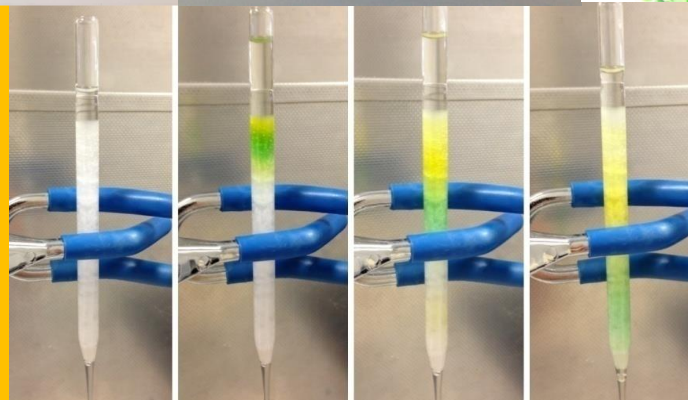
MSCBOT-605L

M. Sc. III Semester

LABORATORY PRACTICAL



DEPARTMENT OF BOTANY

SCHOOL OF SCIENCES

UTTARAKHAND OPEN UNIVERSITY

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BLOCK-1-PLANT PHYSIOLOGY AND BIOCHEMISTRY

UNIT-1: DETERMINE THE OSMOTIC PRESSURE (POTENTIAL) AND CALCULATE THE ISOTONIC COEFFICIENT OF SUGAR

1.1 Objectives
1.2 Introduction
  1.2.1 Osmotic pressure (Potential)
1.3 A method for measuring osmotic pressure of sugar solution
  1.3.1 Equipments
  1.3.2 Procedure
  1.3.3 Problem of osmotic pressure (potential) of sugar solution
1.4 Applications of osmotic pressure
1.5 Procedure of isotonic coefficient
1.6 Other method of isotonic solution
  1.6.1 Water vapor pressure method
  1.6.2 Freezing point depression method of isotonic coefficient
1.7 Example of isotonic cofficient
  1.7.1 Determination of isotonic coefficient and dissociation degree of electrolyte NaCl 0.9%
1.8 Summary
1.9 Glossary
1.10 Self Assessment Questions
  1.10.1 Multiple Choice Questions
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1.12 Suggested Readings
1.13 Terminal Questions

1.1 OBJECTIVES

After reading this unit students will be able-

- To determine the osmotic pressure (potential) and calculate the isotonic coefficient of sugar.

1.2 INTRODUCTION

1.2.1. Osmotic pressure (Potential)

Osmotic pressure can be defined as the minimum pressure that must be applied to a solution to arrest the flow of solvent molecules through a semipermeable membrane as was noted by Donald *et al.,* (2001). It is a colligative property and it is dependent on the concentration of solute particles in the solution. Osmotic pressure (potential) is the pressure required to stop water from diffusing through a membrane by osmosis. It is determined by the concentration of the solute. Water diffuses into the area of higher concentration from the area of lower concentration. When the concentration of the substances in the two areas in contact is different, the substances will diffuse until the concentration is uniform throughout. Osmotic pressure can be calculated using the following equation:

$$\Pi=MRT$$

Where= Π (Capital Pi) denotes the osmotic pressure, M is the molar concentration of the solute, R= is the gas constant, T= is the temperature.

This relationship between the osmotic pressure of a solution and the molar concentration of its solute was put forward by the Dutch chemist Jacobus van't Hoff.

It is also defined as the measure of the tendency of a solution to take in pure solvent by osmosis. Osmotic pressure (potential) is the maximum osmotic pressure that could develop in a solution if it were separated from its pure solvent by a semipermeable membrane. Osmotic pressure is closely related to some other properties of solutions as the colligative properties such as freezing point depression, boiling point elevation & vapor pressure depression**,** all caused by dissolving solutes in a solution. The osmolarity is often determined from vapor pressure depression or freezing point depression, rather than from direct osmotic pressure measurements. The osmolarity is the concentration of a solution necessary to observe these phenomena.

1.3 A METHOD FOR MEASURING OSMOTIC PRESSURE OF SUGAR SOLUTION

1.3.1 Equipments

Osmometer, sample cell, chamber body membrane, meandering dialyzate, base plate, support bracket L-shaped bore, stir rod blade, bushing, pressurized gas source, pressure regulator,

ressure relief valve, handle, bore, concentric channels, transparent dialyzate exit tube, frame, magnetic stirring/hot plate, sample solution (sucrose), meniscus and thermometer etc.

1.3.2 Procedure

- **A.** Placing a sample of a solution into a sample cell above a membrane supported in the sample cell.
- **B.** Sealing the sample in the sample cell.
- **C.** Introducing gas at an elevated pressure to the sample cell to drive a quantity of dialyzed through the membrane and into a transparent dialyzed exit tube adapted to receive the dialyzed which passes through the membrane.
- **D.** Varying the elevated pressure of the gas to yield a substantially stationary dialyzed meniscus in the transparent dialyzed exit tube.
- **E.** Reading the pressure gauge showing the elevated pressure of the gas that yielded the substantially stationary dialyzed meniscus, the elevated pressure of the gas being substantially equivalent to the osmotic pressure of the sample.
- **F.** Opening the sample cell and adding additional sample solution.
- **G.** Weighing the sample cell with the additional sample solution.
- **H.** Repeating steps (b) through (d).

1.3.3 Problem of osmotic pressure (potential) of sugar solution

Example: What is the osmotic pressure of a solution prepared by adding 13.65 g of sucrose ($C_{12}H_{22}O_{11}$) to enough water to make 250 ML of solution at 25°C?

Solution: Osmotic pressure is expressed by following formula:

$\Pi = iMRT$ (note how it resembles the $PV = nRT$ form of the Ideal Gas Law)

Where: Π is the osmotic pressure in atm

i = van't Hoff factor of the solute
M = molar concentration in mol/L
R = universal gas constant = 0.08206 L·atm/mol·K
T = absolute temperature in K

Step 1: Find the Concentration of Sucrose

Look up the atomic weights of the elements in the compound from the **periodic table.**

C = 12 g/mol H = 1 g/mol O = 16 g/mol

Use the atomic weights to find the molar mass of the compound. Multiply the subscripts in the formula times the atomic weight of the element. If there is no subscript, it means one atom is present.

