M.Sc. BOTANY LAB MANUAL

3rd Semester

Prepared By

Biological Science Dept.

Botany

MIDNAPORE CITY COLLEGE

BOT 301
CELL BIOLOGY, GENETICS & BIOTECHNOLOGY

Study of different stages of Mitosis from temporary acetocarmine squash preparation of root-tip cells of onion *(Allium cepa).*

INTRODUCTION:

The reason for using onion root tips for viewing mitosis –

The roots are easy to grow in large numbers, the cells at the tip of the roots are highly dividing and thus many cells will be in stages of mitosis, the chromosomes can be stained to make them more easily observable.

There are three cellular regions near the tip of an onion tip –

i. The **root cap** contains cells that cover and protect the underlying growth region.
ii. The **region of cell division** (or meristem), where cells are actively dividing but not increasing significantly in size.
iii. In the **region of cell elongation**, cells are increasing in size but not dividing.

REQUIREMENTS:

CHEMICALS:

1. 0.1 N HCl (1ml conc. HCl added in 119ml Distilled water).
2. 70 % and 90 % ethanol.
3. Acetic-alcohol fixative (1: 3 acetomethanol fixative). Glacial acetic acid = 1 part
   Ethyl/ methyl alcohol = 3 parts
4. 2 % acetocarmine stain
5. 45 % acetic acid.

MATERIALS:

Slide, Cover slip, Watch glass, squashing needle, spirit lamp, blotting paper, Onion root tips.

PROCEDURE:

Fixation of Root tips :

1. Freshly cut onion root tips (1 cm long) are fixed in acetometanol fixative for overnight in a specimen tube.

2. Then, 90 % ethanol is added after decanting the fixative. Finally 70 % ethanol added after decanting.
   The root tips can be stored in 70 % ethanol for a long period of time in tightly closed container at 40 ^{0}C.

Staining and making squash preparation:

1. Root tips are treated with 0.1%N HCl for 1 min. This will soften the cell wall.
2. Root tips are rinsed in water and transferred to acetocarmine stain for 30 mins.
3. A drop of 45 % acetic acid is taken on a slide having one root tip and left for 1-2 min. If acetic acid drop becomes coloured, it is decanted and a fresh drop is added.
4. A cover glass is placed on the root tip and squashed it using a rubber-end pencil under the folds of a blotting paper.
5. Then edges of the cover glass is sealed with molten was or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
6. Now the slide is observed under microscope.

OBSERVATION:

The following stages of mitosis are observed under microscope.

Interphase : Interphase is also called the resting phase. It is the longest phase.

1. Replication of DNA takes place during this phase.
2. The chromosomes are thinly coiled.
3. Presence of nucleolus and nuclear membrane.

Prophase :

1. The chromatin appears as a network of fine threads. If the cell is in early stage of prophase then the chromatin fibres (chromosomes) are very thin, while the cells at late prophase show comparatively thicker chromatin fibres.
2. Spindle formation is initiated.
3. The nuclear membrane and nucleolus start disappearing at the later stage.

Metaphase :

1. Disappearance of the nucleolus and nuclear membrane.
2. Chromosomes are at their maximum condensed state with two chromatids joined at their centromere.
3. Spindle formation complete.
4. The chromosomes align in the equatorial position of the spindle and form the equatorial pate that is at right angle to the spindle axis.
5. The centromeres are arranged exactly at the equatorial plate.

Anaphase :

1. The centromere of the chromosomes divides and the two chromatids of each pair separate.
2. Each chromatid now represents a separate chromosome and it starts moving towards the opposite poles.
3. The daughter chromosomes assume ‘V’ or ‘J’ shapes or depending upon the position of centromere in them.

Telophase :

1. Chromosomes reach the opposite poles and look like a mass of chromatin.
2. The new nuclear membrane starts to reappear around each set of chromosomes.
3. The nucleolus gets reorganized.

Cytokinesis :

In plants a cell plate is formed in the middle after telophase, finally dividing the cells into two.

