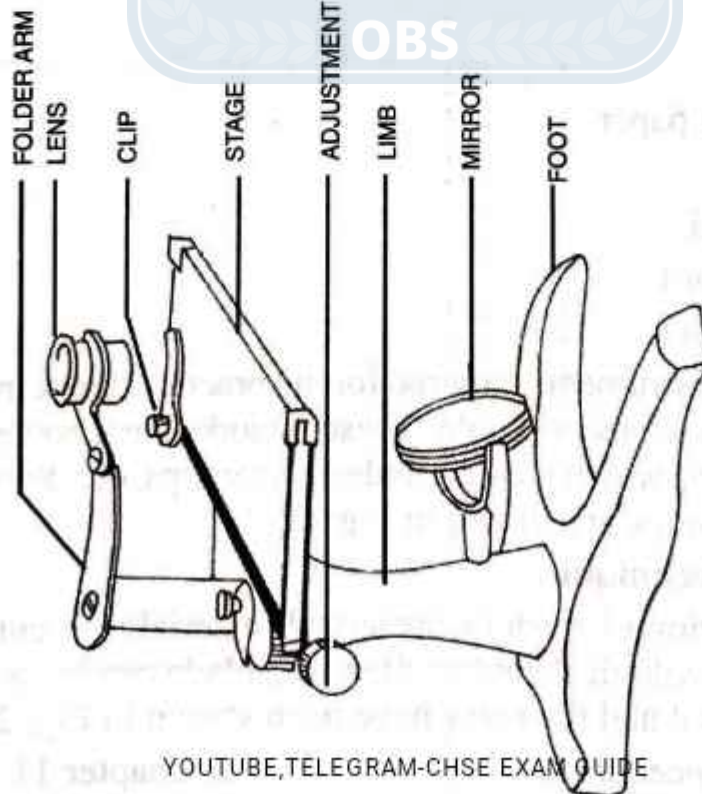
OBSERVATION :

CONCLUSION/IDENTIFICATION :

Signature :

Class : _____ Group _____

Roll No. _____



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OBS

Maintaining Practical Record

After the preparations are ready or practical work is over, those should be carefully observed, salient features / characters should be noted down and labelled diagrams be drawn in the practical record. Practical record is the important record keeping document of a science student. The following instructions must be followed for maintaining a practical record. One must remember that marks are awarded in practical examination for neatness and regularity in maintaining the practical record. One is not allowed to sit in the practical examination without practical record.

- Good quality practical record should be used.
- The record should be well-covered with Name, Roll No. and Class figuring on the cover.
- Record must be maintained neat and clean.
- Experiment No. and date of each experiment should be written as shown on the top and signature and Roll No at the bottom of each experiment (Fig. 3).
- Diagrams must be drawn on the plain sheet in left-hand side and descriptions be written on the ruled sheet in right-hand side facing to diagrams.
- Diagram of different aspects of one specimen should be drawn on the same page. The diagrams of different specimens should not be drawn on one page.
- Pencils of different shades (HB, B, 2B etc.) should be used for light to dark shading and for clarity of the diagram.
- For anatomical studies, outline diagram followed by sector diagram should be drawn.
- All parts of a diagram must be labelled. Labelling should be done on the right side of the diagram in capital letters.
- Labelling lines should not cross each other and should be parallel to each other.
- Each diagram must have caption or title at the bottom.
- Diagram should be drawn by observing the specimen and not from the book.
- Diagram should be scientific and should not be artistic.
- Description must be made in systematic manner as shown.
- Description should be brief and specific.
- Important characters should be underlined.
- Identification or conclusion must be written at the end.
- A new experiment should always be started in a new page.
- Above all, care should always be taken to maintain neatness of the record.
- Record should be regularly corrected and signed by the teacher.
- One can impress the examiner through neat, clean and well-maintained practical record.

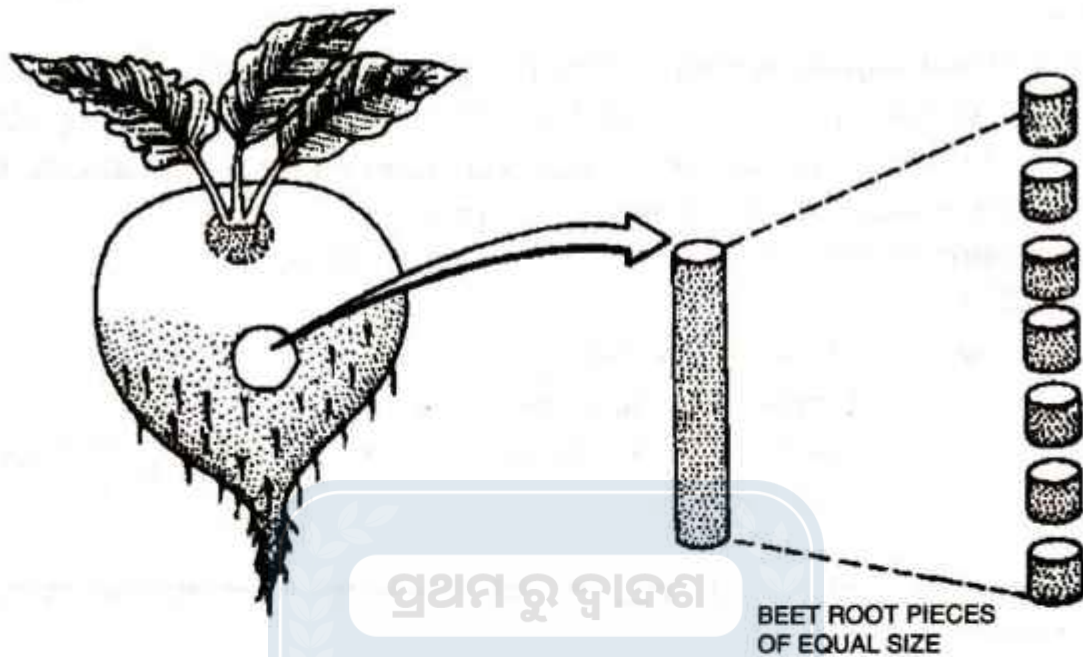


FIG 1.1. METHOD OF TAKING OUT BEET ROOT PIECES.

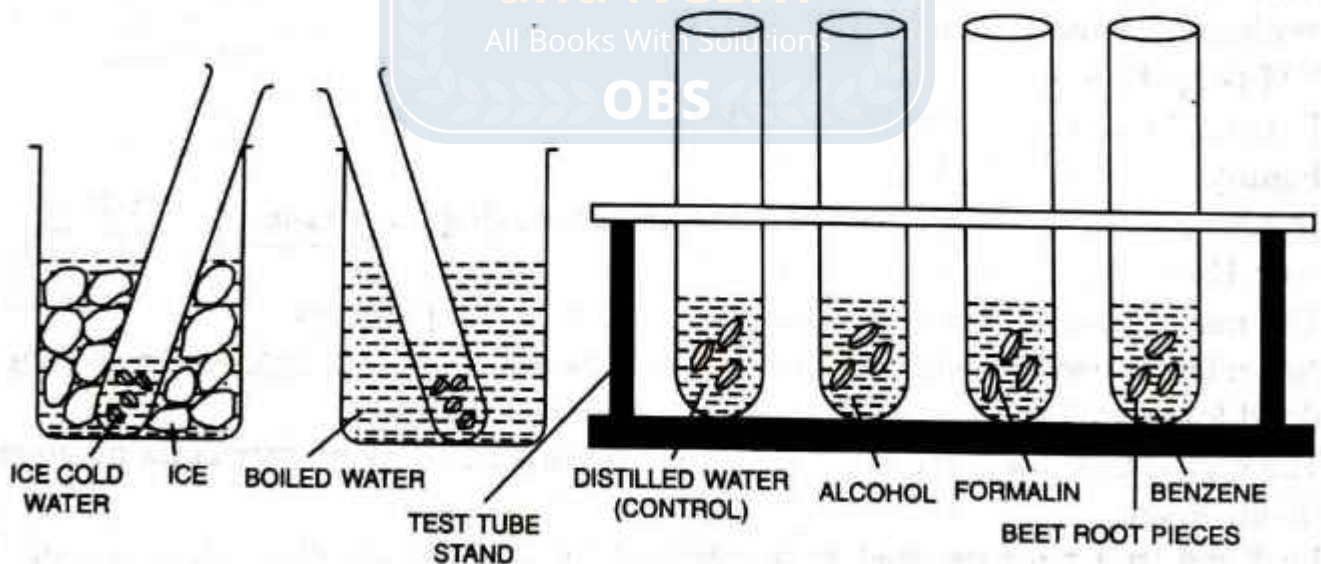


FIG 1.2. EFFECT OF DIFFERENT FACTORS ON THE PERMEABILITY OF CELL MEMBRANE.

Second Year

MAJOR EXPERIMENTS

CHAPTER

1

STUDY OF PLANT PIGMENTS

LEACHING OF PIGMENTS

Experiment No. 1

Date

AIM OF THE EXPERIMENT

To study the effect of temperature and chemicals (ethanol, acetone, formaldehyde) on leaching of pigments in beet root.

REQUIREMENTS

Fresh beet root, cork borer, knife, spirit lamp, test tubes, ice, pipettes, beakers, test tube stand, distilled water.

Chemicals – Ethanol, acetone, formaldehyde.

THEORY

Leaching of the pigments depends upon membrane permeability. The membrane is the thin elastic partition which separates the protoplast or its components from the surrounding. All living cells possess membrane outside the cytoplasm called plasma membrane and around the vacuole called tonoplast. Plasma membrane and tonoplast are selectively or differentially permeable as they allow selective passage of solutes and solvents through them. The cell vacuoles of certain plants contain water soluble pigments like anthocyanin. The tonoplast surrounding the vacuole does not allow the pigments to flow out. However, factors like temperature and various chemicals damage or affect the membrane permeability and thus results in leaching of the pigments.

PROCEDURE

- (i) Fresh beet root is taken and is washed well in water.
- (ii) Cylindrical pieces of this root are taken out with the help of a cork borer and are cut into equal sized slices of about 5 mm in thickness.
- (iii) These pieces are washed thoroughly to remove every trace of purple pigment (anthocyanin) coming out of the cut surfaces.
- (iv) Six test tubes are taken and marked from 1 to 6.

- (v) 10 ml. each of distilled water, ice cold water, boiled water, ethanol, formaldehyde and acetone is poured to test tube No. 1 to 6 separately.
- (vi) 2 or 3 slices of beet root is put into each test tube (The number of slices is maintained constant in each test tube).
- (vii) The test tube containing ice cold water is placed in a beaker containing ice water and the test tube containing boiled water is placed in a beaker containing boiled water so as to maintain the temperature of the test tube. The rest of the test tubes are placed in the test tube stand.
- (viii) The slices of the beet root kept in distilled water is considered as control.
- (ix) The experimental set up is allowed to continue for 15 minutes. The test tubes are taken out and shaken well. The intensity of the colour if any is noted down due to the leaching of anthocyanin pigments from the slices. The observations are recorded in the observation table.

OBSERVATION AND RESULT

Serial No. of Test tubes	Liquid in the test tubes	Change in the colour of the liquid	Result
1	Distilled water (control)	No change	No leaching
2	Ice cold water	No change	No leaching
3	Boiled water	Light Purple	Mild Leaching
4	Ethanol	Dark purple	Maximum Leaching
5	Formaldehyde	Dark purple	Maximum Leaching
6	Acetone	Purple	Medium Leaching

CONCLUSION

The observation table shows that beet root slices placed in distilled water and ice cold water do not show leakage of the pigments. This indicates that low temperature or normal temperature does not affect the membrane permeability. On the contrary, the slices kept in boiled water show leakage indicating the increase in permeability leading to leaching of pigments.

There is also leaching of pigments from the slices kept in different chemicals. The degree of leaching due to various chemicals can be compared looking at the intensity of the colour. Maximum leaching is marked from the slices kept in ethanol and formaldehyde indicating higher permeability of the membrane due to damage of the membrane.

(This experiment can also be performed by taking other coloured plant tissues such as peels of *Tradescantia* leaf.)

SEPARATION OF PIGMENTS

Experiment No. 2

Date

AIM OF THE EXPERIMENT

To separate and study the plant pigments by paper chromatography.

REQUIREMENTS

Fresh Spinach leaves, chromatographic paper (Whatman No. 1), a wide-long test tube, a split cork, mortar and pestle, funnel, beaker, filter paper, capillary tube/micropipette, etc.

Chemicals – Petroleum ether, acetone.

THEORY

Chromatography (Gk – *Chroma* – colour, *graphe* – writing) is a technique used to separate different components/substances present together in a solution based on their mobility on a column of absorbent material (stationary phase), while being carried by another phase called mobile phase. Such a preparation is called chromatogram. In paper chromatography, the mixture containing different compounds is spotted onto a chromatographic paper (Whatman No. 1) and dried. Then a solvent is allowed to flow along the paper sheet by capillary action. As the solvent flows through the paper, it carries the different compounds to different distances and spreads them at different length on the paper. The positions of the different compounds are noted after the paper is dried either directly or by suitable staining.

Different plant pigments (photosynthetic) can be separated by using the technique of paper chromatography.

PROCEDURE

- (i) A few Spinach leaves are taken in about 5 ml of acetone in a mortar and pestle and are grinded thoroughly. It is filtered to get the acetone extract of the leaf pigments.
- (ii) A narrow strip of chromatographic paper (Whatman No. 1) is taken and is cut at one end into a narrow notch as shown in the figure.
- (iii) A line is drawn near the notch with pencil as shown in the figure and a drop of the pigment extract is put exactly in the middle of the strip with the help of a capillary tube/micropipette.
- (iv) The drop is allowed to dry and the process is repeated till four or five drops are placed on the same spot on the paper.
- (v) A wide long test tube is taken and to it 5 ml of petroleum ether-acetone solvent (9 : 1) is poured.

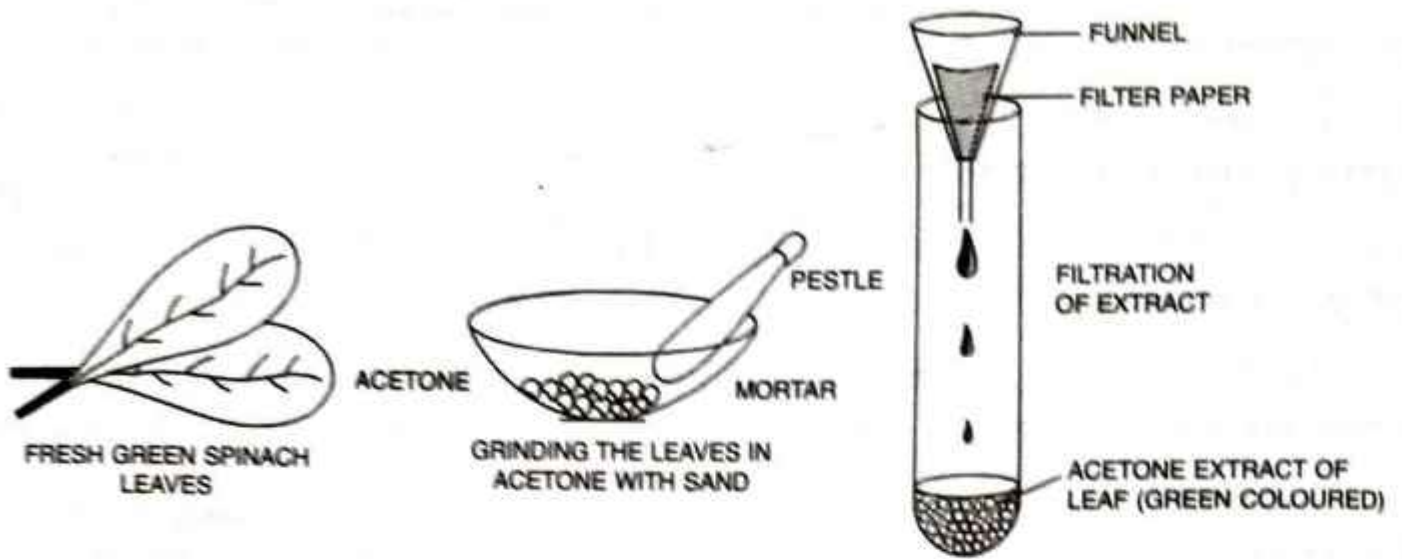


FIG 1.3. PREPARATION OF ACETONE EXTRACT OF LEAF PIGMENTS.