Molar mass of sucrose = 12(12) + 22(1) + 11(16)

Molar mass of sucrose = 144 + 22 + 176

Molar mass of sucrose = 342

$N_{sucrose}$ = 13.65 g x 1 mol/342 g

$n_{sucrose}$ = 0.04 mol

$M_{sucrose} = n_{sucrose}/\text{Volume}_{solution}$

$M_{sucrose}$ = 0.04 mol/ (250 mL x 1 L/1000 mL)

$M_{sucrose}$ = 0.04 mol/0.25 L

$M_{sucrose}$ = 0.16 mol/L

Step 2: Find absolute temperature:

Remember, absolute temperature is always given in Kelvin. If the temperature is given in Celsius or Fahrenheit, convert it to Kelvin.

T = °C + 273

T = 25 + 273

T = 298 K

Step 3: Determine the van't Hoff factor

Sucrose does not dissociate in water; therefore the van't Hoff factor = 1.

Step 4: Find the Osmotic Pressure:

To find the osmotic pressure, plug the values into the equation.

$\Pi = iMRT$

Π = 1 x 0.16 mol/L x 0.08206 L·atm/mol·K x 298 K

Π = 3.9 atm

Answer: The osmotic pressure of the sucrose solution is 3.9 atm.

1.4 APPLICATIONS OF OSMOTIC PRESSURE

Osmotic pressure measurement may be used for the determination of molecular weights. Osmotic pressure is an important factor affecting cells. Osmoregulation is the homeostasis mechanism of an organism to reach balance in osmotic pressure.

A. Hypertonicity is the presence of a solution that causes cells to shrink.

B. Hypotonicity is the presence of a solution that causes cells to swell.

C. Isotonicity is the presence of a solution that produces no change in cell volume.

1. When a biological cell is in a hypotonic environment, the cell interior accumulates water, water flows across the cell membrane into the cell & causing it to expand.
2. In plant cells, the cell wall restricts the expansion, resulting in pressure on the cell wall from within called turgor pressure.
3. Turgor pressure allows herbaceous plants to stand upright.
4. It is also the determining factor for how plants regulate the aperture of their stomata.
5. Osmotic pressure is the basis of filtering (reverse osmosis), a process commonly used in water purification.
6. The water to be purified is placed in a chamber & put under an amount of pressure greater than the osmotic pressure exerted by the water and the solutes dissolved in it.
7. Part of the chamber opens to a differentially permeable membrane that lets water molecules through, but not the solute particles.
8. The osmotic pressure of ocean water is about 27 atm. Reverse osmosis desalinates fresh water from ocean salt water.

1.5 PROCEDURE OF ISOTONIC COEFFICIENT

Isotonic coefficient is the proportionality coefficient between the total concentration of a solute and concentration of solute particles. Particle concentration can be calculated from solute concentration where: i= is the isotonic coefficient.

"i" - **isotonic coefficient** or **coefficient** of Van't - Hoff i = 1 + (n - 1) Where: α = the degree of dissociation; n= the number of particles formed as a result of dissociation (fig.1.1).The phenomenon of osmosis is widespread in living organisms. Their total effect is determined by osmotic pressure. Particle concentration can be calculated from solute concentration Ctotal as:

Cparticles= **i***Ctotal.

Where: **I=** is the isotonic coefficient.

Cparticles =is the concentration of solute particles.

Ctotal = is the total concentration of solute.

In other words, i shows, how many times particle concentration exceeds solute concentration: **i** >1.

i= Cparticles/Ctotal.

In solutions of non-electrolytes, where solute doesn't dissociate into ions, the smallest particle is molecule, therefore particle concentration is equal to solute concentration and i = 1. In solutions of electrolytes molecules of solute dissociate into ions and dissociation is characterized by dissociation.

degree **α**.

α= ndiss/ntotal

α=Cdiss/Ctotal,

Where: ndiss., Cdiss. & ntotal, Ctotal ndiss. & Cdiss. are number and concentration of dissociated molecules respectively, ntotal and Ctotal are total number and total concentration of molecules respectively.

Dissociation degree **α** can be expressed either as usual decimal number. For example both expressions **α** = 0.04 or **α** = 4% mean that 4 molecules out of every 100 molecules are dissociated in ions. Nevertheless, if dissociation degree has to be used in further calculations, it has to be transformed into a decimal number. Using the above expression of **α** the concentration of dissociated molecules is found as the total concentration of solute, multiplied by dissociation degree Cdiss = **α** Ctotal. Our task is to express the isotonic coefficient (i) through dissociation degree **α** and the total concentration of solute. Let us first note that the concentration of particles includes both the concentration of non-dissociated molecules and the concentration of ions:

Cparticles = Cnondiss.mol. + Cions.

The concentration of nondissociated molecules can be expressed as:
Cnondiss = Ctotal - Cdiss = Ctotal - **α***Ctotal

To express the concentration of ions, let us first invent a parameter m, which is the number of ions, formed:

$FeCl_3 \longrightarrow Fe^{3+} + Cl^- + Cl^- + Cl^-$ (1 + 1+ 1+ 1) = 4 = **m** ions

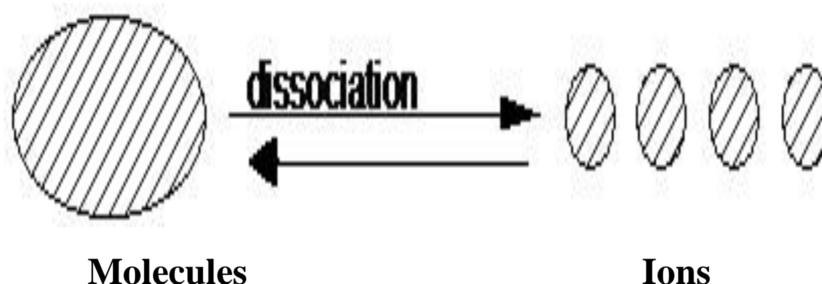

Fig.1.1 Dissociation of molecules

For example, at dissociation of one solute molecule for **NaCl m**=2 one **Na+** and one **Cl-** ion are formed at dissociation of one molecule.