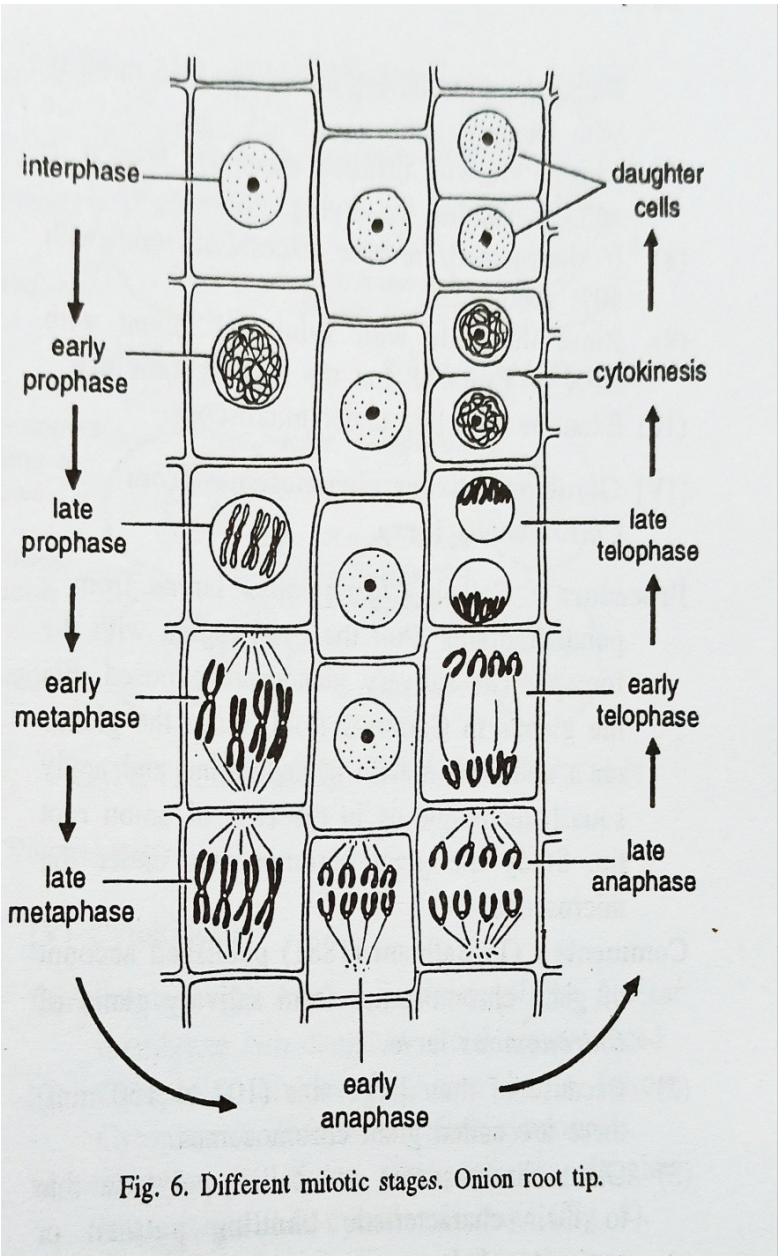


Fig. 6. Different mitotic stages. Onion root tip.

Diagram for practical copy

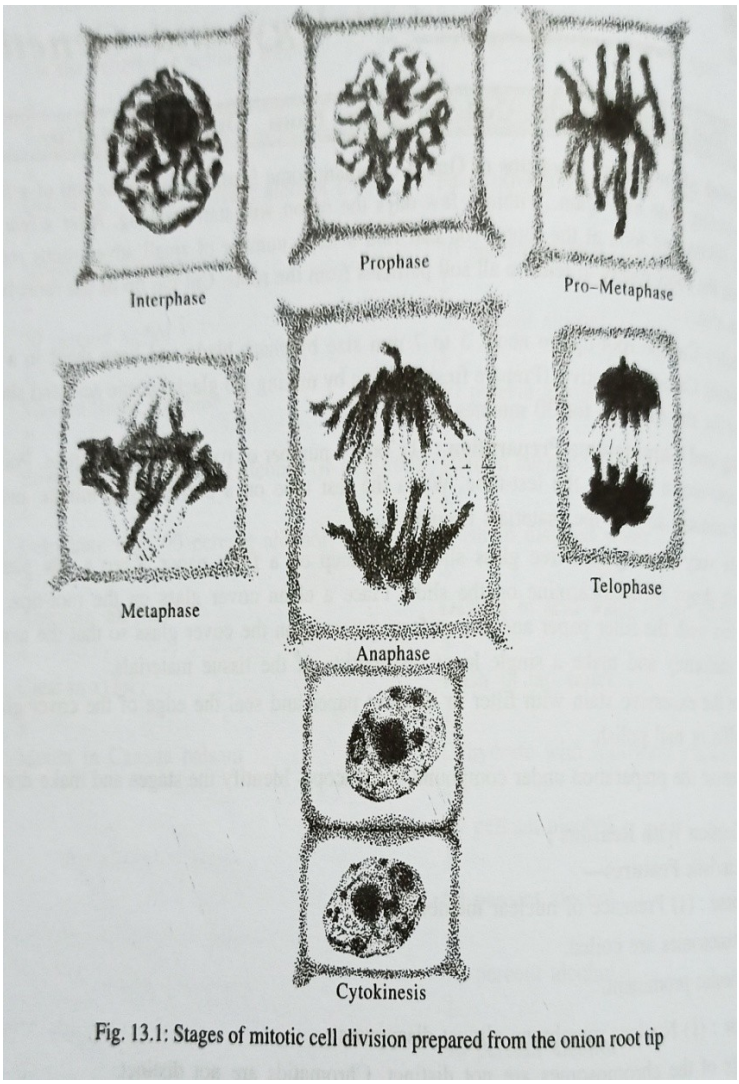
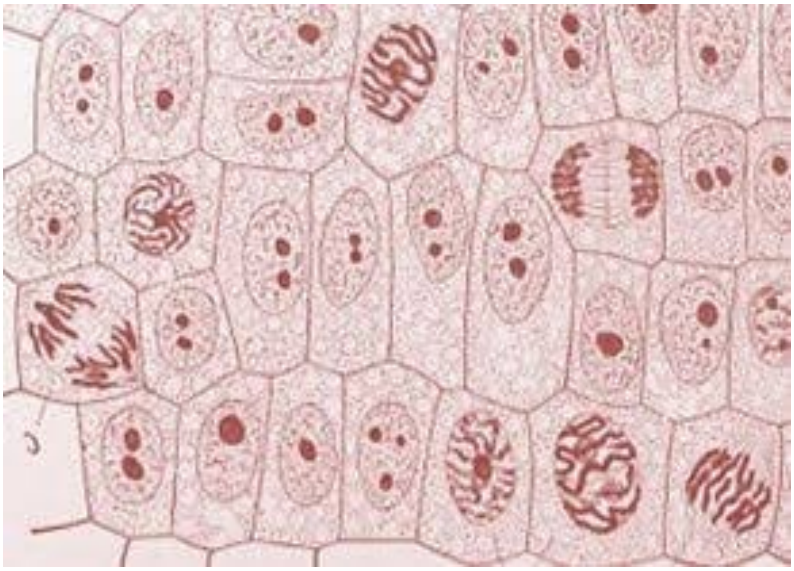
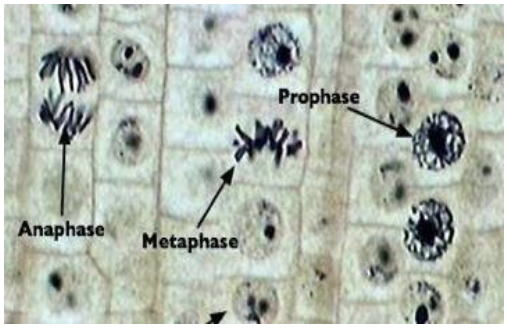
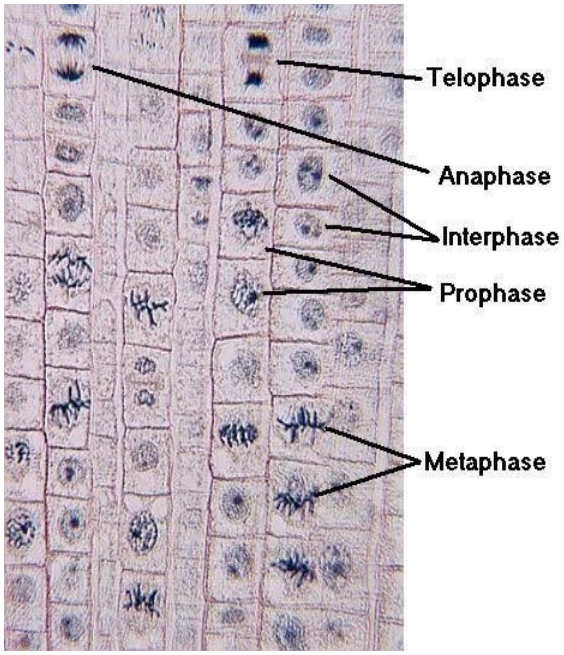