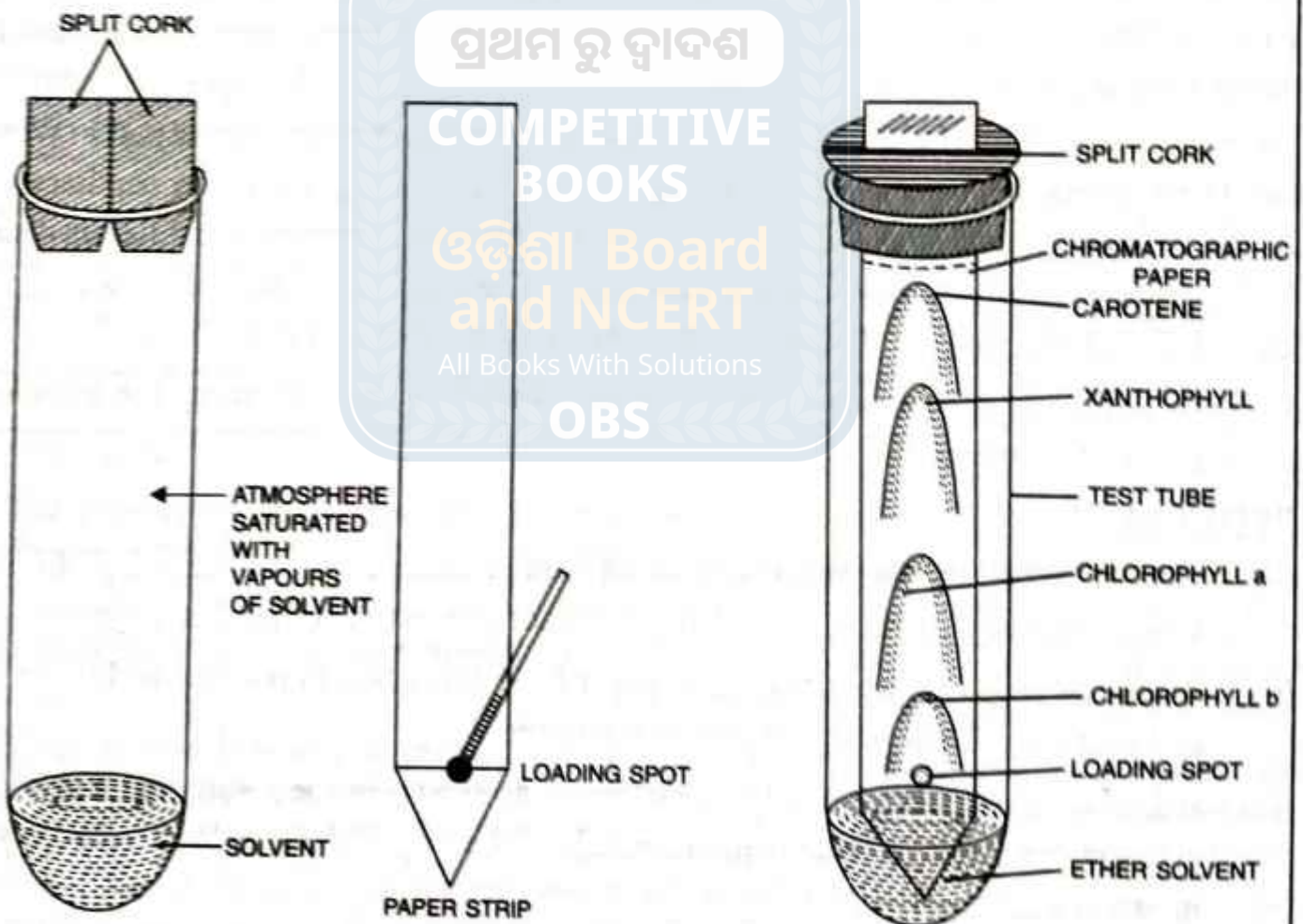


FIG 1.4. SEPARATION OF PIGMENTS BY PAPER CHROMATOGRAPHY.

- (vi) The pigment extract loaded chromatographic paper is now hung in the test tube with the help of the split cork. It is done in such a way that the loading spot lies about 1 cm above the solvent level.
- (vii) The cork is made air-tight and the test tube is placed undisturbed for some time. When the solvent rises about $\frac{3}{4}$ th of the strip, the paper strip is taken out carefully and is allowed to dry.

OBSERVATION AND RESULT

No. of Observation	Colour of the spot	Pigments
1	Orange yellow	Carotene
2	Yellow	Xanthophyll
3	Dark green	Chlorophyll a
4	Yellowish green	Chlorophyll b

CONCLUSION

The different plant pigments are thus separated by paper chromatography. The orange yellow band corresponds to carotene, yellowish band to xanthophyll, dark green band to chlorophyll a and yellowish green band to chlorophyll b.

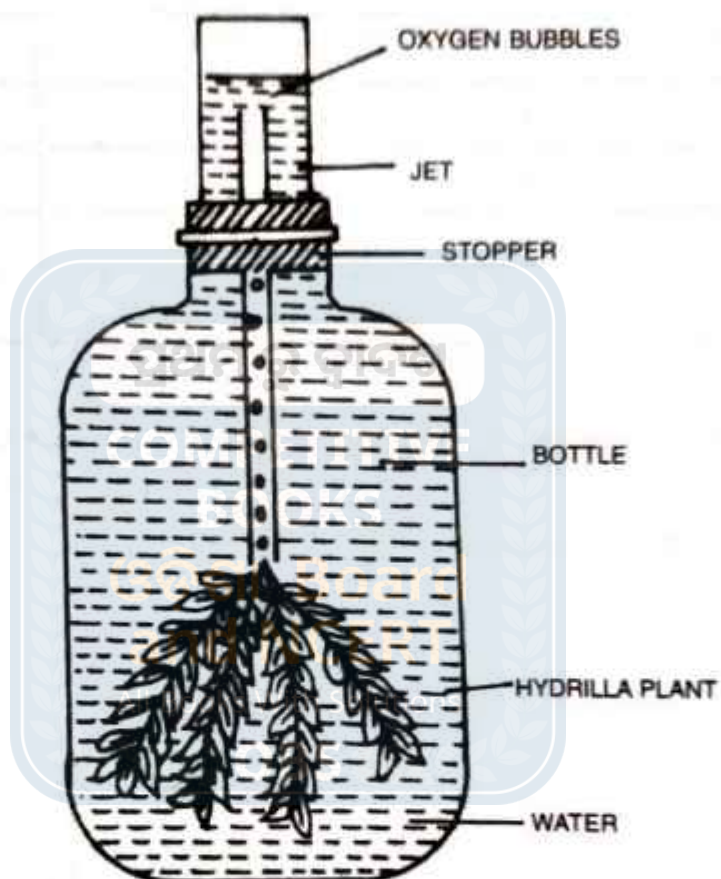


FIG. 2.1. STUDY OF PHOTOSYNTHESIS BY WILMOTT'S BUBBLER.

STUDY OF PHOTOSYNTHESIS

Experiment No. 3

Date

AIM OF THE EXPERIMENT

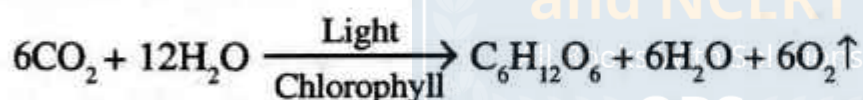
To study the effect of different wavelength of light on photosynthesis by Wilmott's bubbler.

REQUIREMENTS

Wilmott's bubbler, Healthy *Hydrilla* twig, Sodium bicarbonate (NaHCO_3), Vaseline, Bell jar and cellophane papers of different colour (blue, yellow and red).

THEORY

Photosynthesis is a process by which chlorophyll containing green plants manufacture carbohydrates from CO_2 and water absorbed from atmosphere and soil respectively in presence of light.



Photosynthesis is affected by a number of factors, such as light intensity, light quality, wind, temperature, carbon dioxide concentration, etc. The effect of these factors can be studied either in terms of carbon dioxide utilisation or oxygen evolution. The easiest way to demonstrate and measure the rate of photosynthesis is to measure the evolution of oxygen by an aquatic plant *Hydrilla* using Wilmott's bubbler. In the present set-up the number of bubbles coming out per unit time (oxygen evolution) is being calculated to study the rate of photosynthesis. Different coloured light have different wavelengths. The effect of different wavelength of light on photosynthesis can be studied by using difference coloured light.

Description of the apparatus (Fig. 2.1)

Wilmott's bubbler is an apparatus used to demonstrate and measure oxygen that is evolved during photosynthesis from the jet of the apparatus in form of uniform bubbles. It consists of a wide mouth bottle or flask fitted with a Wilmott's apparatus by the help of a cork. The apparatus is having a wide glass tube which acts as water reservoir and inside this there is a narrow glass tube with lower end fitted with a cork and upper end is bent ending in a jet.

PROCEDURE

- (i) Few fresh healthy *Hydrilla* twigs are taken, the ends of which are cut obliquely and inserted through the cork into the lower end of Wilmott's bubbler.
- (ii) The total apparatus is filled with water.
- (iii) The twig is kept inside the bottle /flask and the cork is fitted to the mouth of the bottle/ flask.
- (iv) The apparatus is made perfectly airtight by applying vaseline, if needed.
- (v) The fitted apparatus is kept under sunlight and is allowed to stand for 10-15 minutes.
- (vi) The bubble coming out of the jet is marked and the number of bubbles per unit time is counted.
- (vii) In order to increase the rate, about 25 mg of NaHCO_3 is added into the flask and mixed thoroughly with water.
- (viii) To study the effect of different wavelength of light on photosynthesis, the bubbler is kept under sunlight covered with coloured belljar (blue, yellow and red) time to time or bell jar wrapped with coloured cellophane paper. The time period allowed under a particular colour (light quality) is maintained constant. The number of bubbles per unit time is counted and tabulated.

OBSERVATION AND CALCULATION

Small gas bubbles are seen to accumulate on the top of the bent jet of the bubbler and come out of the jet in form of large bubble. The number of bubbles coming out per unit time is tabulated from which the rate of photosynthesis could be known.

Effect of light quality on photosynthesis

No. of Observation	Colour of light	No. of bubbles evolved				No. of bubbles/ unit time
		1st 5 min	2nd 5 min	3rd 5 min	Mean No. of bubbles	
1	Normal sunlight (control)	42	45	48	45	9
2	Blue	19	20	21	20	4
3	Yellow	9	10	11	10	2
4	Red	28	30	32	30	6

CONCLUSION

Photosynthesis occurred in the *Hydrilla* twig and oxygen is liberated in the form of bubbles. The rate of photosynthesis is seen to be maximum under normal sun light, then in red light followed by blue and minimum under yellow light. As red light has longer wavelength (620–680 nm) and blue light has shorter wavelength (430–470 nm), both these wavelengths are found to be effective to drive photosynthesis. Rate is maximum in normal sun light as it contains lights of both the wavelengths.

Experiment No. 4

Date

AIM OF THE EXPERIMENT

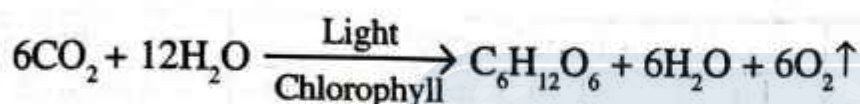
To study the effect of dissolved CO_2 concentration on photosynthesis by Wilmott's bubbler.

REQUIREMENTS

Wilmott's bubbler, Healthy *Hydrilla* twig, Sodium bicarbonate (NaHCO_3) and Vaseline.

THEORY

Photosynthesis is a process by which chlorophyll containing green plants manufacture carbohydrates from CO_2 and water absorbed from atmosphere and soil respectively in presence of light.



Photosynthesis is affected by a number of factors, such as light intensity, light quality, wind, temperature, carbon dioxide concentration, etc. The effect of these factors can be studied either in terms of carbon dioxide utilisation or oxygen evolution. The easiest way to demonstrate and measure rate of photosynthesis is to measure the evolution of oxygen by an aquatic plant *Hydrilla* using Wilmott's bubbler. In the present set-up the number of bubbles coming out per unit time (oxygen evolution) is being calculated to study the rate of photosynthesis.

Description of the apparatus (Fig. 2.1)

Wilmott's bubbler is an apparatus used to demonstrate and measure oxygen that is evolved during photosynthesis from the jet of the apparatus in form of uniform bubbles. It consists of a wide mouth bottle or flask fitted with a Wilmott's apparatus by the help of a cork. The apparatus is having a wide glass tube which acts as water reservoir and inside this there is a narrow glass tube with lower end fitted with a cork and upper end is bent ending in a jet.

PROCEDURE

- (i) Few fresh healthy *Hydrilla* twigs are taken, the ends of which are cut obliquely and inserted through the cork into the lower end of Wilmott's bubbler.
- (ii) The total apparatus is filled with water.
- (iii) The twig is kept inside the bottle /flask and the cork is fitted to the mouth of the bottle/ flask.
- (iv) The apparatus is made perfectly airtight by applying vaseline, if needed.
- (v) The fitted apparatus is kept under sunlight and is allowed to stand for 10-15 minutes.
- (vi) The bubble coming out of the jet is marked and the number of bubbles per unit time is counted.

- (vii) 0.1 gm of NaHCO_3 is added into the flask, mixed thoroughly with water, the apparatus is then kept for 10-15 minutes under sunlight and the number of bubbles coming out of the jet per unit time is counted.
- (viii) Water was removed from the flask, flask was washed and fresh water was taken in it. 0.2 gm of NaHCO_3 is added and number of bubbles per unit time was counted after keeping it for 10-15 minutes under sunlight.
- (ix) The process was repeated with 0.3 gm, 0.4 gm and 0.5 gm of NaHCO_3 .

OBSERVATION AND CALCULATION

Small gas bubbles are seen to accumulate on the top of the bent jet of the bubbler and come out of the jet in form of large bubble. The number of bubbles coming out per unit time is tabulated from which the rate of photosynthesis could be known.

Effect of carbon dioxide concentration

No. of Observation	Amount of NaHCO_3 added (CO_2 conc.)	No. of bubbles evolved				No. of bubbles/unit time
		1st 5 min	2nd 5 min	3rd 5 min	Mean No. of bubbles	
1	Normal condition (control) without NaHCO_3	18	20	22	20	4
2	0.1 gm NaHCO_3	25	24	26	25	5
3	0.2 gm NaHCO_3	28	32	30	30	6
4	0.3 gm NaHCO_3	38	40	42	40	8
5	0.4 gm NaHCO_3	31	30	29	30	6
6	0.5 gm NaHCO_3	27	23	25	25	5

CONCLUSION

Photosynthesis occurs in the *Hydrilla* twig and oxygen is liberated which is released in the form of bubbles. With the addition of sodium bicarbonate, more of CO_2 is utilised and so the rate of photosynthesis is increased that is reflected by increase in number of bubbles per unit time.

The rate of photosynthesis increased with increase in CO_2 concentration (increased conc. of NaHCO_3). However, higher conc. of CO_2 decreased the rate of photosynthesis due to its toxic effect.

STUDY OF TRANSPIRATION

Experiment No. 5

Date

AIM OF THE EXPERIMENT

To study the rate of transpiration by Ganong's potometer.

REQUIREMENTS

Ganong's potometer, beaker, grease/vaseline, freshly cut twig, stopwatch.

THEORY

Transpiration is a physiological process by which water is lost from the aerial parts of the plant in the form of water vapour. Loss of water from the leaf surface causes water potential to become more negative in these cells and so these cells draw water from the neighbouring cells and the process continues. A suction pressure is developed in the xylem of the leaf called transpiration pull which passes downward. Such pressure pulls the water from the reservoir. This principle is applied for measuring rate of transpiration in a potometer.