For example, for **K3PO4** m = 4, as three **K+** ions and one **PO43-** ion are formed. As **m** ions are created at dissociation of one molecule, concentration of ions is **m** times greater, than concentration of dissociated molecules: **Cions= m Cdiss= α m Ctotal**. Inserting the meaning of **Cnondiss** and **Cions** into fig 1.2 we have:

$$\frac{C_{total} - \alpha \bullet C_{total} + m \bullet \alpha \bullet C_{total}}{C_{total}}$$

I = (3.2)
i = 1 – α + m* α
i = 1 + α (m–1)

1.6 OTHER METHOD OF ISOTONIC SOLUTION

1.6.1 Water Vapor Pressure method

Water vapor pressure is the first of the colligative properties of solutions that we have to deal with. Let us compare the vapor pressures of pure water and solution. When pure **water** is in contact with gas phase, two reverse processes proceed at the same time:

1. **Water** molecules leave the surface of liquid phase (evaporate) and transfer into gas phase,
2. As soon as there are **water** molecules in the gas phase, they start to condense and to return back into liquid phase. After some time an equilibrium is reached, at which the rates of evaporation and condensation are equal and a certain value of **water**'s vapor pressure **p°=Keq** is reached and as solute **X** present **pH2O/(1– Nx) =Keq(equilibrium constant).** Fig.1.2.

Now let us consider a solution of a non-fugitive solute ● instead of pure **water**, see fig. (1.2). The same two processes occur, but equilibrium is reached. At this case the upper layer of liquid phase doesn't consist only of **water** molecules ○ (empty dots ○ in fig. 1.2), but solute ● particles (filled dots ●) are present. For this reason the number of **water** molecules in the upper layer of liquid is smaller, than for pure **water**, the rate of evaporation is smaller and the equilibrium will be therefore reached at a smaller vapor pressure. Thus, the vapor pressure **p** above solution is smaller, than vapor pressure **p°** above pure **water** and difference value is depression $(p° - p_{H2O}) = \Delta p$.

Because of the reasons mentioned above the vapor pressure of **water** must be different at different concentrations of solute particles-the greater is the concentration of solute particles, the less **water** molecules remain in the surface layer of solution. **Wate**rs' vapor pressure (fig.1.2) above pure **water** (a) and solution (b). The dependence of **water**s' vapor pressure on the concentration of solute is described quantitatively by Roul's I law, which states, that: *Relative depression of* ***water****'s vapor pressure is equal to molar fraction of solute particles.*

$$\frac{p° - p_{H_2O}}{p°}$$

$$P° > p$$

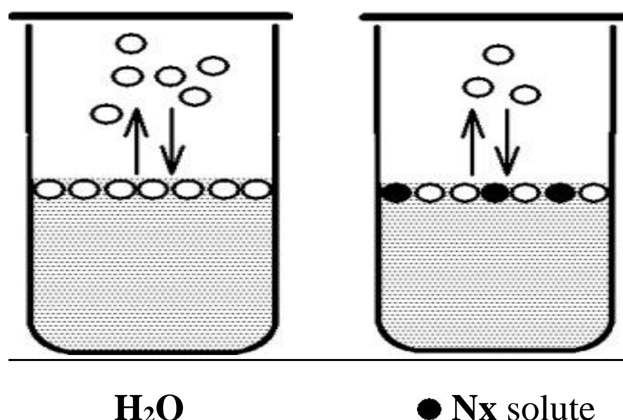

Fig. 1.2 Waters' Vapor Pressure method

Mathematical form of this law is where: p°=pH2O/(1-Nx) is the equilibrium constant Keq,(p°-pH2O)/p° = Δp/p° is called the relative depression of vapor pressure, nx and nH2O are numbers of moles of solute particles and water respectively. If the solute X is a non-electrolyte, Nx=Nsolute as the solute X is present only in molecular form, for electrolytes Nx =i Nsolute, because the number of particles is i times greater than number of molecules. Looking at fig.1.2 one can also understand that the greater is concentration of solute; the lower will be water's vapor pressure

1.6.2 Freezing point depression method of isotonic coefficient

Freezing point of liquid water is the intersection point of vapor pressure curves above liquid water and ice (fig.1.3). In order to see difference between freezing points of pure water (water) and solution, one has to show vapor pressure curves of pure water (water) and solution in the same diagram. As it is clear from previous considerations (see fig. 1.2), vapor pressure of solution is lower, than vapor pressure of pure water. As this is true at any temperature, the entire vapor pressure curve for solution lies lower than for pure water. As the result of this, the intersection point between vapor pressure of solution and vapor pressure of ice (the freezing point of solution) lies at lower temperature, than for pure water-the freezing point is shifted towards lower temperatures (depressed). From considerations, discussed above, one can see that the greater is the concentration of solute, the lower lies the vapor pressure curve of solution and the more freezing point of solution would be depressed when compared to pure water.

Mathematically the connection between freezing point depression and concentration of solute is expressed by II Roul's law states:

Δt freezing = iKcrCm

Freezing point depression is proportional to molality of solute, where **Δt**=0°- t_{fr} is the difference between freezing temperatures of water and solution, **Cm** is molality of solute (number of solute moles in 1000 grams of **water**), **Kcr** =**1.86** is the *cryoscopy constant* of the **water**. Cryoscopy constant of **water** 1.86 shows the freezing point depression in a 1 molal non-electrolyte solution (where **i** = 1) freezes at

temperature –1.86°C less zero 0° C. Cryoscopy constant is a constant value for each given **water** and it doesn't depend on the properties of solute, because the freezing point depression as a colligative property is affected by concentration of particles, but not by their nature. In fact, Roul's laws are strongly valid only for diluted solutions, while it is possible to ignore the interaction of solute particles. For this reason one can use molarity instead of molality for approximate calculations - **Cm≈ CM** in diluted solutions.

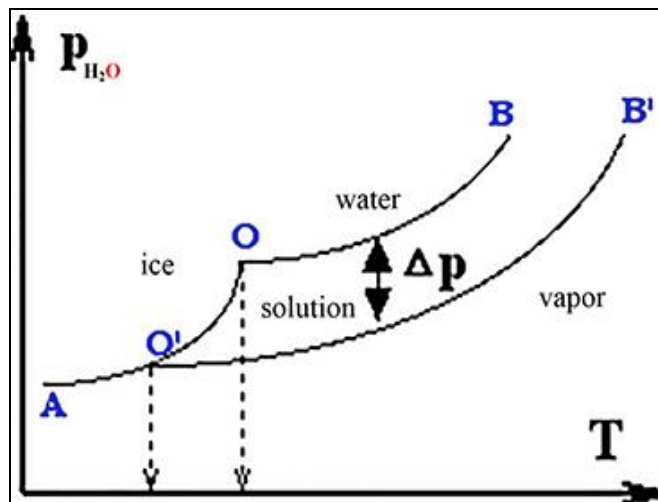

Fig.1.3 Freezing point depression method

1.7 EXAMPLE OF ISOTONIC COFFICIENT

1.7.1 Determination of isotonic coefficient and dissociation degree of electrolyte NaCl 0.9%

To determine the dissociation degree of physiological solution electrolyte NaCl as is prepared known concentration 0.9%, its freezing point depression is measured t freezing=-0.567° C and i is calculated from Roul's 2nd law, knowing the water value of cryoscopy constant Kcr = 1.86.