Fig. 13.1: Stages of mitotic cell division prepared from the onion root tip







Mitotic Aberrations

The purpose of this lab will be to explore the effect of a known mitotic inhibitor on the cell division of onion roots. Vicia faba 2n=12 (or Onion Allium cepa 2n=16) roots will be treated with the chemical and then divided among the students for observation. A report will be handed in by each student in the standard lab report form.

Procedure: *Vicia faba* seeds or onion bulbs are rooted in moistened vermiculite at room temperature until 3-4 cm long. Solutions are prepared of maleic hydrazide (0.1, 0.2, 0.4, 0.8, and 1.6 mM) and distilled water control. The rooted bulbs are placed in beakers so only the roots are immersed in the treatment solutions. The roots are allowed to sit in the solutions for 2 hours at room temperature. The bulbs are then rinsed well with tap water and placed back in the vermiculite for a 24 hour recovery period. Roots are then removed and placed in Farmers Fixative for 16 hours at 4oC. Roots are then transferred to 70% ethanol for storage. Hydrolysis: You will be assigned one treatment to score along with one other person, take these roots and transfer them to 1N HCl for 10-12 minutes at 60oC. Staining: Root tips will be stained as before with aceto-Carmine. Scoring: Carefully scan your root tip at 10X and locate the dividing cells. These are the cells of interest and care should be taken to count a representative sample of them. Count 300 cells per root tip and record the number and type of each mitotic cell. To do this without recounting material over again, start on one side of the dividing group, lets say upper left, and count the cells in your field of view and make note of the dividing cells on the form provided. Keep moving to the right one field of view at a time until you come to the end of the area then move down one field of view and continue counting to the left. Aberrations will be scored in anaphase cells. The type of aberrations may give you a clue to the mode of action of this chemical. Add up the columns on the sheet before handing it in to the demonstrator who will photocopy it and return it to you. All the data will be tabulated and put on the course web site so you can make your final calculations for the report.

Result : Include in the report a short introduction, a materials and methods section should refer to the lab manual with any changes in the procedure, a data section with the mitotic indices and inhibition for all the treatments, include a sample calculation for each using you raw data, and a discussion section. Be sure to include the sources of error in your discussions.

Mitotic Index = No of dividing cells/ Total No of cells

% Mitotic Inhibition = Mitotic Index in Control-Mitotic Index in Treatment X 100 Mitotic Index in Control

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 x concentrated) (2) minor salts (200 x concentrated) (3) iron (200 x concentrated) (4) organic nutrients except sucrose (200 x 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 I-lor m moll-I.
6. Store all the stock solutions in appropriate sterile plastic or glass bottles under refrigeration.
7. The iron stock solution should h., 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 HC!.
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 (tubeslflasks) with nonabsorbent 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 l20°C (1.06 kg/cm2) 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

BOT 302
PLANT PHYSIOLOGY, BIOCHEMISTRY& MOLECULAR BIOLOGY

1. **Determination of percentage seed viability of TTC test.**

Triphenyltetrazolium chloride (TTC) is a clear, water soluble compound which is reduced by respiring tissues to yield triphenylformazan, a water insoluble red pigment.