Description of apparatus (Fig. 3.1).

Ganong's potometer is made of twice bent glass tube one upward and the other downward. The upward bent ends in a wide mouth provided with a cork with a central hole in it, to which the cut twig is inserted. The downward bent end of the tube is provided with a small lateral pore, which is usually kept dipped in a water container. Towards the upward bent end of the horizontal tube there is a reservoir with a stopcock whose extension is mounted on a stand. The horizontal tube is graduated at 1 mm interval.

PROCEDURE

- (i) An obliquely cut fresh twig with sufficient number of leaves is taken.
- (ii) The cut end of the twig is inserted tightly into the hole of the rubber cork of wide mouth tube.

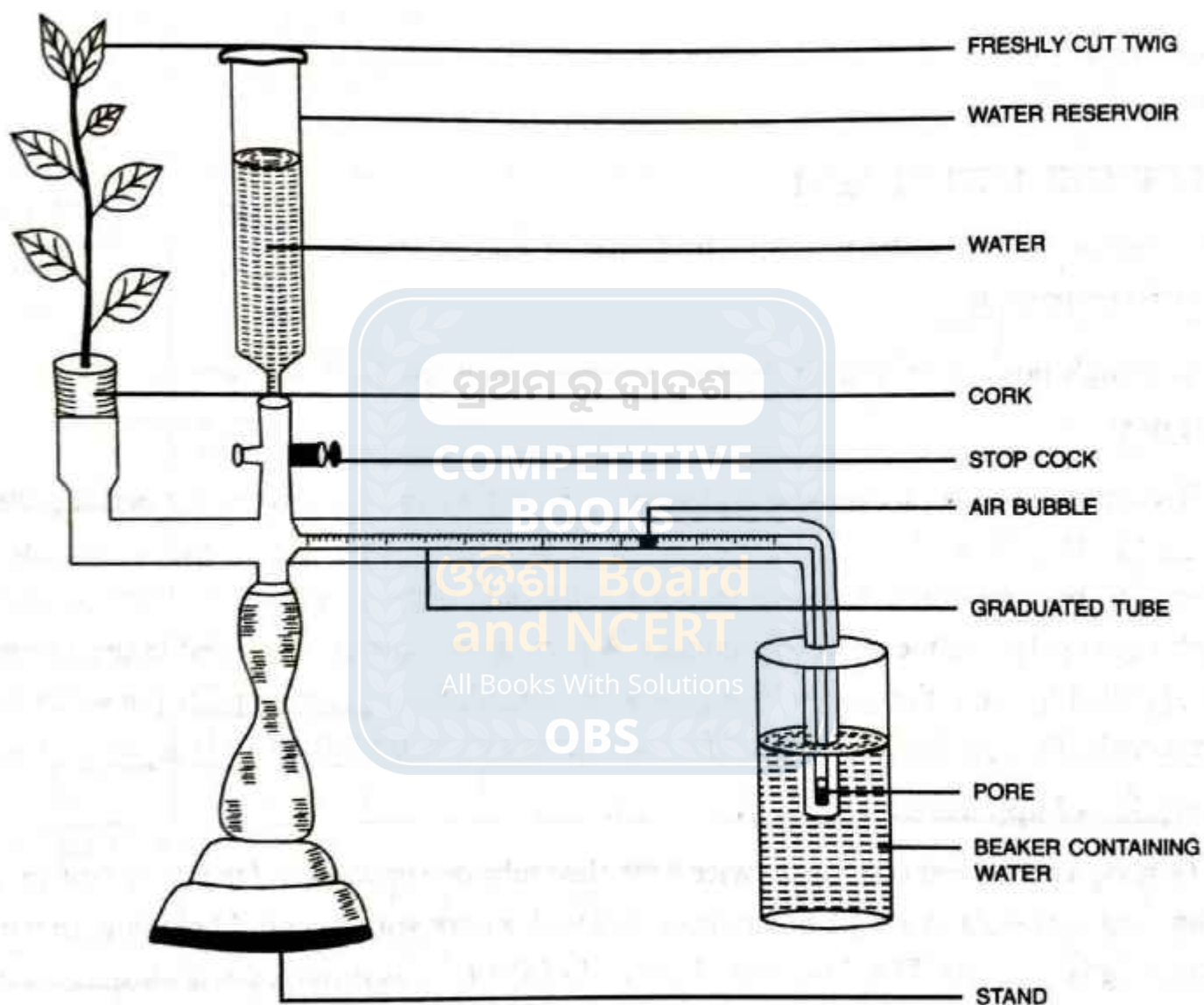


FIG. 3.1. STUDY OF RATE OF TRANSPIRATION BY GANONG'S POTOMETER.

- (iii) The whole apparatus is filled with water through the reservoir by closing the side hole of the bent tube with finger.
- (iv) The cork with the twig is promptly fitted to the wide mouth in such a way that the apparatus is completely airtight. Otherwise it is made airtight by applying vaseline.
- (v) A bubble is inserted through the hole present in the downward bent end by tapping carefully.
- (vi) Then immediately the downward bent end of the horizontal tube is put in a beaker containing water so as to dip the tube in water.
- (vii) The fully fitted apparatus is kept under sunlight.

OBSERVATION AND CALCULATION

The air bubble is found to move in the horizontal tube towards the end bearing the twig. The time is noted by stopwatch and the distance travelled by the bubble per unit time is calculated to know the rate of transpiration. The stopcock is opened gently and the bubble is pushed back certain distance. In order to measure the rate of transpiration, at least the process is repeated thrice and the rate of movement of the bubble is noted in a table. The mean value is calculated and from that the rate of transpiration is known.

No. of Observation	Time period in minutes	Initial level of air bubble in cm.	Final level of air bubble in cm.	Difference in cm.	Mean Difference in cm	Distance Moved/ Unit time in cm (Rate of transpiration)
1	2 min	18	14	4	4.66	2.33
2	2 min	14	10	4		
3	2 min	10	4	6		

CONCLUSION

There is loss of water due to transpiration from the cut twig and the transpiration pull so generated pulled the bubble towards the twig. This experiment has been designed to study and measure the rate of transpiration by Ganong's potometer.

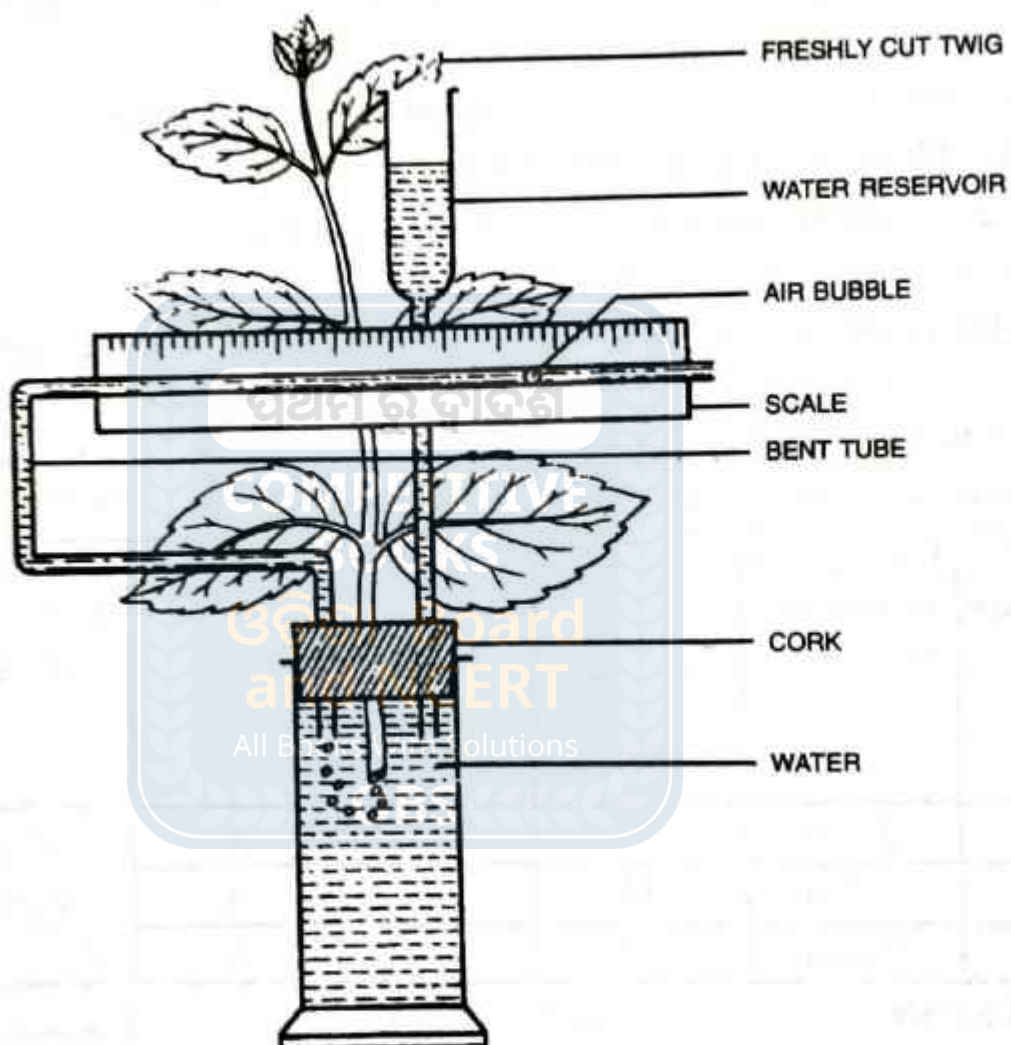


FIG. 3.2. STUDY OF RATE OF TRANSPIRATION BY FARMER'S POTOMETER.

Experiment No. 6

Date

AIM OF THE EXPERIMENT

To study the rate of transpiration by Farmer's potometer.

REQUIREMENTS

Farmer's potometer, cut twig, beaker, vaseline, stopwatch.

THEORY

Same as earlier experiment.

Description of the apparatus (Fig. 3.2)

Farmer's potometer consists of a wide mouth glass bottle tightly fitted with a rubber cork having three holes. To one of the holes a narrow and bent tube is tightly fitted, the horizontal arm of which is attached with a scale. To the second hole, a water reservoir with a stopcock is fitted tightly. The third one is left for the insertion of the twig.

PROCEDURE

- An obliquely cut fresh twig with sufficient number of leaves is taken and fitted to the third hole of the rubber cork.
- The wide mouth bottle and the reservoir are filled with water.
- The stopcock is opened promptly and the rubber cork is fitted to the mouth of the bottle, such that no bubble is left in the apparatus. The stopcock is closed.
- The apparatus is made airtight.
- The apparatus is kept under sunlight.

OBSERVATION AND CALCULATION

Water column in the horizontal arm is seen to be pulled towards the twig. The distance covered per unit time is noted and tabulated. The stopcock is opened and the column is pushed back gently. In order to measure the rate of transpiration at least the process is repeated thrice. The mean value is calculated which gives the rate of transpiration.

No. of Observation	Time period in minutes	Initial level of air column in cm.	Final level of air column in cm.	Difference in cm.	Mean Difference in cm	Distance Moved/ Unit time in cm (Rate of transpiration)
1	2 min	18	16	2	2.66	1.33
2	2 min	16	13	3		
3	2 min	13	10	3		

CONCLUSION

There was loss of water due to transpiration from the cut twig and the transpiration pull so generated pulled the column towards the twig. This experiment is designed to study and measure the rate of transpiration by Farmer's potometer.

Experiment No. 7**Date****AIM OF THE EXPERIMENT**

To study the effect of different environmental conditions on rate of transpiration.

REQUIREMENTS

Ganong's/Farmer's potometer, cut twing, beaker, vaseline, stop watch.

THEORY

Transpiration is a physiological process by which water is lost from the aerial parts of the plant in form of water vapour. The process is affected by various internal and external (environmental) factors, such as temperature, light intensity, wind velocity, etc.

Description of the apparatus (Fig. 3.1 and 3.2)

Same as earlier experiment.

PROCEDURE

Same as earlier experiment but here the fitted apparatus is kept under different environmental conditions, such as shade, sunlight and high wind velocity (under fan) and three readings are taken for each condition.

OBSERVATION AND CALCULATION

Water column in the horizontal arm is seen to be pulled towards the twig. The distance covered per unit time is noted and tabulated. The stopcock is opened and the column is pushed back gently. In order to measure the rate of transpiration at least the process is repeated thrice. The mean value is calculated which gives the rate of transpiration. Same procedure is repeated for each condition.

Environmental conditions	No. of Observations	Time period in min.	Initial level of air columns in cm	Final level of air columns in cm	Difference in cm	Mean difference in cm	Distance moved/unit time in cm. (rate of transpiration)
Shade	1	2 min	18	17	1	1.33	0.67
	2	2 min	17	15	2		
	3	2 min	15	14	1		
Sun light	1	2 min	18	15	3	2.66	1.33
	2	2 min	15	12	3		
	3	2 min	12	10	2		
Wind	1	2 min	18	15	3	3.66	1.83
	2	2 min	15	11	4		
	3	2 min	11	7	4		

CONCLUSION

Rate of transpiration is maximum under high wind velocity, followed by sunlight and it is minimum under shade conditions.

Experiment No. 8

Date

AIM OF THE EXPERIMENT

To study the relation between transpiration (T) and absorption (A) by T/A apparatus.

REQUIREMENTS

A cut twig or a small rooted plant, T/A apparatus, split cork, water, oil/liquid paraffin spring weighing machine/compression balance.

THEORY

Transpiration is the loss of water in the form of water vapour from the aerial portion of the plants. Transpiration mainly occurs through minute pores called stomata present on the surface of leaf and young shoot. Of the total amount of water absorbed by the plants, about 99% is lost to the environment through transpiration. There exists a relationship between transpiration and absorption which equals to almost unity.

Description of the apparatus (Fig. 3.3)

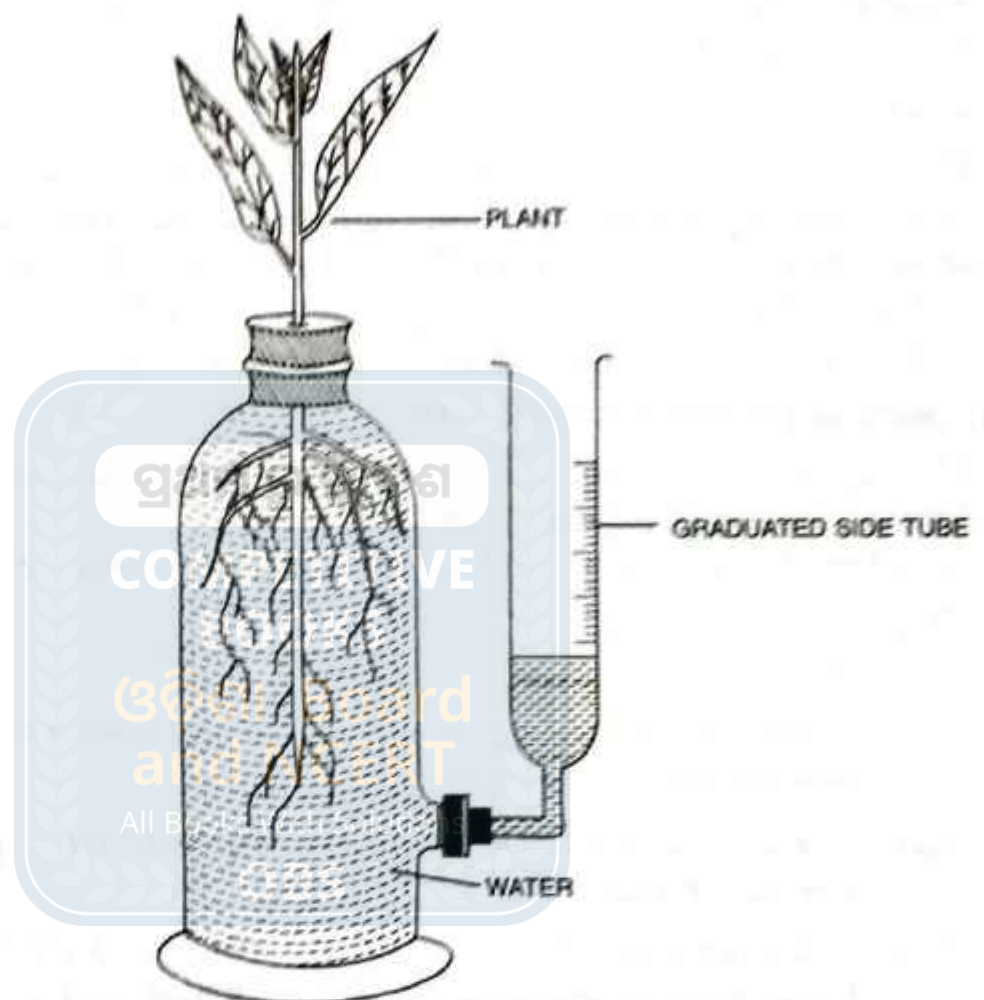
T/A apparatus (Transpiration/Absorption apparatus) is meant for correlating the relationship between transpiration and absorption. It consists of a large wide mouth bottle with a graduated side tube. The side tube is fitted to the bottle through a rubber cork.