As i = 1 + a (m-1), dissociation degree can be expressed as Δtfrezing= i · Cm · Kcr if is 0.9% mass fraction physiology solution of NaCl ρ=1.000 g/mL:

mNaCl = $\frac{w_{\%} \bullet m_{solution}}{100\%} = \frac{0.9\% \bullet 1000g}{100\%}$ = 9 g ;Cm = $\frac{m_{NaCl}}{M_{NaCl} \bullet V} = \frac{9g}{58.5g/mol \bullet 1L}$ = 0.1538

M ;i = $\frac{\Delta t_{freezing}}{C_m \bullet K_{cr}} = \frac{0.567}{0.1538 \bullet 1.86}$ = 1.982 and sodium chloride NaCl dissociation degree

is alpha= $\frac{i-1}{m-1} = \frac{1.982-1}{2-1}$ = 0.982 and in percentage is alpha =98.2%.

1.8 SUMMARY

Regarding the nature of osmotic pressure, widely divergent views have been expressed. One theory places the cause of the phenomenon in the greater bombardment of the membrane by the solute particles than by the solvent particles. The other prominent theory considers the solvent as playing the chief role, and is known as the hydrostatic theory. Osmotic pressure is defined as the difference of pressure on solution and solvent, which produces a condition of equilibrium, such that there is no tendency of the solvent to flow in either direction. Van't Hoff inclined to the kinetic theory, but did not commit himself. He showed the similarity between the mathematical expression of the gas law and the mathematical expression of the osmotic law. The osmotic value of a given cell sap is the molal concentration of an agent which is in dynamic equilibrium with the cell sap.

Plants maintain their upright shape with the help of osmotic pressure. When sufficient water is supplied to the plant, its cells (which contain several salts) absorb water and expand. This expansion of plant cells increases the pressure exerted on their cell walls, causing them to stand upright. When insufficient water is supplied to the plant, its cells become hypertonic. This causes them to wilt and lose their firm, upright posture. Osmotic pressure can also be used to determine molecular weights of compounds and in the purification of seawater, which involves the process of reverse osmosis.

Osmotic pressure is of vital importance in biology as the cell membrane is selective toward many of the solutes found in living organisms. When a cell is placed in a hypertonic solution, water actually flows out of the cell into the surrounding solution thereby causing the cells to shrink and lose its turgidity. Osmosis and dialysis are of prime importance in living organisms, where they influence the distribution of nutrients and the release of metabolic waste products. Osmotic pressure is only permanent if the membrane is truly semipermeable. In case of dialysis, if the collodion or cellophane bag is filled with a solution of a dye with small molecules and placed in contact with water, now water will pass into the dye, but, at the same time, water plus dye will pass out from the bag. As water molecules are smaller than the dye molecules, water will pass into the bag more quickly than water plus dye will leave it.

Therefore, an osmotic pressure will be developed, but it will only be small and transient because the membrane is permeable to both water and dye. If the cell is kept in a hypotonic solution, the cell wall and the vacuolar membrane both will allow water to pass into it and will set up an excess pressure in the interior of the cell causing the cytoplasm to be forced tightly against the cell wall. In normal condition, this is known as “turgor” and the cell is said to be turgid. If the cell is immersed in a concentrated solution (high osmotic pressure), water will pass out of the interior of the cell. The cytoplasm will then shrinks and detach itself from the cell wall. Isotonic coefficient is the proportionality coefficient between the total concentration of a solute and concentration of solute particles.

1.9 Glossary

1) **Diffusion**: A process by which fluids and solutes become evenly distributed through random molecular motion.
2) **Temperature**: A measure of the average kinetic energy of particles in a substance. “Warmer” substances have more particle motion, while “cooler” substances have less.
3) **Water**: The “universal solvent” upon which life on Earth is based. Water’s unique properties make it an ideal carrier for the molecules of life such as sugars, amino acids, and nucleic acids.
4) **Hypertonic and hypotonic solutions:** In a mixture, the solution that has more osmotic pressure is said to be hypertonic and the one with lower is called hypotonic. In other words, hyper tonic solution has high concentration of solute. When the mixture is placed in a membrane tank, flow of solvent is always observed from the hypotonic.
5) **Osmotic pressure and osmotic potential:** Osmotic pressure is the negative pressur**e** applied, while osmotic potential is positive.
6) **Hypertonicity:** is the presence of a solution that causes cells to shrink.
7) **Hypotonicity:** is the presence of a solution that causes cells to swell.
8) **Isotonicity** is the presence of a solution that produces no change in cell volume.
9) **Isotonic coefficient (or Vant Hoff's coefficient):** is the proportionality **coefficient** between the total concentration of a solute and concentration of solute particles. Particle concentration can be calculated from solute concentration as: where: I is the **isotonic coefficient**.
10) **Isotonic Solution:** Two solutions having the same osmotic pressure across a semi permeable membrane is referred to as an isotonic solution. It has the same osmolarity (solute concentration), as another solution. A solution is isotonic when its effective osmole concentration is the same as that of another solution. This state provides the free movement of water across the membrane without changing the concentration of solutes on either side. Some examples of isotonic solutions are 0.9% normal saline and lactated ringers.
11) **Osmosis:** A process by which molecules of a solvent tend to pass through a semipermeable membrane from a less concentrated solution into a more concentrated one.