Tetrazolium (TTC), 2,3,5-tri-phenyltetrazolium chloride, stains living tissues red. This chemical can differentiate between viable and non-viable seeds, and an experienced technician can tell exactly how fresh and vigorous a seed is and whether or not vigor is decreasing. Highly viable seeds are uniformly red, whereas seeds of reduced vigor are white and/or speckled white. Deeper red spots, especially on low viability seeds, may represent microbial respiration.

Requirements:

1. 1% TTC solution
2. Seed lots
3. Beaker
4. Test tubes

Procedure:

Triphenyltetrazolium chloride (TTC) is a clear, water soluble compound which is reduced by respiring tissues to yield triphenylformazan, a water insoluble red pigment.

a) Remove the seed coats from six seeds of your assigned seed group.

b) Cut seeds in half and place in container of TTC.

c) Container will be incubated in warm (30°C [86°F]) water for 1 hour.

d) After incubation period, decant off the liquid.

e) Rinse seed halves with distilled water until water is clear.

f) Blot seeds on dry towel; don't crush seeds.

g) Observe colour. Actively respiring tissue will stain red.

Results & Conclusion:

Seed Lot No	No of total seeds	No of Viable seed	% of Viability

Conclusion on the % of viability should be drawn on the basis of Results.

2. Determination of effect of respiratory inhibitor on the rate of Respiration

Requirements:

1. Respiroscope
2. KOH pellets
3. Cotton, Beaker
4. NaF
5. Weigh machine
6. Brine water
7. Eosin
8. Thermometer
9. Gram seeds.

Experiment:

This is a simple apparatus fixed in a wooden frame (Figure 26) which consists of a pair of vertical tubes. The upper end of the tube is wider than the lower end which is graduated. The lower end of the graduated tube dips into a beaker containing coloured brine solution.

The upper bulb-like wider ends of the respiroscopes are closed by means of corks through which pass two small tubes having bent ends. The upper ends, i.e., the outside ends of the bent tubes are fitted with stopcocks.

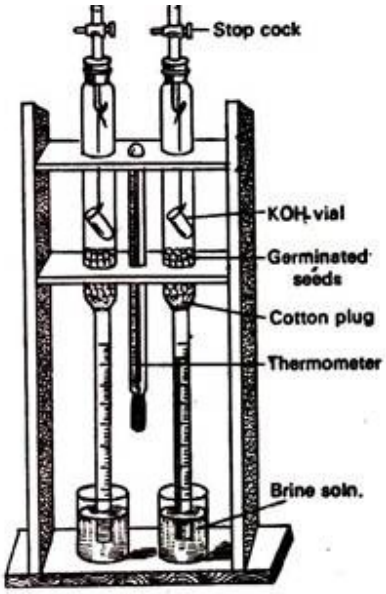


Fig. 26.

From the lower bent ends of the two tubes, two small vials one containing KOH pellets hang within the bulbs.

Equal amounts of germinated seeds are placed within the wider bulbs of the respiroscope on a soaked cotton plug.

Experimental seed lots are pre socked in different concentration of NaF solutions for 30 mins.

The stopcock is then turned to make connection with atmospheric air. The brine solution rises through the graduated end of the respiroscope and becomes stationary at the brine level of the beakers. The stopcock is closed and the level of brine solution in both the tubes is recorded. The rise of brine columns in the tubes is recorded at an interval of 15 minutes for 2 hours.

Results:-

Seed lot type	Conc. of NaF (M)	Time (Mins.)	Initial reading	Final reading	Difference	Avg.	Rate of respiration
Normal	0	15	0				
Experimental 1	0.5	15	0				
Experimental 2	1	15	0				

Conclusion:-

Should be drawn on the basis of results.

4. Determination of isotonic concentration and osmotic pressure of cell sap.

Procedure:

20 ml each of 0 15, 0-20, 0-25, 0-30, 0 35 and 0.40 molar concentrations are prepared from 1 M stock solution of sucrose. All suberized peripheral layers from a fresh potato tuber or beet root are removed and cut into 1 cm x 2 cm pieces using a cork borer and a scalpel.

Each piece is washed in distilled water, blotted well, weighed (initial weight) and transferred to each of the graded solutions of sucrose. These are then left for about an hour. After the stipulated period the pieces are taken out, blotted carefully and reweighed (final weight).

In the above way, the osmotic concentration of fully turgid potato tuber tissue (made turgid by keeping the tissue in water for half an hour previously) and that of partially dehydrated tissue (made partially dehydrated by keeping the tissue in open air for half an hour) are separately determined.

The difference between the osmotic pressures obtained in case of dehydrated and normal tissues and that between normal and turgid tissues give the D.P.D), of dehydrated and normal tissues respectively.