PROCEDURE

- (i) The wide mouth bottle is filled with water.
- (ii) A small rooted plant or a twig cut obliquely under water is taken and introduced into the bottle through the split cork.
- (iii) The apparatus is made air-tight, if required by applying grease at the joint and around the cork.
- (iv) The free surface of water in the graduated side tube is covered by a drop of oil or liquid paraffin to prevent direct evaporation from it.
- (v) The water level in the graduated side tube is recorded to note the initial level of water.
- (vi) The whole apparatus is weighed by a compression balance to note the initial weight.
- (vii) The experimental set-up is allowed to stand as such in sunlight for 1 hour. (Transpiration is allowed to continue).
- (viii) After 1 hour the final level of water is marked and final weight of the apparatus is taken.

OBSERVATION AND CALCULATION

Water column in the side tube is seen to be decreased. The initial and final volume of water is marked on the side tube to know the amount of water absorbed. The initial weight and the final weight of the entire apparatus were noted and the difference was calculated to know the amount of water loss by transpiration.



**FIG. 3.3. TRANSPIRATION-ABSORPTION TOWER.
(T/A APPARATUS)**

Study of Relation between Transpiration and Absorption

Initial wt. of the Apparatus W_1	Final wt. of the Apparatus W_2	Initial volume of water in the side tube V_1	Final volume of water in the side tube V_2	Amount of Transpiration 'T' $(W_1 - W_2) = W$ gms.	Amount of Absorption 'A' $(V_1 - V_2) = V$ ml (equivalent to V gms.)
850.5 gm	848.0 gm	0.5 ml	3.2 ml	2.5 gm	2.7 ml $\equiv$ 2.7 gm

$$\text{So } T/A = \frac{W}{V} = \frac{2.5}{2.7} = 0.93$$

The loss of weight is due to transpiration loss of water (T). The decrease in water level is due to the absorption (A) of water by the cut twig or potted plant. Since the open surface of the water in the side tube is covered with oil droplets the entire loss in volume is due to absorption.

The T/A is almost equal to unity or slightly less than it. If it becomes more than one, (that means transpiration is more than absorption), the plant will show wilting symptom and die.

- (i) The apparatus must be completely airtight.
- (ii) The free surface of the water in the side tube must be covered properly by oil or liquid paraffin.
- (iii) If twig is taken, it should be cut obliquely under water.

Date

AIM OF THE EXPERIMENT

Comparative study of the rate of transpiration from upper and lower surface of dicot leaf.

REQUIREMENTS

REQUIREMENTS
A potted dicot plant, filter paper, slides, clips or leaf-clasp, desiccator, stopwatch, punching machine.

THEORY

Transpiration is the loss of water in the form of water vapour from the aerial parts of the plants. Transpiration mainly occurs through minute pores called stomata present on the surfaces of the leaves and young stems. The number of stomata present on the two surfaces of the leaves differ in most of the plants. In most of the dicot plants more number of stomata are present on the lower surface than the upper surface of the leaf. Therefore, the loss of water from the lower surface is more than the upper surface.

The rate of transpiration from the two surfaces of a leaf can be studied by comparing the loss of water vapours from the two surfaces of the leaf.

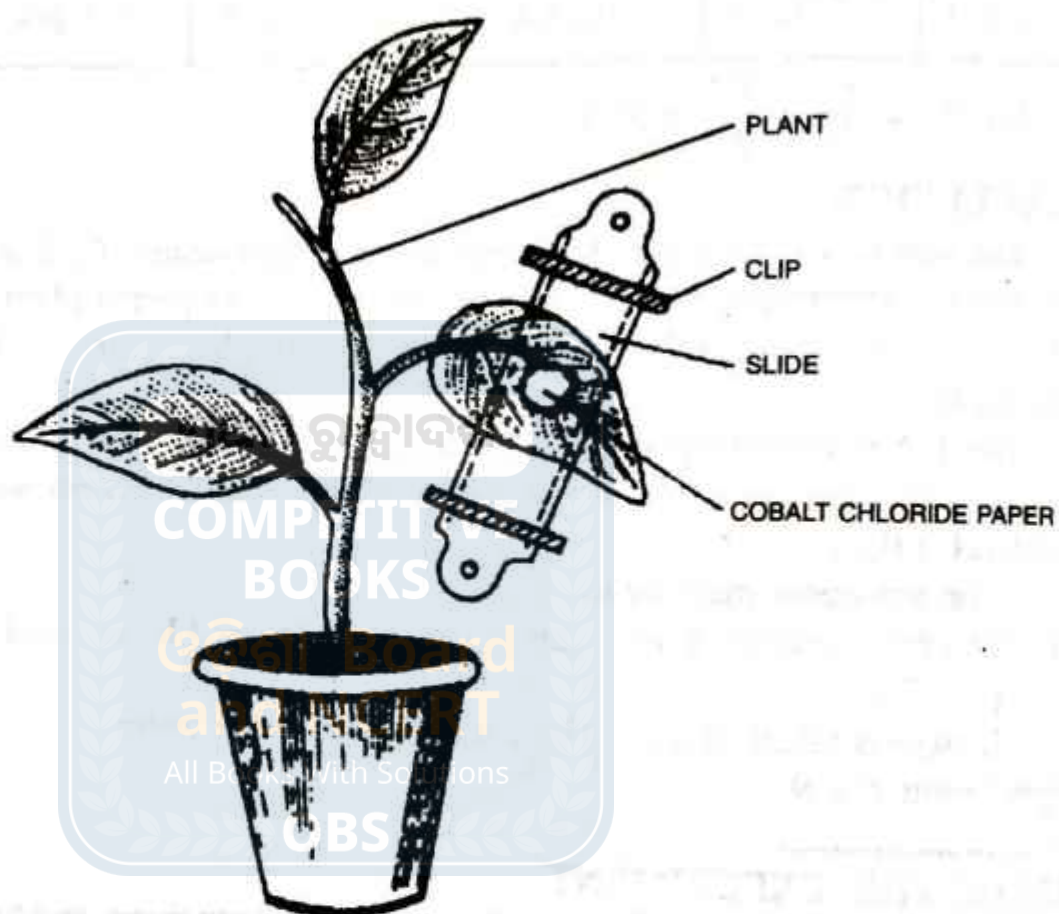


FIG. 3.4. COBALT CHLORIDE PAPER EXPERIMENT TO COMPARE THE RATE OF TRANSPIRATION FROM TWO SURFACES OF THE LEAF.

PROCEDURE

- (i) A healthy potted dicot plant is chosen.
- (ii) 3% cobalt chloride solution is prepared.
- (iii) Cobalt chloride paper is prepared by dipping a filter paper in the prepared cobalt chloride solution.
- (iv) The filter paper is dried and paper discs are cut from it with the help of punching machine.
- (v) These paper discs are stored in a desiccator. The cobalt chloride papers are pink in colour when wet but become blue on drying.
- (vi) The dried discs of cobalt chloride paper are placed one on the upper and one on the lower surface of a leaf of the potted plant. They are pressed close to surface of glass slides and the slides are clipped together as shown in the fig. 3.4. The paper discs can also be fixed by means of leaf-clasp.
- (vii) Such arrangements are repeated at least in 3 to 5 leaves of the potted plant.
- (viii) The potted plant is kept under sunlight and transpiration is allowed to continue.
- (ix) The time taken by the cobalt chloride paper to change from blue to pink colour is noted in each case both on the upper and lower surface of the leaf.

OBSERVATION AND RESULT

The time taken for the change in colour of the cobalt chloride paper from blue to pink is noted and tabulated as follows.

Surface of the leaf	No. of observation	Time taken for change in colour in min.	Mean time in min.
Upper surface	1	3	4
	2	4	
	3	5	
Lower surface	1	2	3
	2	3	
	3	4	

The time taken for this change in colour on the lower surface of the leaf is less than the upper surface.

CONCLUSION

The quick change in the colour of the cobalt chloride paper on the lower surface indicates higher rate of loss of water vapour from this surface than the upper surface. This is due to the presence of more number of stomata per unit area on the lower surface of dicot leaf than the upper surface.

PRECAUTIONS

- (i) Always a well-watered, healthy plant should be chosen for the experiment.
- (ii) The dried cobalt chloride paper should be handled with dry hand or dry forceps.
- (iii) The leaf surface should not be wet while putting the cobalt chloride paper disc.

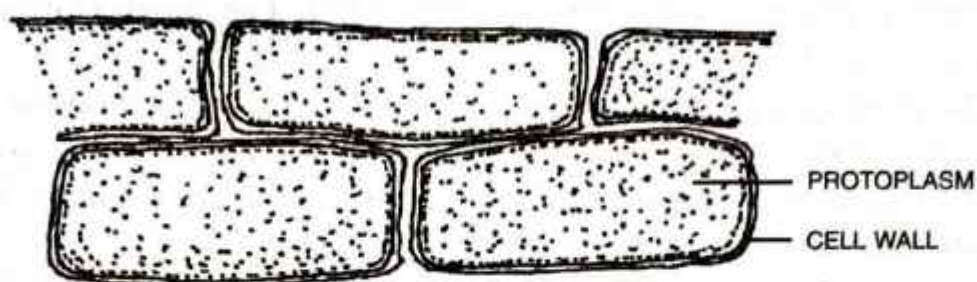


FIG. 4.1. CELLS OF *Rhoeo discolor* IN NORMAL WET MOUNT.



FIG. 4.2. CELLS OF *Rhoeo discolor* IN HYPERTONIC SOLUTION (SHOWING PLASMOLYSIS).

CHAPTER

4

STUDY OF PLASMOLYSIS

Experiment No. 10

Date

AIM OF THE EXPERIMENT

Study of plasmolysis in cells of epidermal cells of *Rhoeo discolor* and to find out the concentration of isotonic condition.

REQUIREMENTS

Leaves of *Rhoeo discolor*, slide, coverslip, needle, forceps, scissors, microscope, NaCl (1 M)/Sucrose (1M) solution.

THEORY

Cell is surrounded by a membrane called cell membrane or plasma membrane which acts as a semipermeable membrane. It controls the in and outflow of water and other materials in solution to the cell. The cell protoplasm has a particular concentration of water. When a cell is placed in a solution having higher concentration than protoplasm (hypertonic solution) the water moves out of the cell due to exosmosis and the protoplasm shrinks. This phenomenon is called plasmolysis. On the other hand, if the cell is placed in a solution having lower concentration than the protoplasm (hypotonic solution), water enters the cell due to endosmosis and the cell becomes turgid.

Thus, plasmolysis can be defined as the shrinkage of the protoplasm due to exosmosis of water when the cell is placed in a hypertonic solution. Percentage of plasmolysis can be studied with different concentration of sucrose/NaCl solutions.

Preparation of sucrose solution

34.2 gm of sucrose is dissolved in 80 ml of water and the volume is made up to 100 ml to prepare 1M sucrose solution. Different molar concentration of sucrose solution 0.1M to 0.5M can be prepared to study percentage of plasmolysis.

Preparation of NaCl solution

5.8 gm of NaCl is dissolved in 80 ml of water and the volume is made up to 100 ml to prepare 1M NaCl solution. Different molar concentration of NaCl from 0.1M to 0.5M can be prepared to study percentage of plasmolysis.

PROCEDURE

- (i) Small piece of epidermal peel of *Rhoeo discolor* leaf is taken and is cut into sizes of 1 sq cm.
- (ii) A piece is taken and a wet mount with drop of water is prepared (as described for onion scale leaf) and is observed under microscope.
- (iii) Another piece is taken and is mounted with drop of 0.5 NaCl or 0.5 M sucrose solution. A clear cell is focussed under high magnification and is observed with an interval of five minutes upto twenty minutes. The sequential change in the shape of the protoplasm is observed time to time and the diagram is drawn.

- (iv) Pieces of epidermal peels are taken and placed in different molar concentration of sucrose/NaCl 0.1, 0.2, 0.3, 0.4, 0.5 M solution one in each watch glass and the watch glass is covered.
- (v) The experiment is allowed to continue for 30 minutes (The pieces of epidermal peel are allowed to remain in a particular concentration for 30 minutes).
- (vi) The peels are taken out, mounted on glass slides and are observed under microscope.
- (vii) The total number of cells under focus and also the number of cells plasmolysed are counted. From this the percentage of plasmolysis is calculated.

OBSERVATION AND RESULT

- (i) The peels when placed in distilled water exhibited fully expanded protoplasm without any plasmolysis.
- (ii) When kept in different concentration of sucrose/NaCl solution, the percentage of plasmolysis gradually increased with increase in concentration of the solution.
- (iii) The starting of shrinkage of protoplasm is called **incipient plasmolysis**.
- (iv) The concentration of the solution where 50% plasmolysis occurs is called the **isotonic** solution where the concentration of the cell sap and that of the external solution are almost same. In the given example it is 0.3 M (approximately).

TABLE – 1

Time '0' (Before putting in sucrose/NaCl solution)	Conc. of sucrose or NaCl solution	No. of cells under focus	No. of cells plasmolysed
5 min treatment	0.5 M	20	Nil
10 min treatment		20	01
15 min treatment		20	08
20 min treatment		20	15

TABLE – 1

No. of observation	Conc. of sucrose/NaCl	No. of cells under focus	No. of cells plasmolysed	Percentage of plasmolysis
1	0 (control)	15	0	0
2	0.1 M	15	2	13.3
3	0.2 M	16	4	25.0
4	0.3 M	15	7	46.7
5	0.4 M	16	10	62.5
6	0.5 M	16	15	93.8

CONCLUSION

The percentage of plasmolysis increases with increase in concentration of the solution. At a particular concentration all the cells under focus show plasmolysis *i.e.* 100% plasmolysis. In the given experiment it is 0.5 M (approximately). Isotonic solution (50% plasmolysis) in this case is 0.3 M approximately. The exact isotonic concentration can be calculated by plotting the above data in a graph.

N.B. Epidermal peel of *Tradescantia* may be taken in place of *Rhoeo discolor*.



MINOR EXPERIMENTS

CHAPTER

5

QUALITATIVE TEST FOR BIOMOLECULES

TEST FOR CARBOHYDRATES

Experiment No. 11

Date

AIM OF THE EXPERIMENT

Qualitative test for the presence of carbohydrates (glucose, starch, cellulose) in rice/wheat/potato/cotton.

(A) TEST FOR GLUCOSE

REQUIREMENTS

Potato tuber, test tube, mortar and pestle, distilled water, Fehling's solution/Benedict's solution.

1. FEHLING'S TEST

PROCEDURE

- (i) Potato tuber extract is prepared and 4 ml of the clear extract is taken in a test tube.
- (ii) To it 2.0 ml of Fehling's solution (A) and 2.0 ml of Fehling's solution (B) is added.
- (iii) The solution is boiled.

OBSERVATION

Orange or brick-red precipitate appears in the test tube.

CONCLUSION

The carbohydrates has a reducing group, so the cupric ion is reduced to cuprous for which an rusty brown or brick-red colour precipitate is formed. It indicates the presence of glucose.