1.10 SELF ASSESSMENT QUESTIONS

1.10.1 Multiple Choice Questions:

1. The membrane that allows some of solute molecules to pass through it and prevent others is called:

a) Permeable membrane b) Semi permeable membrane

c) Selectively differentially permeable membrane d) Impermeable membrane

2. The external solution having more concentration then the cell sap is called:

a) Hypertonic solution b) Isotonic solution

c) Hypotonic solution d) None of the above

3. The external solution having same concentration as that of cell sap is called:

a) Hypertonic solution b) Isotonic solution

c) Ultratonic solution d) None of the above

4. The external solution having less concentration than the cell sap is called:

a) Hypertonic solution b) Isotonic solution

c) Hypotonic solution d) Ultratonic solution

5. The pressure exerted by the swelling protoplast on the walls of the cell is:

a) Wall pressure b) Osmotic pressure

c) Suction pressure d) Turgor pressure

6. The pressure exerted by wall of the cell on the protoplast is:

a) W.P b) T.P

c) D.P d) O.P

7. O.P of cell can be measured by:

a) Manometer b) Photometer

c) Calorimeter d) Plasmolysis

8. O.P of a solution can be measured by:

a) Photometer b) Osmometer

c) Calorimeter d) Plasmolysis

9. The common material used in demonstrating plasmolysis in the laboratory is:

a) Garden nasturtium b) Balsam

c) Banyan d) Tradescantia

10. When cell is placed in 0.25 M concentrated sugar solution; there is no change in it. So the external solution is called:

a) Hypertonic b) Isotonic

c) Hypotonic d) None of the above

11. When cell is placed in 0.25 M concentrated sugar solution; there is no change in it. The concentration of cell sap would be:

a) 0.025 M b) 0.25 M

c) 0.25 M d) None of the above

12. When a cell is placed in 0.5 M concentrated solution of sugar, there is no change in its volume. But if the same cell is placed in 0.5 M concentrated solution of sodium chloride there will be:

a) Decrease in volume
b) Increase in volume
c) No change in volume
d) None of the above

13. The selectively permeable membrane of the cell is:

a) Plasmalemma
b) Cytoplas
c) Cell wall
d) None of the above

14. A wooden peg inserted in a rock causes its breaking during the rainy season. It is due to development of:

a) Turgor pressure
b) Osmotic pressure
c) Matric potential
d) Plasmolysis

15. 1 gm molar solution is:

a) 1 gm mole of solute dissolved in 1000 ml of solvent
b). 1 gm mole of solute dissolved in 1000 ml of solution
c) 1 gm of solute dissolved in 1000 ml of solvent
d) 1 gm of solute dissolved in 1000 ml of solution

16. Osmotic potential of pure water is:

a) One
b) Zero
c) Less than zero
d) Between zero and one

17. The process in which loss of water occurs in the form of water vapour is:

a) Respiration
b) Guttation
c) Transpiration
d) Exosmosis

18**.** Consider the following pairs of liquids. Which pairs are **miscible**?

1. Benzene, C_6H_6, and hexane, C_6H_{12}
2. Water and methanol, CH_3OH
3. Water and hexane

a) 1, 2 only
b) 2 only
c) 1 only
d) 1, 2, 3

1.10.2 Answer keys: 1-c, 2-a, 3-b, 4-c, 5-d, 6-a, 7-d, 8-b, 9-d, 10-b, 11-b, 12-a, 13-a, 14-c, 15-b, 16-b, 17-c, 18-a.

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1.13 TERMINAL QUESTIONS

1. What is isotonic coefficient?
2. How do you know if a solution is isotonic?
3. What is an example of an isotonic solution?
4. How do you determine if a solution is isotonic hypertonic or hypotonic?

5. How do you calculate isotonic osmolarity?
6. How do you calculate osmolarity of IV fluids?
7. What is the tonicity of the solution?
8. Which solution has a higher osmotic pressure?
9. What are the factors affecting osmotic pressure?
10. What happens when osmotic pressure increases?
11. What is the importance of isotonic solution?
12. What is isotonic coefficient?
13. What is “semi permeable membrane”?
14. What is osmotic pressure and why is it important?
15. What is osmotic pressure?
16. Discuss the effects of a solute on the osmotic pressure of a solution.

UNIT-2: DETERMINE THE DIFFUSION PRESSURE DEFICIT OF PLANT CELLS

2.1 Objectives
2.2 Introduction
2.2.1 Diffusion
2.2.2 Importance of diffusion
2.2.3 Permeability
2.2.4 Diffusion Pressure Deficit (DPD)
2.2.5 Turgor pressure
2.2.6 Water Potential
2.3 Summary
2.4 Glossary
2.5 Self Assessment Questions
2.5.1 Multiple Choice Questions
2.6 References and suggested readings
2.7 Terminal Questions

2.1 OBJECTIVES:

After reading this unit students will be able:

- To determine the diffusion pressure deficit of plant cells.

2.2 INTRODUCTION:

2.2.1 Diffusion

If a small bottle filled with some gas or vapors is opened at a certain place in the room, very soon its molecules become evenly distributed throughout the available space in that room. Similarly, if a solute is placed in its solvent, it is dissolved and its particles move so that they are evenly distributed throughout the container. This movement of particles or molecules from a region of higher concentration to a region of lower concentration is called as diffusion (Fig. 2.1). The rate of diffusion of gases is faster than liquids or solutes.

The diffusing particles have a certain pressure called as the diffusion pressure which is directly proportional to the number or concentration of the diffusing particles. Therefore, the diffusion takes place always from a region of higher diffusion pressure to a region of lower diffusion pressure i.e., along a diffusion pressure gradient.

The rate of diffusion increases if:

(I) The diffusion pressure gradient is steeper.

(II) The temperature is increased.

(III) The density of the diffusing particles is lesser.

(IV) The medium through which diffusion occurs is less concentrated.

Diffusion of more than one substance at the same time and place may be at different rates and in different directions, but is independent of each other. A very common example of this is the gaseous exchange in plants.

2.2.2 Importance of diffusion

(1). Gaseous exchange during the process of photosynthesis and respiration takes place by the principal of independent diffusion.

(2). The process of diffusion is involved in the transpiration of water vapours.

(**3**)**.** The ions are absorbed by the simple diffusion during passive salt uptake.

(4). Diffusion is an effective means of transport over very short distances and helps in translocation of food materials.