Results:

Conc. Of solution	Initial weight (W1)	Final Weight (W2)	Difference (W2-W1)	%

The percentage increase or decrease in weight of the tissue is determined for each concentration of sucrose solution. Results are plotted on a graph paper taking sugar concentrations as abscissa and the percentage change in weight as ordinate. The concentration of sucrose corresponding, to no-change-in-weight is found out from the graph. This is the isotonic concentration.

This value gives the osmotic concentration of the cell sap when osmotic pressure is equal to the suction pressure (D.P.D.), turgor pressure being zero.

The OP can be calculated using either of the following formulas:

$$(a)\ \text{O. P.} = \frac{22{\cdot}4 \times M \times T°C.}{273}$$

where · O. P. = osmotic pressure in atmosphere,
M = osmotic concentration of the cell sap in molarity,
T°C. = 273 + laboratory temperature,
273 = absolute temperature,
22·4 = osmotic pressure of 1 M sugar solution at N.T.P.

$$(b)\ \text{O. P.} = C \times R \times T$$

where O. P. = osmotic pressure in atmosphere,
C = osmotic concentration of the cell sap in molarity,
R = universal gas constant (0:082),
T = 273 + laboratory temperature.

5. Isolation of chloroplasts and demonstration of Hill reaction.

Introduction:

The splitting of water molecules during the light reactions is a crucial step in photosynthesis. The electrons released from the water molecules will ultimately be used by the photosystems of the light reactions to supply ATP and NADPH, key materials for the synthesis of sugar in the dark reactions. In 1938 Robert Hill of Cambridge University demonstrated that isolated chloroplasts, in the presence of light and a suitable electron acceptor, can split water molecules. We can easily use the classic Hill Reaction to demonstrate:

(1) The release of electrons from split water molecules

(2) The need for light as an energy source in this process and hence photosynthesis in general; (3) the efficiency of different wavelengths of light in carrying out this process.

Requirements:

1. 500 ml beaker
2. Blender
3. spinach leaves (enough to fill beaker)
4. cheesecloth "miracloth"
5. Centrifuge
6. Four 10 ml test tubes
7. Foil cellophane- green and red.

Prepare the following solutions:

1. 0.25M sucrose in tap water

2. 0.02% 2, 6 Dichloroindophenol (the sodium salt) in tap water.

Procedure for Isolating Chloroplasts:

Place 200 ml of 0.25M sucrose solution in the beaker and fill it with loosely packed spinach leaves. Pour the contents of the beaker into a blender and blend at low speed for 10 seconds. Filter the solution of homogenized leaves through a filter made of two layers of cheesecloth and one layer of "miracloth." This filtration will remove the plant fibres but allow the chloroplasts to pass through. Centrifuge the chloroplast solution in an ordinary clinical centrifuge at medium speed for three to five minutes. Pour off the supernatant and suspend the chloroplast pellet in 0.25M of sucrose. Dilute the suspended chloroplast pellet solution to a final volume of 200 ml.

6.Demonstration of the Hill Reaction:

Place 2 ml of the chloroplast suspension in each of the four test tubes. Cover one tube with foil leaving the top open. Cover one tube with green cellophane and one tube with red cellophane, also leaving the tops open. Allow one tube to remain uncovered. Add 0.2 ml of the .02% 2, 6 Dichloroindophenol solution to each tube and mix. Expose the four tubes to light. After an exposure of one to two minutes, the solution in the foil covered tube should be a dark blue-green, the solution in the tube covered with green cellophane should be a light blue-green, and the solutions in the tube covered with red cellophane and the uncovered tube should be very light blue-green to green. 2,6 Dichloroindophenol is a blue dye that accepts electrons given off when water is split. After accepting these electrons, the dye goes from a blue to a colourless state. This experiment, therefore,

demonstrates not only the light requirement for the Hill Reaction, but also the particular effectiveness of those wavelengths of light absorbed by chlorophyll.

7. Extraction and comparative study of chlorophyll levels in leaves of different Chronological ages

Introduction:

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots. From formation to senescence the content of chlorophyll in leaves vary variously. The chlorophyll content of different ages of leaves will be studied here.

Requirements:

1. Leaves of chronological ages i.e. young, mature and old
2. Mortar and pestle
3. Acetone
4. $MgCo_3$
5. Test tubes
6. Funnel
7. Flask
8. Centrifuge
9. Weigh machine
10. Spectrophotometer.

Procedure:

i. One gram of each leaf sample was finely cut and gently mixed with a clean pestle and mortar.

ii. To this homogenized leaf material, 20ml of 80% acetone and 0.5gm MgCO3 powder was added.

iii. The materials were further grind gently. The samples were then put into a refrigerator at 40^0C for 4 hours.

iv. Thereafter, the samples were centrifuged at 500 rpm for 5 minutes.

v. The supernatant was transferred to 100 ml volumetric flak.

vi. The final volume was made up to 100 ml with addition of 80% acetone.

vii. The colour absorbance of the solutions were estimated by a spectrophotometer using 645 and 663nm wavelength against the solvents.

viii. Acetone (80%) was used as a blank.

Results & Conclusion:

Formula:

Chl a= 11.75×A662.6 – 2.35×A645.6

Chl b=18.61×A645.6 – 3.96× A662.6

Where, Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance.

The Chlorophyll contents of the leaves should be calculated and inference from the results will be drawn.

8. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Principle:

The blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components in the Folin –ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's method.