Fehling's solution

It is prepared by mixing equal volume of Fehling's solution (a) with Fehling's solution (B).

Fehling's solution (A)

34.65 gms of CuSO_4 is dissolved in distilled water and the volume is made upto 500 ml with distilled water.

Fehling's solution (B)

125 gms of KOH and 175 gms of sodium potassium tartarate are dissolved in about 100 ml of distilled water and the final volume is made upto 500 ml with distilled water. These solutions must be prepared fresh before use.

2. BENEDICT'S TEST

(This test can also be performed in place of Fehling's test.)

PROCEDURE

- (i) 1 ml of potato extract is taken in a test tube.
- (ii) To it 1 ml of distilled water is added followed by 2 ml of Benedict's solution.
- (iii) It is boiled and then cooled.

OBSERVATION

Green precipitate appears which later turn to orange or brick-red.

CONCLUSION

The given sugar has a reducing group for which a brick-red colour precipitate is formed. It indicate the presence of glucose.

Benedict's Reagent

17.3 gm of sodium citrate and 10 gm of sodium carbonate are dissolved in about 75 ml of water. 1.73 g of $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ is dissolved in about 20 ml water and is slowly added with stirring to the alkaline citrate solution. The volume is made up to 100 ml.

(B) TEST FOR STARCH**REQUIREMENTS**

Rice grains, wheat grains, potato tuber, test tubes, mortar and pestle, distilled water.

Reagent/Chemical – Iodine solution.

THEORY

Food stuffs contain different components such as carbohydrates, fats, proteins, minerals, vitamins and water. Carbohydrates are the basic component of our food and are the principal source of energy. The common carbohydrates in plants are cellulose, starch and sugars. The presence of starch can be easily studied in rice, wheat and potato as starch is the main storage food in these plant materials.

PROCEDURE**Preparation of Iodine solution**

5 gms of Iodine (I_2) and 10 gms of potassium Iodide (KI) are added to distilled water to make the final volume 100 ml. If a less intense colour of the solution is desired then 1 gm of Iodine and 29 gm of potassium Iodide are added to distilled water to make the final volume 300 ml.

- (i) Rice grain/ wheat grain/ potato tubers are taken and grinded with distilled water separately to make their paste.
- (ii) The paste can be taken directly or the contents can be filtered and kept in separate test tubes.
- (iii) A few drops of Iodine solution are added to the paste/filtrate.

OBSERVATION

The paste turned deep blue/black indicating the presence of starch.

Starch + Iodine $\longrightarrow$ Starch Iodide (Blue/Black colour)

CONCLUSION

The presence of starch (polysaccharide) in rice, wheat and potato is confirmed as starch developed blue/black colour with iodine due to formation of starch iodide. Starch can be hydrolysed to release simple sugars by boiling it with few drops of conc. HCl. Presence of sugar can be studied by Benedict's test or Fehling's test.

(C) TEST FOR CELLULOSE**REQUIREMENTS**

Cotton fibre/small piece of cotton, chlor-zinc-iodine solution, test tube or slide, pipette.

THEORY

Cellulose is one of the most important constituents of the plant cell, particularly the cell wall. It is complex insoluble carbohydrate forming the main framework of the plant which on hydrolysis gets converted to starch. With iodine, starch forms starch iodide, that gives blue colouration.

PROCEDURE**Preparation of chlor-zinc-iodine solution**

20 gm of $ZnCl_2$ is dissolved in 8.5 ml of water and is allowed to cool. Iodine solution is prepared separately by dissolving 3 gm of KI in 60 ml of water and then 1.5 gm of iodine is dissolved in the solution. Iodine solution, so prepared, is then added dropwise to cool $ZnCl_2$ solution until iodine begins to precipitate.

- (i) Cotton fibre (or a small piece of cotton is so torn that the fibres are exposed) is collected.
- (ii) A small amount of cotton fibre is taken either in a test tube or on a slide with drop of water.

- (iii) Few drops of chlor-zinc-iodine reagent is added to it.
- (iv) The colour change is observed.

Cotton fibre in test tube/slide



Few drops of chlor-zinc-iodine solution is added.



Change in colour is observed.

OBSERVATION

The cotton fibres turn deep blue.

CONCLUSION

Zinc chloride reacts with cellulose present in the cotton fibre and converts it to simple carbohydrate called amyloids, which with iodine forms blue colour. Presence of cellulose in plant material is indicated.

Presence of cellulose in plant material can also be tested by hydrolysing cellulose with conc. H_2SO_4 and then adding few drops of iodine solution. The procedure is as follows :

- (i) Few cotton fibres are taken in a test tube.
- (ii) Few drops of conc. H_2SO_4 is added to it and is heated on a spirit lamp.
- (iii) Cotton fibre is removed from the test tube and is kept on a slide.
- (iv) Few drops of iodine is added to the slide.

The material turns blue indicating the presence of cellulose.

Conclusion :

Cellulose on hydrolysis with conc. H_2SO_4 releases starch, the later with iodine forms starch iodide that imparts blue colour.

TEST FOR PROTEIN**Experiment No. 12**

Date

AIM OF THE EXPERIMENT

To test the presence of protein in pea/gram/bean seeds

(A) XANTHOPROTEIC TEST**REQUIREMENTS**

Pea/gram/bean seeds, mortar and pestle, conc. HNO_3 , conc. NaOH /conc. NH_4OH , test tube, test tube holder, spirit lamp, pipette.

THEORY

Proteins are the most important and essential constituents of the living organisms. As enzymes they participate in all biochemical reactions of the cell, as reserve products they are stored in different plant parts and as building blocks they participate in cell repair. In seeds proteins are stored as reserve materials that are utilised during the process of seed germination. Of the various methods known for qualitative test for protein, xanthoproteic test is the most suitable one.

Proteins are composed of amino acids. Xanthoproteic reaction is due to the presence of phenyl groups in certain amino acids that form the protein.

PROCEDURE**Preparation of $\text{NaOH}/\text{NH}_4\text{OH}$ (40% solution)**

40 gm of $\text{NaOH}/\text{NH}_4\text{OH}$ is dissolved in water to have 100 ml of the solution.

- (i) Gram/pea/bean seeds are soaked in water for about twelve hours. About 10 seeds are homogenised with few drops of water by mortar and pestle. Then 5 ml of water is added to it.
- (ii) 2 ml of seed homogenate is taken in a test tube.
- (iii) 1 ml of conc. HNO_3 is added to it. A white precipitate is formed.
- (iv) The solution is heated gently. The colour change is observed. It changes to yellow colour.
- (v) The test tube is cooled under tap water.
- (vi) 4 ml of 40% $\text{NaOH}/\text{NH}_4\text{OH}$ is added to the tube. The colour changes to orange.

Seed homogenate in test tube
↓
1 ml of conc. HNO_3 is added (white precipitate)
↓
Mixture is heated gently (colour changes to yellow)
↓
Test tube is cooled under tap
↓
4 ml of 40% $\text{NaOH}/\text{NH}_4\text{OH}$ is added
↓
Yellow colour changes to orange.

CONCLUSION

When protein reacts with conc. HNO_3 , the phenyl groups of amino acids produce the nitro derivatives which give yellow colour on heating. When these products react with alkali, bright orange colour is produced. The development of orange colour confirms the presence of protein in the seed.

(B) BIURET TEST

REQUIREMENTS

Plant material (pea/gram/bean seeds), mortar, pestle, 10% NaOH solution, 0.5% CuSO_4 solution, test tube, test tube holder, spirit lamp, pipette.

PROCEDURE

(i) Preparation of NaOH (10% solution)

10 gm of NaOH is dissolved in water to have 100 ml of the solution.

(ii) Preparation of CuSO_4 (0.5% solution)

0.5 gm of CuSO_4 is dissolved in water to have 100 ml of the solution.

- (i) Gram/pea/bean seeds are soaked in water for about twelve hours. About 10 seeds are homogenised with few drops of water by mortar and pestle. Then 5 ml of water is added to it.
- (ii) 2 ml of seed homogenate is taken in a test tube.
- (iii) 4 ml of 10% NaOH is added to it and the solution is shaken well.
- (iv) 1 drop of 0.5% CuSO_4 solution is added and it is shaken.
- (v) The process of adding CuSO_4 and shaking is repeated gently (in controlled way) till light blue or violet colour developed.
- (vi) It is heated to deepen the colour.

2 ml Seed homogenate in test tube



4 ml of 10% NaOH is added



0.5% CuSO_4 is added drop by drop



Light blue/violet colour developed



Test tube is heated gently



Light blue/violet colour intensified

CONCLUSION

It is a test for peptide bonds and hence for proteins. Nitrogen atoms in the peptide chain formed a light blue/violet complex with copper ions (Cu^{+2}) in alkaline solution. As biuret is a compound derived from urea which contains the $-\text{CONH}-$ group, it also gives a similar result. Hence the test is called Biuret test.

TEST FOR FAT

Experiment No. 13

Date

AIM OF THE EXPERIMENT

To test the presence of fats in plant materials like coconut milk/groundnut oil.

REQUIREMENTS

Groundnut oil/coconut milk or oil, distilled water, test tube

Reagents – Sudan III Or Sudan IV.

THEORY

Fats are esters of higher fatty acids (like oleic, palmitic, etc.) and glycerol. These compounds exist in both solid and liquid forms depending on their molecular composition. In solid forms they are called fats and in liquid forms as oils. Many plant materials such as mustard seeds, castor seeds, groundnut seeds, coconut milk, etc. contain fat. Fats and oils are the storage product providing energy to plants. They have even more caloric value than the carbohydrates.

PROCEDURE

Preparation of reagent

1. Sudan IV solution is prepared by dissolving 0.5 gm of Sudan IV in 50 ml of 70% ethanol and stirring continuously for 30 minutes with addition of 50 ml of acetone.
- Or, By dissolving 0.5 gm. of Sudan IV in 100 ml of chloroform.
2. Sudan III solution is prepared by dissolving 0.1 gm of Sudan III in 50 ml of 95% ethanol (warming if necessary and then cooling) and mixing properly with 50 ml of glycerine (filtration if necessary).

- (i) A little amount of groundnut oil/coconut milk is taken in a test tube and water is added such that the test tube is half filled up.
- (ii) The mixture is boiled over a burner to form an emulsion.
- (iii) A few drops of Sudan III or IV are added to it and the test tube is allowed to stand for sometime.
- (iv) The fat particles are seen to settle down.

Groundnut oil/coconut milk in a test tube



Water added till the tube is half filled.



Mixture is boiled to form an emulsion



A few drops of Sudan III or IV is added



Tube is allowed to stand for few minutes.



Settling of red particles.

OBSERVATION

The settling of red particles indicated the presence of fat.

CONCLUSION

Oil globules turned red by the action of Sudan III or IV.

PRECAUTIONS

- (i) Thoroughly washed test tubes and glasswares should be used.
- (ii) The standard reagents and chemicals must be used or reagents should be freshly prepared before use.

N.B.

Ethanol solubilizes the dark red coloured Sudan dye which partition into the fat molecules, thus giving a pinkish-red colouration.



STUDY OF OSMOSIS

Experiment No. 14

Date

AIM OF THE EXPERIMENT

Study of osmosis by potato osmometer.

REQUIREMENTS

A large potato, knife, 10-20% sugar solution, petridish, beaker, water, pins.

THEORY

Osmosis is a type of diffusion in which solvent molecules move from a region of their higher concentration to the region of their lower concentration through a semipermeable membrane till a state of equilibrium is reached. The living cells are surrounded by plasma membrane which is selectively permeable and thus show osmosis when placed in different solutions. Water moves into the cell when the cell sap is in higher concentration and the cell is placed in a solution less concentrated than cell sap (hypotonic). It is called endosmosis. Water moves out of the cell when the cell is placed in a solution having higher concentration (hypertonic) than cell sap. The process is called exosmosis. The phenomenon of osmosis can be demonstrated with the help of osmometer.

PROCEDURE

- (i) The outer skin of potato tuber is peeled off. One end is cut flat as shown in the figure 2.1. A cavity is made in the centre of the potato opposite to the flat side almost upto the bottom.
- (ii) The potato tuber is placed by its flat end on a petridish half-filled with water.
- (iii) Half of the cavity of the potato is filled with 10-20% of sugar solution and level of the sugar solution is marked in the cavity with the help of a pin. This now acts as a osmometer.
- (iv) The osmometer is left undisturbed for 30 minutes and then the level of sugar solution in the cavity is marked. The final level is marked with the help of another pin.

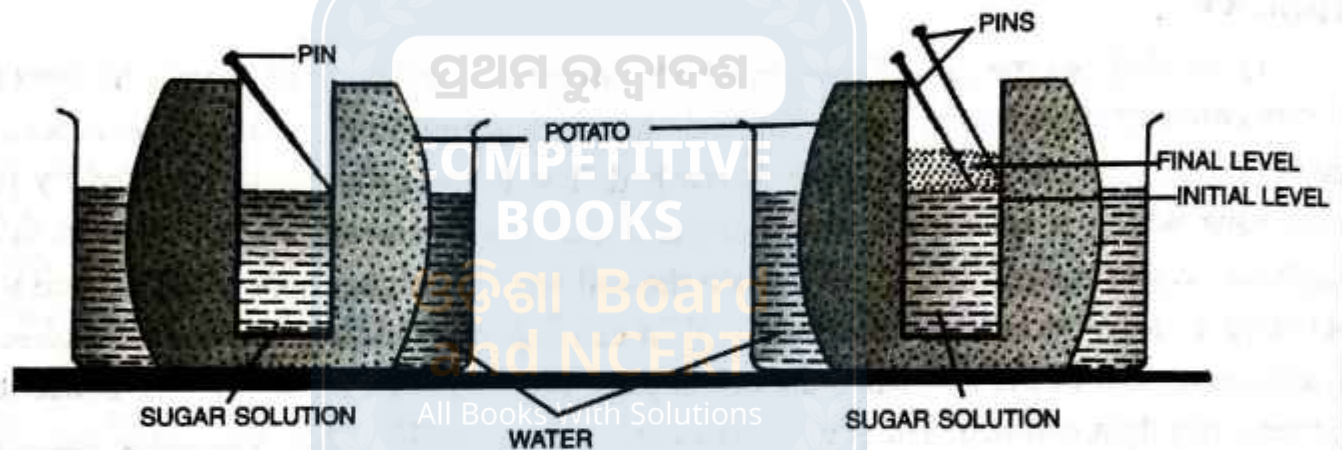


FIG 6.1. DEMONSTRATION OF OSMOSIS BY POTATO OSMOMETER.

OBSERVATION

The level of sugar solution raised after sometime due to endosmosis of water.

CONCLUSION

Water moved from the petridish into the potato tuber because of the difference in concentration of solvent (water) molecules in the two regions. The outer membrane enclosing the cells of the potato acted as the selectively permeable membrane.

PRECAUTIONS

- (i) The cavity in the potato tuber should be deep enough leaving only a thin layer of tissue at the base.
- (ii) The lower end of the potato should be flat to keep it stable in water on the petridish.
- (iii) There should not be any leakage in the potato tuber.