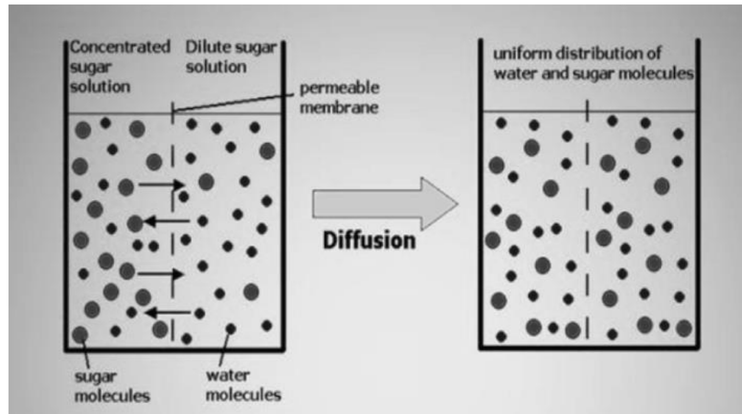

Fig. 2.1 Process of diffusion.

2.2.3 Permeability

A membrane which allows both water as well as solute particles to pass through is called permeable. The membrane which allows the passage of only the solvent particles and not the solute particles is called semipermeable or differentially permeable. Impermeable membranes are those which prohibit the entry of both solvent and solute particles. A plant cell normally has a differentially permeable membrane (the plasma-membrane). The permeability of the complex cell membrane depends on the nature of the surrounding particles as well as on the changing conditions inside and outside the cell.

Among all substances, water has the most rapid rate of diffusion through cell membranes. The membrane is very much permeable to gases like CO_2, O_2, N_2, and fat-solvents like alcohol, ether, chloroform, etc., relatively less permeable to some organic substances like mono- and disaccharides, fatty acids, amino acids, strong electrolytes, salts, bases, etc., and relatively impermeable to polysaccharides, phospholipids, proteins, etc.

Diffusion also plays a very important role in the life of the plants:

(i). It is an essential step in the exchange of gases during respiration and photosynthesis.

(ii). During passive salt uptake, the ions are absorbed by simple process of diffusion.

(iii). Last step in stomatal transpiration is the diffusion of water vapors from the intercellular spaces into the outer atmosphere through open stomata.

2.2.4 Diffusion Pressure Deficit (DPD)

The term diffusion pressure deficit (DPD) was proposed by **Meyer (1938).** DPD was also called suction pressure, coined by Renner. The tendency of water to move is used to be measured by in terms of diffusion pressure. The pure solvent has the maximum diffusion pressure.

Usually DPD=OP-TP

OP=Osmotic pressure and TP=Turgor pressure

Diffusion Pressure Deficit (DPD) is the pressure difference between pure water and solution. Mathematically, DPD= Osmotic pressure-Turgor pressure. Osmotic pressure is the pressure which needs to be applied to a solution to stop the flow of its pure solvent whereas turgor pressure is the pressure inside a cell that pushes the cell wall against plasma membrane. When a plant cell is kept in a hypotonic solution, the cell absorbs water through the process of osmosis and becomes fully turgid (no water can be absorbed). Cell membrane gets fully stretched and hence, osmotic pressure decreases and become equals to turgor pressure of cell. Thus, DPD becomes zero. The pressure needs to be applied to a solution to stop the flow of its pure solvent is called osmotic pressure whereas turgor pressure is the pressure inside a cell that pushes the cell wall against plasma membrane i.e.-no water can be absorbed.

When a plant cell is kept in a hypotonic solution, the cell absorbs water through the process of osmosis and becomes fully turgid. Cell membrane gets fully stretched and hence, osmotic pressure decreases and become equals to turgor pressure of cell thus DPD becomes zero. Diffusion pressure of a solution is always lower than its pure solvent. The difference between the diffusion pressure of the solution and its solvent at a particular temp, and atm. conditions is called as Diffusion Pressure Deficit (D.P.D). If the solution is more concentrated its D.P.D. increases but it decreases with the dilution of the solution.

- **D.P.D. is directly proportional to the concentration of the solution:**

  In case of plants the cell sap is a watery solution of many inorganic and organic substances; i.e., its pure solvent is water. If these cells are placed in pure water the water will enter into the cells due to higher D.P.D. of the cell sap or water deficit.

  In other words, the D.P.D. of the cell sap or the cells is a measure of the ability of the cells to absorb water and hence it is often called as the Suction Pressure (S.P.). It is related with osmotic pressure (O.P.) and turgor pressure (T.P.) of cell sap and also the wall pressure (W.P.) as follows:

  D.P.D (S.P.) = O.P. – W.P.

  But (W.P.) = T.P.

  Therefore, D.P.D. (S.P.) = O.P. – T.P.

  Due to the entry of the water the osmotic pressure of the cell sap decreases while its turgor pressure is increased so much so that in a fully turgid cell turgor pressure equals the osmotic pressure:

  O.P = T.P. (in fully turgid cell)

  Hence, D.P.D. (S.P) = O (zero)

On the other hand, the removal of water from the cell sap (ex-osmosis) results in an increase of its O.P. and decrease of the turgor pressure so much so that in fully plasmolysed cells the value of turgor pressure becomes zero.

T.P. = O (in fully plasmolysed cell) and hence, S.P. = O.P.

In case, the cell is placed in a hypotonic solution instead of pure water, the suction pressure of the cell sap will be:

S.P. = (O.P. – O.P.$_1$) – T.P.

Where O.P$_1$. is the osmotic pressure of outer hypotonic solution?

Thus it is quite obvious that the D.P.D. or S.P. in case of plant cells is not directly proportional to their osmotic pressure or the concentration of the cell sap but depends both on O.P. and T.P. Higher osmotic pressure of the cell sap is usually accompanied by lower turgor pressure so that its D.P.D. or S.P. is greater and water enters into it (Fig. 2.2 A).

But, sometimes it is possible that two cells are in contact with each other one having higher osmotic pressure and also higher turgor pressure than the other cell (Fig. 2.2 B), and still its does not draw water. It is because of its lower D.P.D or suction pressure (S.P)., no matter its O.P. is higher.

	Cell a	→	Cell b
A	O.P. = 25 atm. T.P. = 15 atm.		O.P. = 30 atm. T.P. = 10 atm.
	S.P. = 10 atm.		S.P. = 20 atm.

	Cell a	←	Cell b
B	O.P. = 35 atm. T.P. = 10 atm.		O.P. = 40 atm. T.P. = 20 atm.
	S.P. = 25 atm.		S.P. = 20 atm.

Fig.2.2 Entry of water into the cell depends on D.P.D. or Suction Pressure and not on O.P. only.