Reagents:

i. Folin –ciocalteau reagent (reagent D)-reflux gently for 10 hours a mixture consisting of 100g Sodium tungstate (Na2WoO4.2H2O), 25g Sodium molybdate (Na2WoO4.2H2O), 700ml water, 50ml of 80% phosphoric acid, and 100ml of concentrated hydrochloric acid in a 1.5L flask. Add 150g lithium sulfate, 50ml water and a few drops of bromine water. Boil the mixture for 15 min without condenser to remove excess bromine. Cool, dilute to 1L and filter. The reagent should have no greenish 20% Sodium carbonate in 0.1N sodium hydroxide (Reagent A).

ii. 0.5% Copper Sulphate (CuSO4.5H2O) IN 1% potassium sodium tartrate (Reagent B).

iii. Alkaline copper solution.: Mix 50ml of A and 1ml of B prior to use (Reagent C)

iv. Protein Solution (Stock Standard):

Weigh accurately 50mg of bovine serum albumin (fraction V) and dissolve in distilled water and make up to 50ml in a standard flask.

v. Working Standard Solution:

Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask. 1.0ml of this solution contains 200µg protein.

Procedure

Extraction of protein from Sample:

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind well with a pestle and mortar in 5-10mL of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of Protein:

1. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard into a series of test tubes.

2. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes.

3. Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0ml of water serves as the blank.

4. Add 5.0 ml of reagent C to each tube including the blank. Mix well and allowed to standing for 10mins.

5. Then add 0.5 ml of reagent D, Mix well and incubate at room temperature in the dark for 30min, blue colour is developed.

R & C: Take the reading at 660nm. Draw a standard graph and calculate the amount of protein in the sample.

9. ESTIMATION OF AMINO ACIDS (Ninhydrin method)

Principle

Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intencsly coloured bluish purple product which is colormetrically measured at 570 nm.

Reagents

i. Dissolve 50mg leucine in 50ml of water in a volumetric flask. Take 10ml of this stock standard and dilute to 100ml in another volumetric flask for working standard solution. A series of volume from 0.1-1 ml of this standard solution gives a concentration range 10 µg-100µg. Proceed as that of the sample and read the colour.

ii. Ninhydrin: Dissolve 0.8 stannous chloride in 500 ml of 0.2 M citrate buffer (pH 5.0). Add this solution to 20g of ninhydrin in 500ml of methylcellosolve (2 methoxyethanol)

iii. 0.2M Citrate buffer pH 0.5

iv. Diluent solvent: Mix equal volumes of water and n-propanol and use.

Procedure

1. To 0.1 ml of extract, add 1ml of ninhydrin solution

2. Make up the volume to 2ml with distilled water

3. Heat the tube in a boiling water bath for 20min.

4. Add 5ml of the diluents and mix the contents.

5. After 15min reat the intensity of the purple colour against a reagent blank in a colorimenter at 570 nm. The colour is stable for 1h.

6. Prepare the reagent blank as above by taking 0.1ml of 80% ethanol instead of the extract.

Results & Conclusion:

Plot the obtained OD of the unknown sample on the Standard curve (Y axis) and draw a perpendicular line on the X axis. This will give you the concentration of the unknown sample.

10. ESTIMATION OF CARBOHYDRATE

The total carbohydrate content can be estimated by the method of Hedge and Hofreiter, 1962.

Principle

Carbohydrate is first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxmethyl furfural. This compound forms with anthrone a green coloured product with absorption maximum at 630 nm.

Reagents

1. Glucose stock standard: 100 mg of glucose was dissolved in 100 ml of water in a standard flask.

2. Working standard: 10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose.

3. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use.

4. 2.5 N HCl.

Procedure

Weighed 100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceas made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.2 to 1.0 ml for analysis. Prepared the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm.

Calculation

A standard graph will be drawn by taking the concentration of glucose on X axis and spectrophotometer reading (OD value) on Y axis. From the graph the concentration of glucose in the sample can be calculated.

BOT 396.1: ECOLOGY & ENVIRONMENTAL BIOLOGY Marks: 25

1. Study of Raunkier's life forms and biological spectrum.

The species of a community can be grouped into several life-forms on the basis of general appearance and growth. The life-forms of the vegetation, to a certain extent act as indicators of the climate.

This idea has been extensively developed by a Danish botanist Raunkiaer (1934). He considered that it is the un-favourable environmental condition which exerts a major control over the growth form and he stressed the significance of the adaptations of buds and shoot tips for overcoming adverse temperatures and survival in droughty A classification of plants on the basis of the types and kinds of perennating organs seems to be natural Raunkiaer called the kinds and types of these organs as life-forms.

Raunkiaer employed three guiding rules in the selection of life-form characteristics:

(1) The character must be structural and essential and must represent important morphological adaptation.

(2) The character must be sufficiently clear so that one can easily see in nature.

(3) All the life-forms employed must be of such nature as could constitute a homologous system and represent a single point of view or aspect of plants and thus enable a comparative statistical treatment of flora of different communities.

On the basis of these principles, he recognized live life-form groups.

These groups are as follows:

(1) Phanerophytes (2) Chamaephytes (3) Hemicryptophytes (4) Cryptophytes(5) Therophytes

(1) Phanerophytes: In these, the perennating buds are located on the twigs and branches and are exposed to the atmosphere during un-favourable periods. Plants are mostly woody (trees and shrubs), although lianas, epiphytes and some large perennial herbs can be described under this group.