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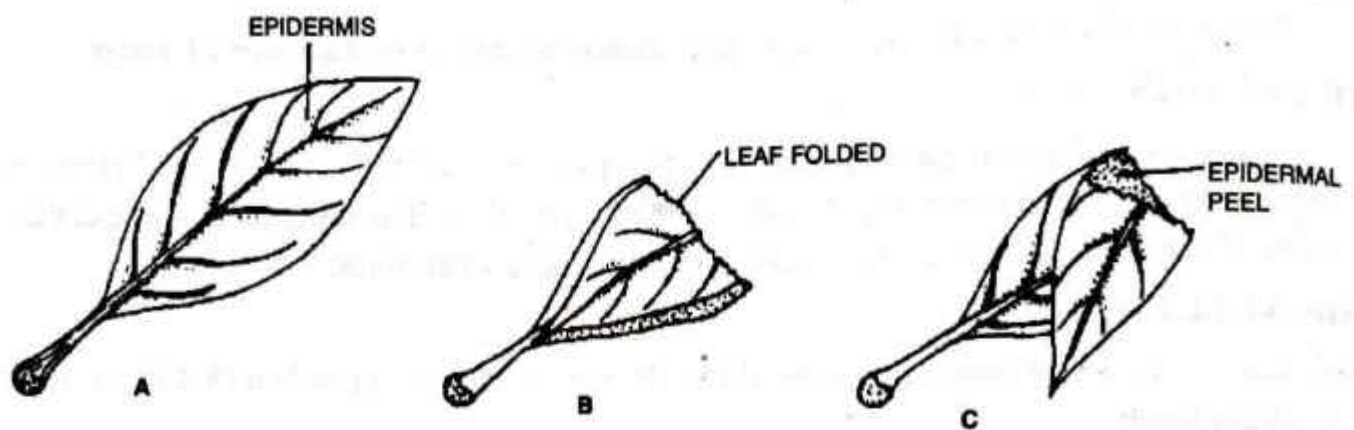
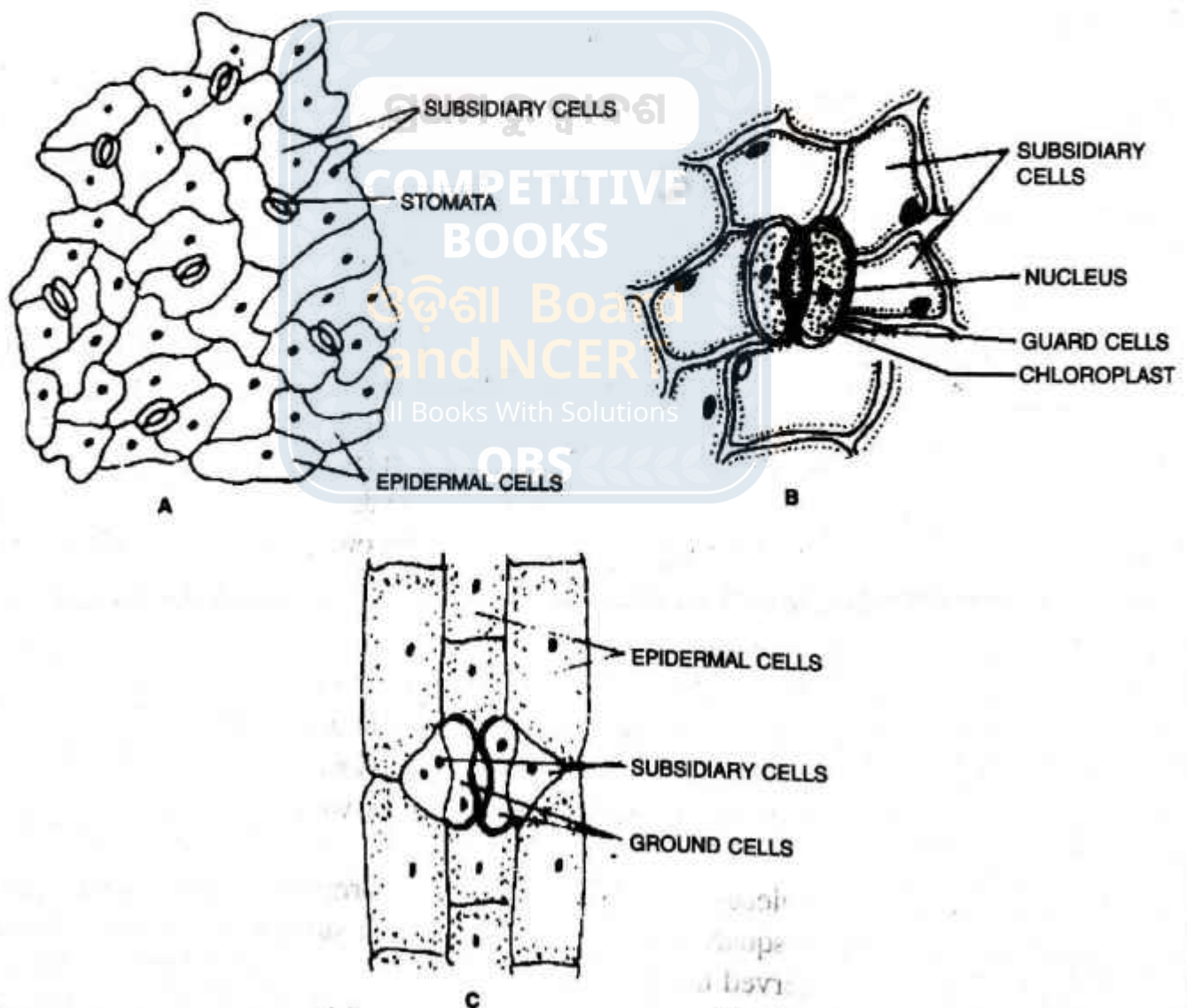


FIG 7.1. TAKING OUT OF EPIDERMAL PEEL OF A LEAF.



**FIG 7.2. STOMATA A. STOMATA ON THE LOWER EPIDERMIS OF A DICOT LEAF
B. SINGLE STOMA. C. STOMATA ON THE EPIDERMIS OF A MONOCOT LEAF.**

CHAPTER

7

STUDY OF STOMATAL DISTRIBUTION

Experiment No. 15

Date

AIM OF THE EXPERIMENT

To study the distribution of stomata on upper and lower surfaces of a dicot and monocot leaf.

REQUIREMENTS

Fresh leaf of *Petunia/Nerium* or any other dicot leaf and *Polyanthes* or any other monocot leaf, forceps, needle, blade, brush, watch glasses, petridish, beaker, slides, coverslips, safranin, glycerine, water.

THEORY

Stomata are microscopic openings present in the epidermis of leaves and young shoots. Each stoma is surrounded by two kidney-shaped cells in case of dicot and two dumb-bell shaped cells in case of monocot leaves called guard cells. The guard cells are surrounded by two or more specialised cells called subsidiary or accessory cells. Stomata are concerned with loss of water during transpiration and exchange of gases during photosynthesis and respiration. Stomata are mostly restricted to lower surface of the leaf in the dicots and in monocots they are distributed equally on the two surfaces of the leaves. The distribution of the stomata on the upper and lower surfaces of the leaf can be studied by taking out the peels of the leaf from the two surfaces and observing the same under microscope.

PROCEDURE

- (i) Epidermal peels from the upper and lower surfaces of the leaves are taken and are kept in separate watchglasses containing water. The peels are taken by tearing the leaf obliquely with a single jerk or scraping it with blade as shown in the figure 7.1.
- (ii) Peels are taken from both the surfaces of the dicot as well as monocot leaves to see stomata.
- (iii) Peels are cut into small pieces and are stained with few drops of safranin in a watchglass.
- (iv) A small rectangular or square piece of peel from each surface is mounted separately with glycerine and observed under microscope.
- (v) The number of stomata in the peels under focus both in upper and lower epidermis is counted and noted (Counting is done separately for dicot and monocot leaves).

OBSERVATION AND RESULT

In dicot leaf

The number of stomata on the upper surface – Nil.

The number of stomata on the lower surface – 07.

In monocot leaf

The number of stomata on the upper surface – 02.

The number of stomata on the lower surface – 03.

CONCLUSION

In dicot leaves the stomata are mostly restricted to the lower epidermis whereas the monocot leaves possess almost equal number of stomata on both the epidermis.

PRECAUTIONS

- (i) Curling of the peels should be avoided.
- (ii) Thin and uniform peels must be taken.
- (iii) Excess of glycerine should be removed by using blotting paper.

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CHAPTER

8

VERIFICATION OF MENDELIAN RATIOS

Experiment No. 16

Date

AIM OF THE EXPERIMENT

To analyse the supplied seed samples for verification of Mendelian monohybrid and dihybrid ratios.

REQUIREMENTS

Pea seeds collected from different sources.

THEORY

Inheritance of characters from generation to generation is called heredity and the principles behind it as genetics. Mendel is known as Father of Genetics as he for the first time formulated certain laws to explain the inheritance of characters in garden pea. His monohybrid and dihybrid experiments and the results derived from these experiments are given below.

(A) MONOHYBRID CROSS

In this cross only one character with two alternative forms was considered. These two alternative forms appeared in the F_2 generation were in the ratio of 3 dominant : 1 recessive. From this he formulated the law of segregation.

PROCEDURE

Samples of seeds of peas supplied were separated on the basis of their shape, that is smooth (dominant) and wrinkled (recessive). These seeds were counted and tabulated as shown in the table. Three samples were analysed in the similar manner. The ratio of smooth and wrinkled seeds was calculated for each sample as well as for total number of seeds and recorded in the table.

Samples	Total No. of seeds	No. of smooth seeds	No. of wrinkled seeds	Smooth:Wrinkled
1	85	64	21	3.04 : 1 or 3 : 1 (approx.)
2	87	65	22	2.95 : 1 or 3 : 1 (approx.)
3	94	71	23	3.05 : 1 or 3 : 1 (approx.)
Total	246	200	66	3.03 : 1 or 3 : 1 (approx.)

CONCLUSION

In monohybrid cross the F_2 ratio of smooth and wrinkled seeds in different seed samples is 3 smooth : 1 wrinkle.

N.B. Teacher can mix-up glass beads of two different sizes or of two different colours in approximate proportion and use in place of seeds.

(B) DIHYBRID CROSS

In this cross two characters, each with alternative traits were considered. The traits appeared in F_2 were in the ratio of 9 : 3 : 3 : 1 from which he derived the law of independent assortment.

PROCEDURE

Supplied samples of seeds of peas were collected and two characters, such as shape (smooth and wrinkled) and colour (yellow and green) of seeds were observed. Four types of seeds observed were smooth yellow, smooth green, wrinkled yellow and wrinkled green. These seeds were separated, counted, tabulated and their ratios for each sample as well as for total number of seeds were calculated as shown in the table.

Samples	Total No. of seeds	No. of smooth and yellow seeds	No. of smooth and green seeds	No. of wrinkled and yellow seeds	No. of wrinkled and green seeds	Ratio
1	85	46	18	16	5	9.2 : 3.6 : 3.2 : 1 or, 9 : 3 : 3 : 1 (approx)
2	87	49	16	17	5	9.8 : 3.2 : 3.4 : 1 or, 9 : 3 : 3 : 1 (approx)
3	94	53	18	17	6	8.83 : 3.0 : 2.83 : 1 or, 9 : 3 : 3 : 1 (approx)
Total	266	148	52	50	16	9.25 : 3.25 : 3.13 : 1 or, 9 : 3 : 3 : 1 (approx.)

CONCLUSION

In dihybrid cross the F_2 ratio of smooth-yellow, smooth-green, wrinkled-yellow and wrinkled-green seeds in different seed samples is 9 : 3 : 3 : 1.

N.B. Teachers can use glass beads of two different sizes (one slightly bigger than other) and of two different colours, say red and blue, so that four types of beads – big-red, big-blue, small-red and small-blue can be available. These can be mixed-up in approximate proportions and given to the students instead of seeds.



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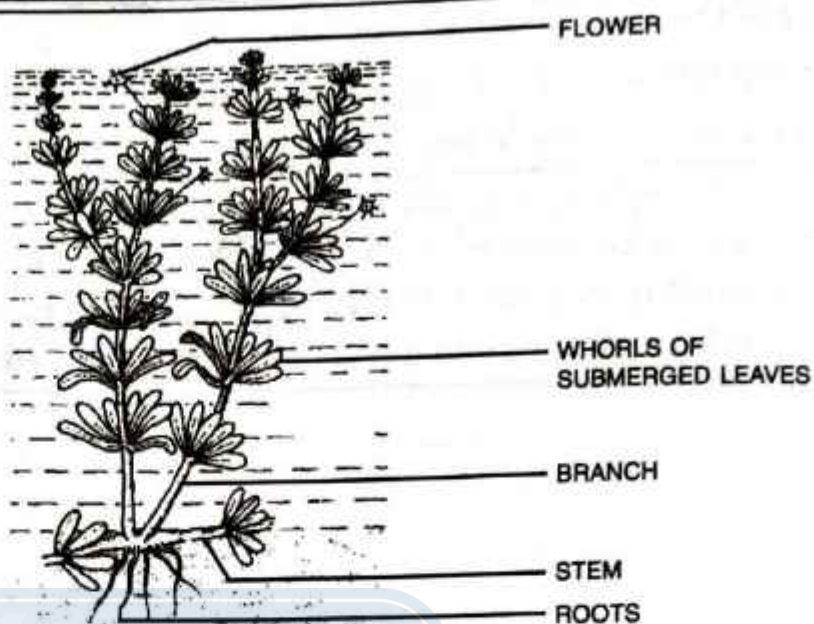


FIG 9.1. HYDRILLA – A SUBMERGED HYDROPHYTE

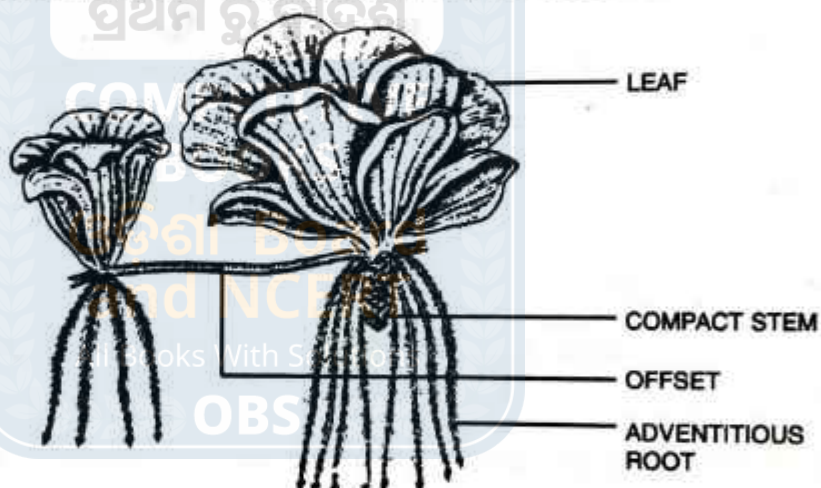
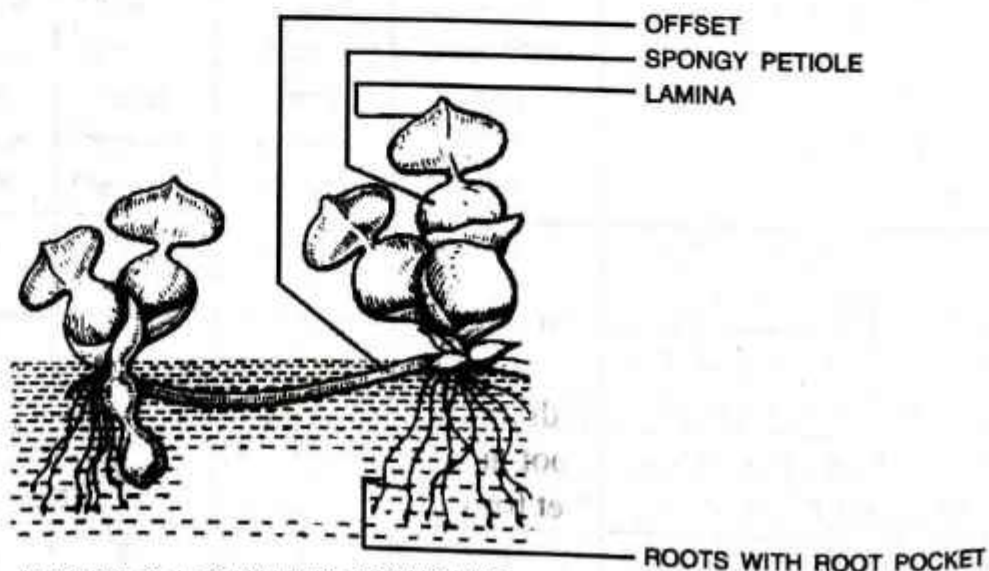


FIG 9.2. PISTIA – A FREE FLOATING HYDROPHYTE



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 FIG 9.3. EICHHORNIA – A FREE FLOATING HYDROPHYTE

STUDY OF ECOLOGICAL ADAPTATIONS OF PLANTS

Experiment No. 17

Date

AIM OF THE EXPERIMENT

To study, draw and describe the morphological adaptations and identify the supplied specimens.