2.2.5 Turgor pressure

When a cell is placed in pure solvent (water), it shows endosmosis because of higher osmotic pressure of the cell sap. As a result, the vacuole increases in its size and presses the protoplasm. The pressure with which the cell sap presses the protoplasm against the cell wall is called turgor pressure (TP). The cell wall, on the other hand, being almost rigid exerts a counter pressure, known as wall pressure (WP). At any given movement TP = WP and the cell is in equilibrium. In other words, turgor pressure is the positive hydrostatic pressure developed within the cell. It is indicated as Ψ p and also called pressure potential.

- **Example of turgor pressure**

Think of a balloon that is being filled up with water as a **turgor pressure example**. The balloon swells as more water draws in. The **pressure** that the water exerts against the walls of the balloon is similar to the **turgor pressure** exerted against the wall.

2.2.6 Water Potential

1. It is modern term coined by **Slatyer** and **Taylor (1960)** which is equivalent to DPD.

2. It has a negative value e.g. -5 atm.

3. Water potential is reduction in free energy of solvent in a system over its pure state.

4. Water is absorbed by a system with lower Ψw from another system with higher Ψw.

5. $\Psi w = \Psi s + \Psi p$ where Ψs has a negative value while Ψp has a positive value.

6. Matric potential or Ψm is considered wherever it is appreciable.

- **Instruments used:**

  Sugar, water, cork borer (Hollow cylinder with piston), petridishes, pipette, scale, watch glass, balance with weighing box.

- **Measurement of Diffusion Pressure Deficit or Suction pressure of Plant Cells:**

  1. Take a potato tuber and remove the skin .Then bore a hollow cylinder into the tuber and with the piston take out a cylinder of tissue .Cut the cylinder into about 20 equal sized slices (Fig. 2.3).
  2. Also prepare 0.10M, 0.20M, 0.25M and 0.30M solutions of sugar. Pour approximately equal quantity of each solution into four different petridishes and mark them as A, B, C and D respectively.
  3. Weigh the cylinder or slices of tuber. Place the slices into the petridishes containing different molar concentrations of sugar solutions. Cover the petri dishes.
  4. After some time (approx 24 hours), remove the cylinders, blot away the excess solution and reweigh. Record the gain or loss of weigh.

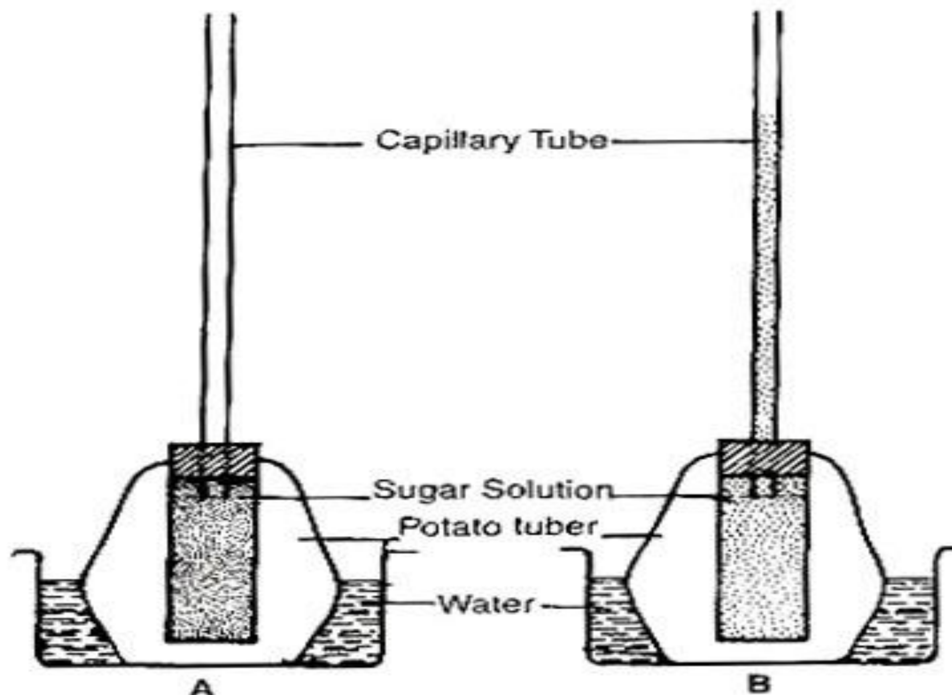

Fig.2.3 Potato osmometer: (A). At start: (B). A few hours later.

2.3 SUMMARY

From the above experiment, you now understand how to measure the diffusion pressure deficit from a given plant cell. It needs careful observation during solution preparation and weighing. In petri dishes A and B weight increases because the volume of the cell increases when placed in a hypotonic solution. In petri dishes D the weight decreases due to water moves from inside to outside of the cell hence cell volume also decreases. For petri dish C there is no movement of water molecules so the weight is unchanged. Thus, the sugar solution (0.25M) should be isotonic with the cell sap. This indicates that the osmotic concentration of the cell sap is equal to 0.25M.

Plant cells maintain a delicate balance of water, various dissolved salts and sugars. If the fluid inside the plant cells is "saltier" than the surrounding fluid, water molecules move in to try to reach equilibrium. If there is no cell membrane, then at the same time the salty water would diffuse out, until the salt concentration inside and outside the cell were equal.

2.4 GLOSSARY

- **Diffusion Pressure Deficit:** Diffusion pressure deficit is the reduction in diffusion pressure of solvent in a system over its pure state. It is an old term coined by **Meyer (1938).** (DPD, suction pressure) In older terminology, the net force or pressure that causes water to enter a plant cell. Its magnitude is the difference between the osmotic pressure and the turgor pressure (TP), i.e. DPD = II-TP.

- **Osmotic Pressure:** The maximum amount of pressure that can be developed in a solution separated from pure water by a semi-permeable membrane is called osmotic pressure.
- **Turgor Pressure:** If a plant cell is put in a medium of pure water or solution of lower concentration then the sap inside the cell, water enters the cell as a result of osmosis. Such an entry of water result in hydrostatic pressure called turgor pressure, which presses on the cell wall.
- **Water potential, Suction pressure or formerly diffusion pressure deficit (DPD):** The tendency of a cell to draw in water from outside by osmosis, the water moving from a higher to a lower water potential. Since pure water at one atmosphere has water potential zero, cells drawing in water have a water potential of less than zero. Thus water potential is measured as a negative value, which can be confusing.
- **Diffusion:** The movement of molecules or ions in a fluid from areas of high concentration to areas of low concentration. In a closed system this will continue until the solution or gas mixture is evenly mixed.
- **Cork borer:** Metal tool for cutting a hole in a cork.
- **Hypertonic solution:** A greater concentration of solutes on the outside of a cell from inside.
- **Hypotonic solution:** A solution that has less solute and more water than other solution.
- **Isotonic solution:** A solution that has the same solute concentration as another solution.
- **Plasmolysis:** It is the withdrawal of protoplast of a plant cell from its wall due to excessive loss of water from cell due to the exosmosis. It occurs when a cell is placed in a hypertonic solution.
- **Osmosis:** The passage of solvent molecules from a region of their higher concentration to a region of their lower concentration through a semi-permeable membrane. It occurs spontaneously due to the pressure gradient.