According to their heights, phanerophytes are divided into the following classes:

(i) Megaphanerophytes; Perennial parts including buds are more than 30 metres in height. (ii) Mesophanerophytes; Between 8 and 30 metres height.

(iii) Microphanerophytes; Between 2 and 8 metres height.

(iv) Nanophanerophytes; Under 2 metres and over 25 cm tall.

The above height classes are further divided according to the conditions whether the meristematic tissue of buds are protected by bud scales or they are naked and whether the plants are evergreen or deciduous.

(2) Chamaephytes: Buds or shoot apices are situated on shoots that lie on or near the ground surface. Plants of this class are less than 25 cm in height but their perennating buds are found

definitely above the soil surface. During the un-favourable seasons, the buds are protected by the dead fallen leaves and on higher altitudes they are protected by snow.

Chamaephytes are divided into the following classes:

(i) Subfructicose chamaephytes:

Buds are protected by dead plant remains. Examples— Caryophyllaceae, Labiatae, Leguminosae, etc.

(ii) Passive chamaephytes:

Shoots lie on the ground surface. Examples: *Sacxifraga, Polygonum, Sedum,* etc.

(iii) Active chamaephytes:

Buds lie on the ground and are protected by snow under the unfavourable conditions.

Example: *Vinca*.

(iv) Cushion chamaephytes:

They represent transition stage from chamaephytes to hemicryptophytes. In this group the shoots are closely packed, for example, *Azorella.*

(3) Hemicryptophytes:

They are found on the soil surface and buds and shoots are protected by soil and dead leaves.

They are herbaceous and spread on the surface horizontally as runners. The group is divided into protohemicryptophytes, partial rosette plants, and rosette plants.

(4) Cryptophytes:

In this group, the buds and shoots of plants are underground. Reserved food material is stored in the subterranean perennating organs.

The class is divided into the following categories:

(i) Geophytes (ii) Helophytes (iii) Hydrophytes

(i) Geophytes:

They are land plants with subterranean perennating buds.

They are of following types:

(I) Rhizome geophytes. (II) Stem tuber geophytes, (III) Bulb geophytes, (IV) Root tuber geophytes, (V) Root geophytes.

(ii) Helophytes:

These are the plants of water saturated soil. The leaves and flowers emerge above the ground but the rhizomes are subterranean.

(iii) Hydrophytes:

They are water plants, for example—Eichhomia, Nelumbium, etc.

(5) Therophytes:

This group includes those annual summer plants which survive under un-favourable seasons in the form of seeds. In summer, all the pats of plants die except seeds and fruits. Thus, the perennating bud is actually the embryo of the seed.

Biological Spectrum or Phytoclimatic Spectrum:

Raunkiaer's system is unitary being based completely on the position and protection of perennating buds during un-favourable seasons. The characters are structural, essential and adaptive. Furthermore, they provide simple basis for statistical treatment. The array of percentages of life- form classes of a flora of any area composing any floristic community is called the biological spectrum or phytoclimatic spectrum. Since each life-form class is related to the environment, the biological spectrum is direct indicator of its environment.

In biological spectrum, the life-form classes are represented by their percentages. Raunkiaer prepared a normal spectrum based on sampling of world flora using one thousand entities. The normal spectrum provides a base line from which the departure of percentages of any class in any given flora can be ascertained.

The normal spectrum has:

Phanerophytes 46%

Chamaephytes 9%

Hemicryptophytes 26%

Cryptophytes 6%

Therophytes 13%

The biological spectra of a given area is worked out and compared with Raunkiaer's normal spectrum. The percentage of phanerophytes in different floras ranges from zero to over 74% in the tropical rain forest. The higher percentage of phanerophytes is indicative of phanerophytic climate. The higher percentage of therophytes (above 40%) indicates therophytic climate (desert). High percentage of chamaephytes (above 50%) indicates an extremely cold climate.

High percentage of hemicryptophytes in temperate forest vegetation indicates the conditions favourable for the development of extensive grassland. Geophytes (cryptophytes) are abundant in the regions of Mediterranean climate and in the broad leaf deciduous forest. The biological spectra of different areas differ from one another and there is no base line for comparison.

[There are certain limitations to usefulness of biological spectrum as are indicative of climatic condition, because at certain places it does not indicate the environmental conditions, for example, therophytes are abundant in Indogangetic planes where phanerophytic vegetation should actually be dominant. Biotic agencies are the chief causes for changing the biological spectrum in a given floristic zone. Thus comparison of biological spectrum with normal spectrum may sometimes create confusions and may also lead to wrong conclusions.]

Results:

Table-1: List of the plants and their category

Table 2: Distribution of plants in different categories.

+ Comparison of Obtained spectra with the Normal spectra.

Conclusion: Draw conclusion as per results.

2. Study of frequency, abundance and density, IVI of plants following standard method.

Aim of the Experiment:

To study communities by quadrat method and to determine % Frequency, Density and Abundance.

Finally, to calculate the importance values of the species within the community.

Requirements:

Metre scale, string, four nails or quadrat, notebook.

(i) Frequency:

Frequency is the number of sampling units or quadrats in which a given species occurs.