REQUIREMENTS

Hydrophytes : *Hydrilla*, *Pistia* and *Eichhornia* plants

Xerophytes : *Opuntia* phyllacode and *Casuarina* twig.

(1) HYDRILLA

- (i) Supplied specimen is a completely submerged plant with roots, stem and leaves.
- (ii) Roots are few, very much reduced and fixed to substratum.
- (iii) Stem is soft, spongy and with long internodes.
- (iv) Leaves are whorled, reduced, linear and fleshy.
- (v) Soft and spongy nature of stem and leaves are due to the presence of air storage tissue for floating purpose.

Identification :

Hence, it is a rooted submerged hydrophyte.

(2) PISTIA

- (i) A free floating plant with poorly developed adventitious root system.
- (ii) Roots are few, developed at nodes, less branched, root hairs and root caps absent, root pockets are present in place of root caps.
- (iii) Shoot system is also reduced, stem soft, spongy with condensed and short internodes.
- (iv) Lateral branches modified into stolon for vegetative propagation.
- (v) Tuft of floating leaves with waxy surface are present at the tips of lateral branches.

Identification :

Hence, it is a free-floating hydrophyte.

(3) EICHHORNIA

- (i) A free floating plant with poorly developed root system.
- (ii) Roots without having root hairs and root caps. Root pockets replace the root caps.
- (iii) Stem modified into offset for vegetative propagation. Offset horizontal with short, thick and soft internode.

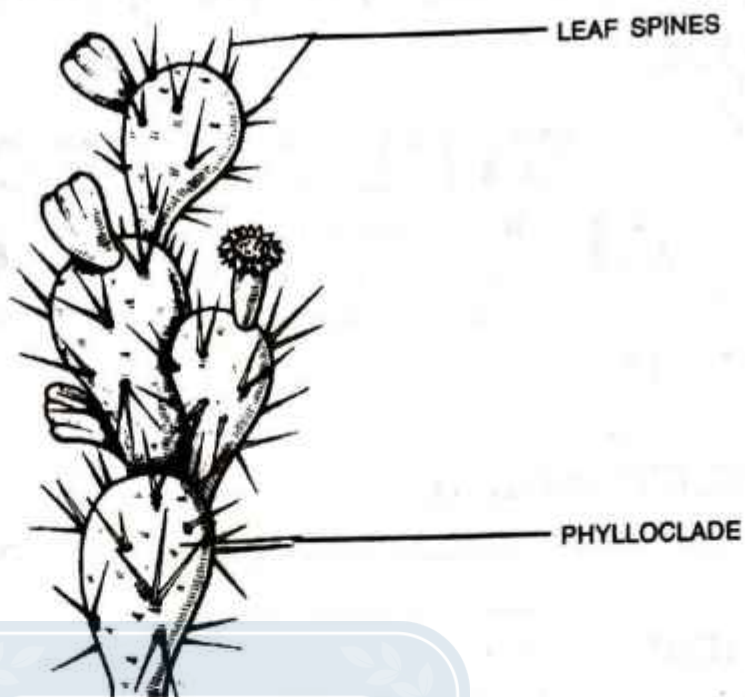


FIG. 9.4. OPUNTIA – A SUCCULENT XEROPHYTE

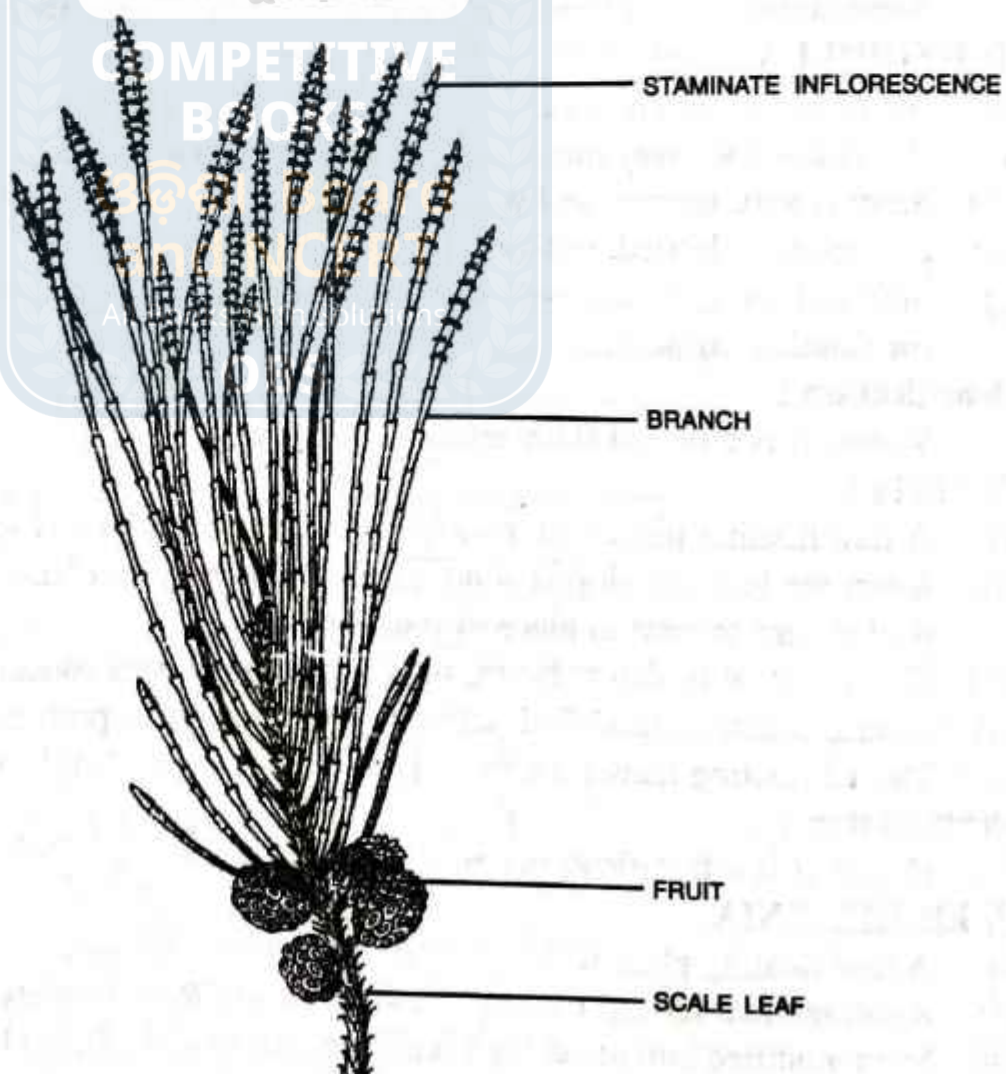


FIG. 9.5. CASUARINA – A XEROPHYTE

- (iv) Internode bears a tuft of leaves and a cluster of adventitious roots at the tip.
- (v) Petioles are swollen into spongy and bulbous structure for storage of air for the purpose of floating.

Identification :

Hence, it is a free-floating hydrophyte.

(4) OPUNTIA

- (i) It is a spiny shrub growing in xeric conditions.
- (ii) Stem is modified into a green, flat, fleshy and jointed structure called phylloclade.
- (iii) Phylloclade bears succession of nodes and internodes and performs the functions of leaf as well as stores water.
- (iv) Leaves are reduced into spines to check transpiration.
- (v) Root system is well developed with profusely branched and deep seated roots.

Identification :

Hence, it is a succulent xerophytic plant of draught resistant type.

(5) CASUARINA

- (i) It is an ever-green woody tree with xerophytic habits.
- (ii) The stem is grooved with jointed and grooved branches arranged in whorls.
- (iii) Branches are reduced into green structures and perform the functions of leaf.
- (iv) Leaves are reduced to scale-like structures to minimise transpiration.
- (v) Root system is well developed and deep-seated to avail water in xeric condition.

Identification :

Hence, it is a xerophytic plant.



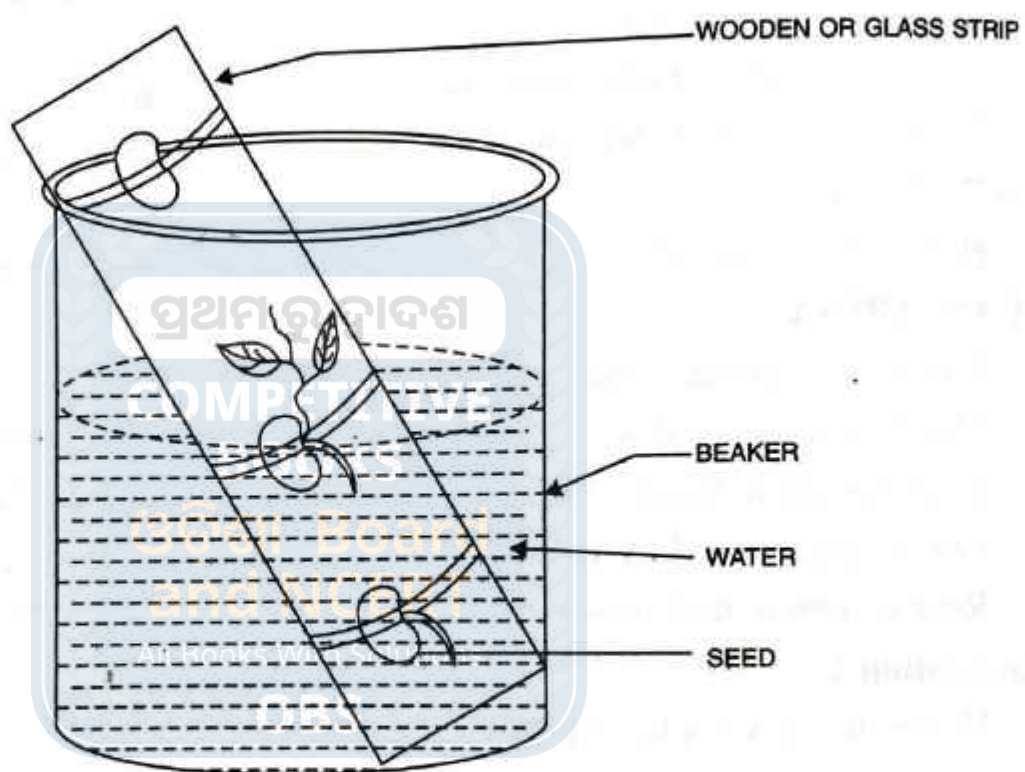


FIG. 10.1. EXPERIMENT SHOWING CONDITIONS ESSENTIAL FOR SEED GERMINATION.

CHAPTER

10

STUDY OF SEED GERMINATION

Experiment No. 18

Date

AIM OF THE EXPERIMENT

To demonstrate the conditions necessary for seed germination (air and water).

REQUIREMENTS

Bean or Gram seeds, wooden or glass strip, beaker, water.

THEORY

Seed germination is the sprouting of the seed and growth of the embryo present inside the seed into a seedling and subsequently young plant. A number of conditions are essential for the process of seed germination. The most important ones are air, water and temperature. Oxygen is required for respiration that releases energy for various metabolic activities during the process of germination and growth of seedling. Water softens the seed coat, causes hydrolysis of reserve food and helps in their transport. Temperature is essential for normal metabolism of the embryo and the seedling. All these three factors are equally important for the process of seed germination.

PROCEDURE

- (i) Three healthy bean/gram seeds are taken.
- (ii) They are tied on a wooden or glass strip at three different places in a line, one in the middle and one at each end, upper and lower.
- (iii) The glass or wooden strip is placed in a beaker and water is poured into it until the middle seed is half immersed in water.
- (iv) The set up is left at room temperature for 3-4 days till the seeds start germinating.

OBSERVATION

Only the middle seed germinated perfectly and started sprouting. The topmost seed did not germinate. The lowermost seed started germinating but aborted halfway, giving out only the tip of the radicle.

CONCLUSION

The experimental set-up demonstrated that air and water are essential for seed germination. The middle seed which received both the essential conditions showed sprouting.

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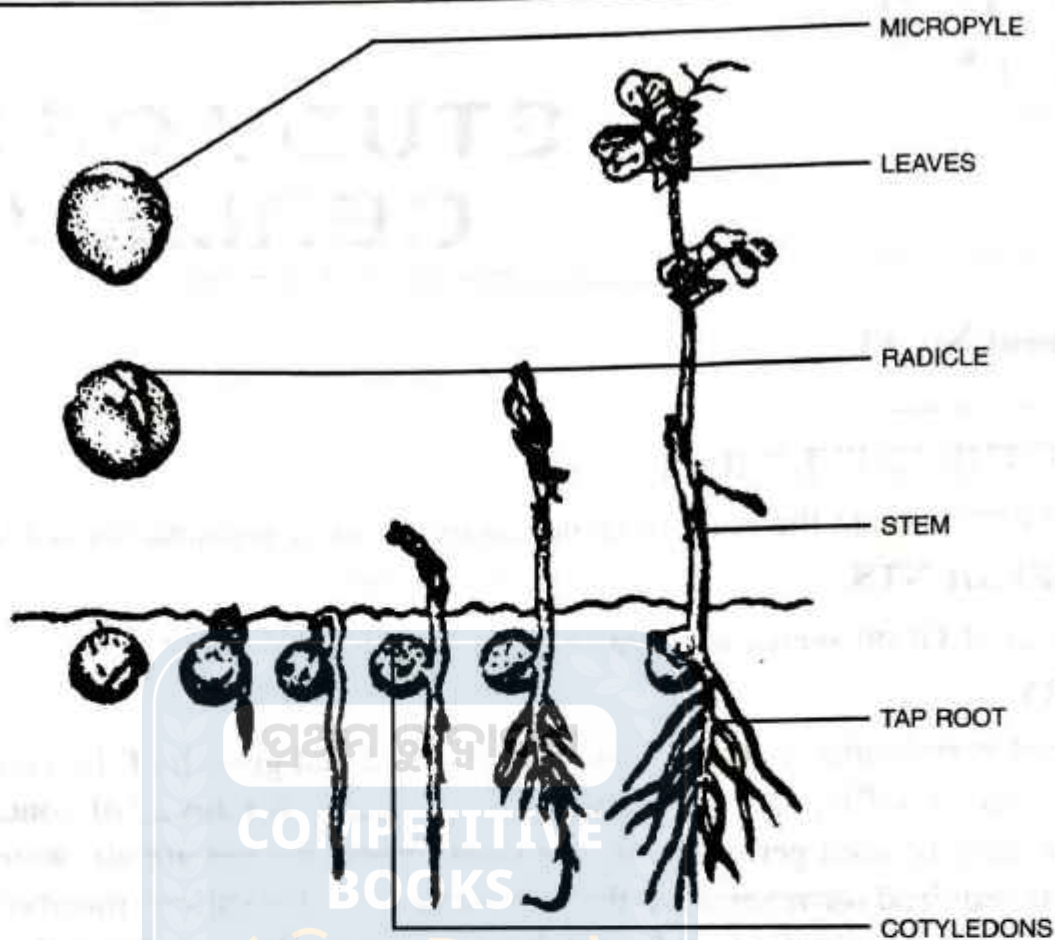


FIG. 10.2. STAGES OF GERMINATION SHOWING HYPOGEAL GERMINATION (PEA SEEDS).

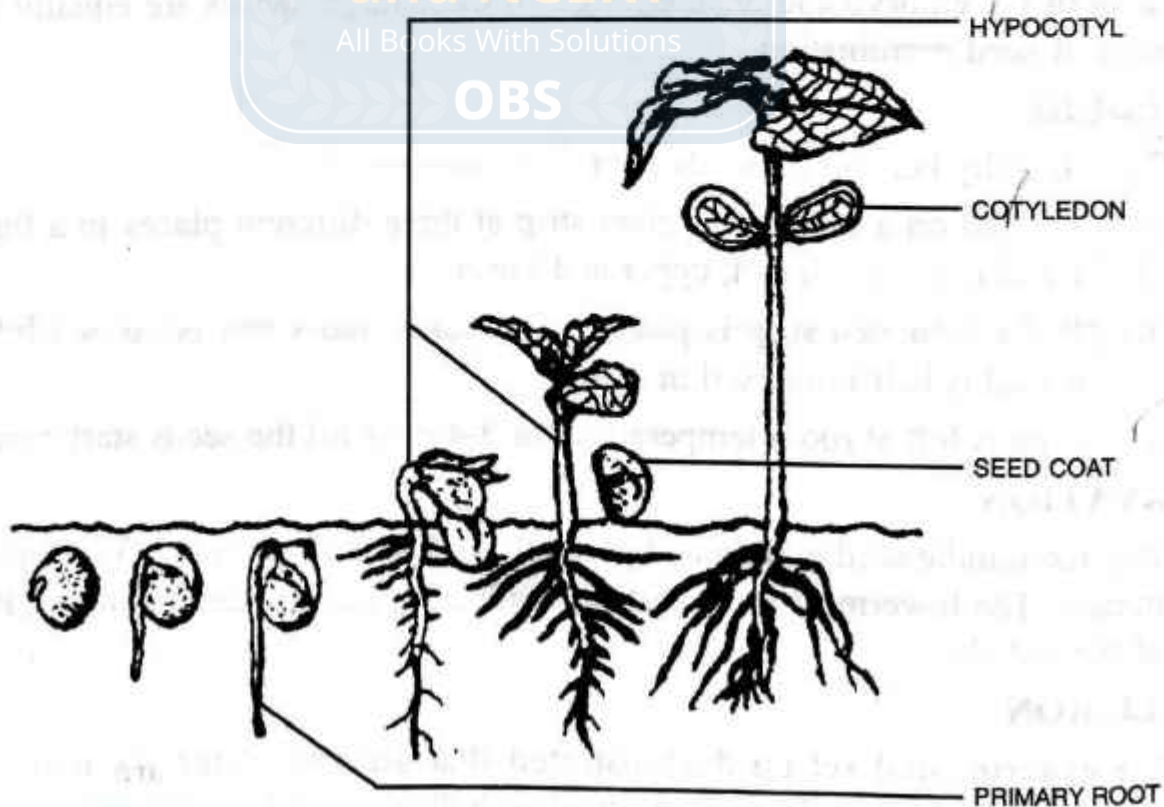


FIG. 10.3. STAGES OF GERMINATION SHOWING EPIGEAL GERMINATION (BEAN SEEDS).

The topmost one failed to receive water and so did not germinate. The lowermost seed got enough water but failed to receive enough air, thus started germinating but aborted halfway giving out only tip of radicle.

PRECAUTIONS

- (i) Healthy seeds should be selected for germination.
- (ii) The experimental set-up should be left at a warm place for germination.

Experiment No. 19

Date

AIM OF THE EXPERIMENT

To demonstrate epigeal and hypogeal germination.

REQUIREMENTS

Glass vessels/beakers, sterile sand, water, bean/caster seeds for epigeal germination and pea/gram/maize seeds for hypogeal germination.

THEORY

The region of the axis between the point of attachment of cotyledon and the plumule is called epicotyl (as shown in Fig. 10.2 & 10.3). The region of the axis below the cotyledon is called hypocotyl. Both epicotyl and hypocotyl of a seed never elongate together during germination. It is either epicotyl or the hypocotyl that elongates. If the epicotyl elongates, the cotyledons remain underground (or on the ground if the seed is on the ground) and the germination is hypogeal as seen in pea, gram, etc. If the hypocotyl elongates, the cotyledons are pushed above the ground and the germination is epigeal, as in castor, bean, etc.

PROCEDURE

- (i) Two glass vessels/beakers are taken and half filled with sterile sand.
- (ii) Few presoaked bean/caster seeds and pea/gram/maize seed are placed inside the sand of separate vessels/beakers.
- (iii) Water is sprinkled and the two experiment set-ups are kept in well lighted and aerated place for couple of days.
- (iv) Water was sprinkled at regular intervals and stages of germination was observed every day.

OBSERVATION AND RESULT

In case of bean seeds, the arched hypocotyl grew and became straight, bringing the cotyledons above the soil. Thus, the germination was epigeal. In case of pea seeds, the hypocotyl did not elongate, rather the epicotyl grew, the cotyledons remained under the ground, hence the germination was hypogeal.

CONCLUSION

Pea/maize seeds show hypogeal type of germination whereas bean/caster seeds show epigeal germination.



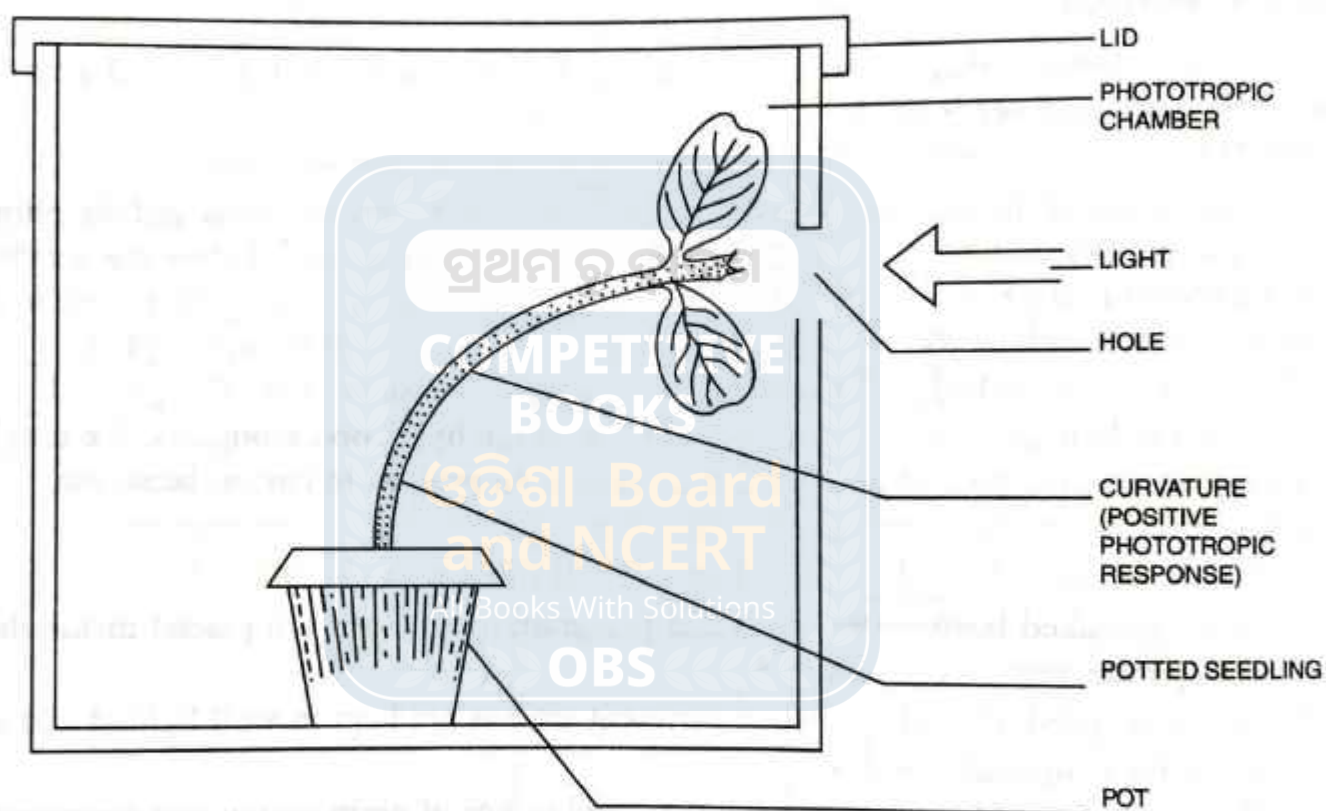


FIG. 11.1. EXPERIMENT TO DEMONSTRATE PHOTOTROPISM.

STUDY OF PLANT MOVEMENT

PHOTOTROPISM

Experiment No. 20

Date

AIM OF THE EXPERIMENT

Demonstration of experimental set up showing phototropism.

REQUIREMENTS

Potted seedling of a plant, phototropic chamber with a small hole on one side, black cloth.

THEORY

Phototropism is a tropic movement induced by light (unidirectional light). The direction of response varies in different parts of the plant. Generally the stem grows towards the source of light (positive phototropism) and roots grow away from light (negative phototropism). The phototropic response is marked by the growth of the plant part towards light.

PROCEDURE

A potted seedling of a plant is taken and is placed in a phototropic chamber with a small hole on one side. The top of the chamber is also closed. The chamber is covered with black cloth except at the hole. The potted plant is placed in such a position that sufficient light is allowed to pass through the hole to the plant. The experimental set up is kept for a couple of days.

OBSERVATION AND RESULT

The seedling showed the bending (curvature) of the stem towards the hole or light.

CONCLUSION

The phototropic effects are caused by unequal growth of the plant in response to light. As stem shows positive phototropism the plant shows bending towards light.

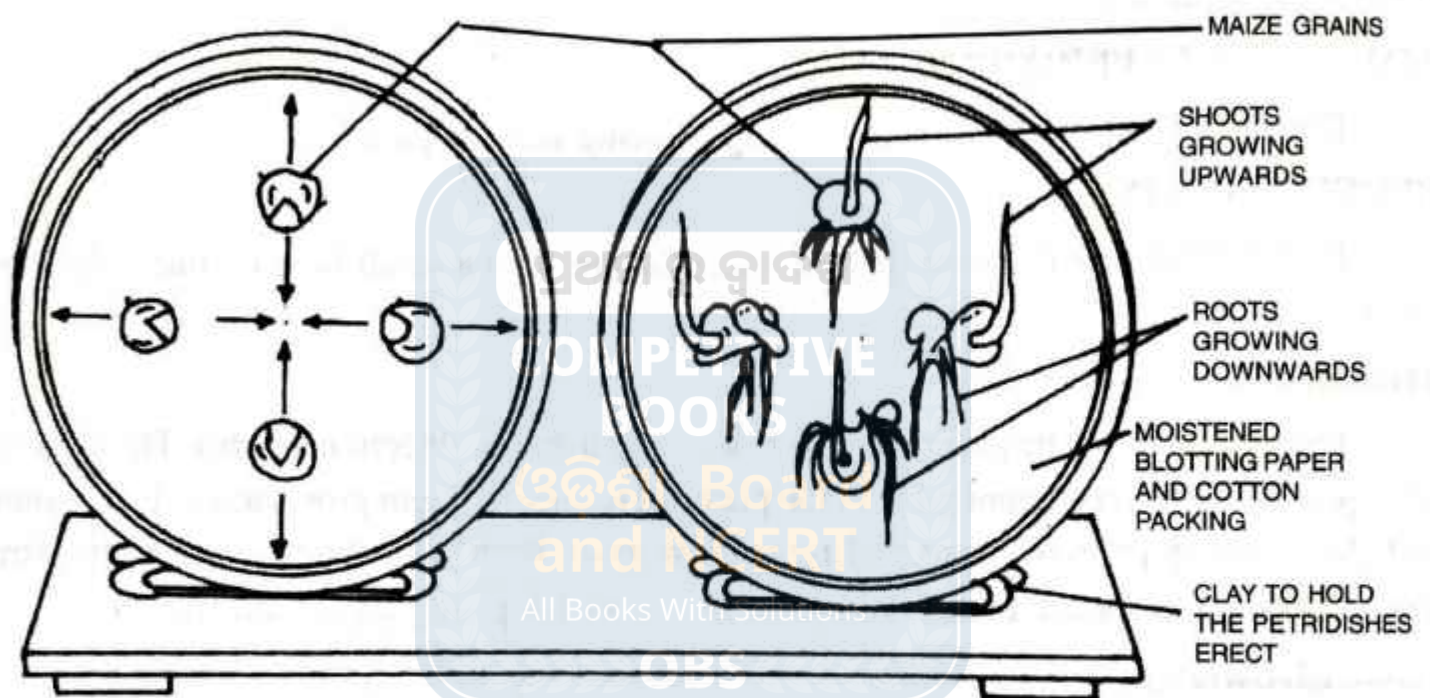


FIG. 11.2. EXPERIMENT TO DEMONSTRATE GEOTROPISM IN MAIZE SEEDLINGS.

GEOTROPISM**Experiment No. 21****Date****AIM OF THE EXPERIMENT**

Demonstration of experimental set up showing geotropism.

REQUIREMENTS

Maize grains/germinating seeds, petridish, blotting paper, cotton pad.

THEORY

Geotropism is a type of tropic movement produced in response to the force of gravity. Normally the roots grow towards the force of gravity and the main stem grows away from it. Thus, roots exhibit positive geotropism and the stems show negative geotropism.

PROCEDURE

- (i) Four maize grains/germinating seeds are taken and are placed in a vertically placed petridish containing moist blotter and cotton pad to hold the grains in position (as shown in the fig. 11.2).
- (ii) The grains are placed in such a way that their radicles lie in different directions.
- (iii) The experiment is allowed to stand like that for a couple of days.
- (iv) Suitable conditions (air, water and light) are provided for the growth of the seedlings.

OBSERVATION AND RESULT

After a few days it is seen that irrespective of their position, the radicles/roots of all the germinating seeds grew downward and the coleoptiles/shoots grew upward.

CONCLUSION

The roots show positive geotropism. Therefore irrespective of the position of the seed, the roots of all the grains grew downward. The shoots, on the other hand, exhibit negative geotropism (phototropic rather) and so they grew upward.



HOW TO APPEAR AT PRACTICAL EXAMINATION

In a practical examination, the knowledge of the candidate is tested from his practical performance and efficient reproduction in the examination. Many students get nervous before practical examination. Therefore some of the basic points are to be followed so that the candidates can appear the examination with spirit and impress the examiner with the performance.

- One must prepare thoroughly by going through own practical record, rough practical record, textbook and practical book before the practical examination.
- One should recollect the memory on the work done in different practical classes (Regular attendance and maintaining a rough practical record become most helpful for this purpose).
- One should come for examination being neatly dressed and should enter the practical room with confidence.
- One must carry the practical record (well covered with CHSE Roll No. written on it), dissection instruments, pencils of different shades (HB, B, 2B etc.), eraser, admit card and identity card to the practical examination room. No objectionable paper should be carried by the candidate.
- The practical record must be submitted after entering the room and one must occupy the seat allotted to him/her after submitting the practical record.
- The answer book be collected and the requirements be filled up on the answer book.
- One must go through the question paper thoroughly and answer as per the requirement of the question.
- For anatomy question, thin, uniform, complete transverse sections of the specimen supplied be cut. Good sections should be selected and stained as described in Chapter 7. The section should be mounted with glycerine. A small slip should be pasted on the prepared side indicating the name of the slide and Roll No. of the candidate.
- The candidate must draw neat diagram (A. Ground plan; B. Portion enlarged) and label the diagram neatly.
- Write only the identifying characters and identify with reasons.
- Submit the preparation for evaluation.
- For taxonomy question, describe the characters of the supplied twig in technical terms. The diagram of the floral parts should be drawn neatly and labelled. Floral diagram be drawn. Floral formula should be written. Underline the important identifying characters of the family. Assign the specimen to the family it belongs to.
- For Biochemistry work, follow the procedure as described earlier and complete the work, show at least one observation to the examiner, write the theory procedure and conclusion drawn from the experiment.
- For cytology or temporary mounts follow the procedure described earlier and prepare the slide. Draw neat labelled diagram. Write identifying characters and identify with reasons and submit the preparation to the examiner for evaluation.
- For spotting, three minutes time will be given for each spot. Carry the pencil, eraser etc. before you go for spotting. Draw neat labelled diagram (diagram need not be artistic but should be scientific). Comment on the spot and identify with reasons. Try to complete each spotting within the three minutes schedule.
- One physiology experiment is given alongwith spot identification. Write the aim of the experiment, theory and procedure. Draw neat, labelled diagram of the experimental set-up. Write the inference or conclusion drawn from the experiment.
- During the entire period of examination whenever the candidate faces any problem he/she may seek the help of the Internal / External examiner (one must not try to copy or discuss with other co-candidates).
- Discipline, dedication and determination always bring the candidate all success in examination.