Percentage frequency (%F) can be estimated by the following formula:

$$\%\ \text{frequency (F)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

(ii) Density:

Density is the number of individuals per unit area and can be calculated by the following formula:

$$\text{Density (D)} = \frac{\text{Total number of individuals}}{\text{Total number of quadrats studied}}$$

(iii) Abundance:

Abundance is described as the number of individuals per quadrat of occurrence.

Abundance for each species can be calculated by the following formula:

$$\text{Abundance (A)} = \frac{\text{Total number of individuals}}{\text{Number of quadrats of occurrence}}$$

Method:

Lay a quadrat in the field or specific area to be studied. Note carefully the plants occurring there. Write the names and number of individuals of plant species in the note-book, which are present in the limits of your quadrat. Lay at random at least 10 quadrats (Fig. 69) in the same way and record your data in the form of Table 1.

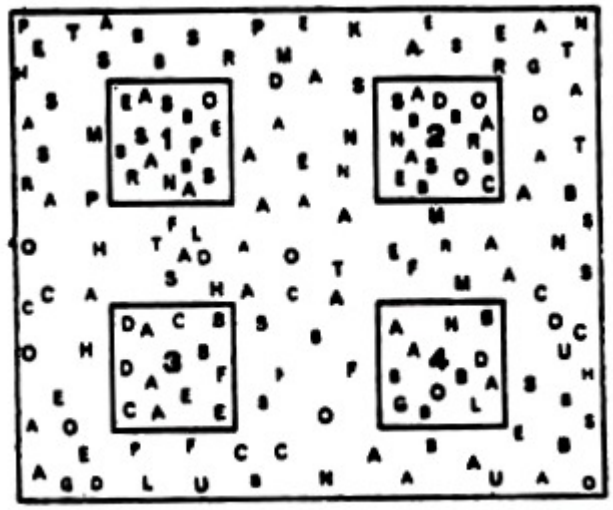


Fig. 69. Sketch of an artificial field showing four quadrats (1–4).

Observation:

Put all the observation in a table (Table 1) to calculate F, A, and D directly.

Table 1:

S. No.	Name of plant species	Number of individuals in quadrat number										Total number of quadrats of occurrence	Total number of quadrats studied	Total number of individuals	% Frequency (F)	Density (D)	Abundance (A)
		1	2	3	4	5	6	7	8	9	10						
1.	*Cyperus*	10	9	7	0	0	3	8	15	0	7	7	10	60	70%	6	8.57
2.	*Cassia*	0	0	2	0	3	0	5	0	6	10						
3.	*Cynodon*	50	0	7	41	6	0	0	8	0	5						
4.	*Eclipta*	0	0	4	0	3	0	0	1	0	2						
5.	A	0	0	0	0	2	0	0	1	3	0						
6.	B	5	10	1	0	0	0	3	1	0	2						
7.	C	3	5	0	0	2	1	8	0	2	0						

Results:

Arrange percentage frequency of different species of the above in the five frequency classes (A-E) as formulated by Raunkiaer (1934) in Table 4.4.

Table 4.4 : Classes of species in a community according to Raunkiaer (1934).

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

Draw a histogram (Fig. 70) with the percentage of total number of species plotted on Y-axis and the frequency classes (A-E) on X-axis. Compare your data with the Normal frequency.

This is the frequency diagram (Fig. 70):

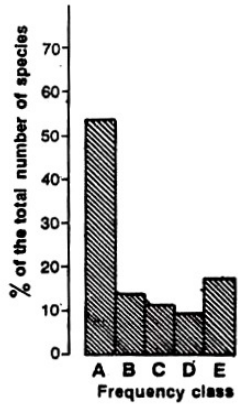


Fig. 70. Histogram showing the normal frequency diagram.

Calculation of IVI:

Importance Value Index (IVI) gives the overall picture of ecological significance of any plant species in a community. The percentage values of relative frequency, relative density and relative dominance are added together and this value out of 300 is taken as the IVI o f a species (Curtis, 1959; Misra, 1968).

$$\text{Relative Frequency} = \frac{Number\ of\ occurrence\ of\ the\ species}{Number\ of\ occurrence\ of\ all\ species} \times 100$$

$$\text{Relative Density} = \frac{Number\ of\ Individuals\ of\ the\ species}{Number\ of\ individuals\ of\ all\ species} \times 100$$

Relative Dominance = $\frac{Total\ Basal\ area\ of\ the\ species}{Total\ basal\ area\ of\ all\ the\ species} \times 100$

IVI= RF + RD+ RDo

---

3. Ecological study on plant adaptation.

Morphological adaptive features should be pointed out from the given specimen and thin section of the supplied specimen should be made to observe the anatomical details.

Some of the prominent anatomical adaptive features are:

i. Presence of thick cuticle

ii. Sunken stomata

iii. Multi-layered epidermis & hypodermis

iv. Presence of mechanical tissue

v. Specialised water retention tissue

vi. Well-developed vascular tissue

vii. Prominent secondary growth

…….Hence the specimen shows Xerophytic adaptation

i. Thin cuticle layer/ Wax layer

ii. Single layered epidermis

iii. Less no of stomata

iv. Presence of arenchyma

v. Poorly developed vascular tissue

vi. Minimum secondary growth

.....Hence the specimen shows Hydrophytic adaptation

(Respective diagrams should be drawn with features pointed out)