2.5 SELF ASSESSMENT QUESTIONS

2.5.1 Multiple Choice Questions

1. In a plant cell, the diffusion pressure deficit (DPD) is zero when it is:

a) Plasmolysed b) Turgid

c) Flaccid d) Incipient

2. The movement of water in plant cell from:

a) Higher to lower b) Lower to higher

c) Higher to higher d) Lower to lower

3. Phenomena through which water is absorbed by solids such as colloids leading them to increase in volume are:

a) Diffusion b) imbibitions

c) Facilitated diffusion d) osmosis

4. Passage of water across a selectively permeable membrane is:

a) Osmosis b) Active transport

c) Facilitated diffusion d) Pinocytosis

5. Wall pressure will______________ with rise in turgidity:

a) Increase b) Decrease

c) Remain unaffected d) Fluctuate

6. Transpiration is a phenomenon pertaining to:

a) Activated transport b) Diffusion

c) Osmosis d) Facilitated diffusion

7. Phenomena through which water is absorbed by solids such as colloids leading them to increase in volume is:

a) Diffusion b) Imbibitions

c) Facilitated diffusion d) osmosis

8. Passage of water across a selectively permeable membrane is:

a) Osmosis b) Active transport

c) Facilitated diffusion d) Pinocytosis

9. What is the measure of the osmotic pressure gradient of solutions separated by a semipermeable membrane?

a) Water potential b) Ficks law

c) Turgor pressure d) Tonicity

10. The external solution having more concentration then the cell sap is called:

a) Hypertonic solution b) Isotonic solution

c) Hypotonic solution d) None of the above

11. The pressure exerted by the swelling protoplast on the walls of the cell is:

a) Wall pressure b) Osmotic pressure

c) Suction pressure d) Turgor pressure

12. Net movement of water is form:

a) Low DPD to high DPD b) High DPD to low DPD

c) DPD gradient plays no role d) None of the above.

13. Cell turgidity is caused by:

a) Endosmosis b) Exosmosis

c) Plasmolysis d) Diffusion.

14. Which helps in maintaining from and structure of cells?

a) Wall pressure b) Turgidity atmospheric pressure

c) D.P.D. d) None

15. O.P of cell can be measured by:

a) Manometer b) Photometer

c) Calorimeter d) Plasmolysis

16. O.P of a solution can be measured by:

a) Photometer b) Osmometer

c) Calorimeter d) Plasmolysis

17. When cell is placed in 0.25 M concentrated sugar solution, there is no change in it. So the external solution is called:

a) Hypertonic b) Isotonic

c) Hypotonic d) None of the above

18. When a cell is placed in 0.5 M concentrated solution of sugar, there is no change in its volume. But if the same cell is placed in 0.5 M concentrated solution of sodium chloride there will be:

a) Decrease in volume b) Increase in volume

c) No change in volume d) None of the above

19. Osmotic potential of pure water is:

a) One b) Zero

c) Less than zero d) Between zero and one.

20. Photometers are made on the principle that:

a) The amount of water transpired is approximately equal to amount of water absorbed.

b) The amount of water transpired is more than the amount of water absorbed.

c) The amount of water transpired is less than the amount of water absorbed.

d) Humidity causes reduction in transpiration.

2.5.2 Answer keys: 1-b, 2-a, 3-b, 4-a, 5-a, 6-b, 7-b, 8-a, 9-d, 10-a, 11-d, 12-a, 13-a, 14-b, 15-d, 16-b, 17-b, 18-a, 19-b, 20-a.

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2.7 Terminal Questions

1 What is diffusion pressure deficit?

2 Who coined the term diffusion pressure deficit?

3 What is osmotic pressure and turgor pressure?

4 What's an example of turgor pressure?

5 What is the relation between diffusion pressure (DP) and turgor pressure (TP)?

6 When the Diffusion Pressure Deficit becomes zero?

7 In which case the diffusion pressure will be maximum?

8 What is the hypotonic solution?

9 What is plasmolysis and osmosis?

10 What is the water potential?

UNIT-3: STUDY THE STRUCTURE OF STOMATA AND FIND OUT THEIR FREQUENCY ON THE ADAXIAL AND ABAXIAL SURFACES OF LEAVES.

3.1 Objectives
3.2 Introduction
3.2.1 Stomata
3.2.2 Structure of Stomata
3.2.3 The mechanism of stomatal opening and closing
3.2.4 Functions of Stomata
3.2.5 Factors influence the opening and closing of stomata
3.3 Find out the stomatal frequency on the adaxial and abaxial surfaces of the leaves
3.4 Adaxial and abaxial surface of leaves
3.5 Stomatal Frequency
3.6 Microscopic method of analysis
3.7 Distribution and types of stomata
3.8 Summary
3.9 Glossary
3.10 Self Assessment Questions
3.10.1 Multiple Choice Questions
3.11 References and Suggested Readings
3.12 Terminal Questions

3.1-OBJECTIVES:

After reading this unit students will be able:

- To Study the structure of stomata and find out their frequency on the adaxial and abaxial surfaces of leaves.

3.2-INTRODUCTION:

3.2.1 Stomata

Stomata were discovered by Pfeffer & name 'stomata' was given by Malphigii. Stomata cover 1-2% of leaf area. It is minute pore present in soft aerial parts of the plant. Algae, fungi and submerged plants do not possess stomata. The stomata are apertures in the epidermis, each bounded by two guard cells. In Greek, *stoma* means "mouth", and the term is often used with reference to the stomatal pore only. Esau (1965, p. 158) uses the term stoma to include the guard cells and the pore between them, and we will use her definition. The plural of stoma is *stomata*. There is no such word as "stomates".

Stomata occur in vascular plants. Vascular plants include the lower vascular plants such as horsetails (*Equisetum*), ferns (class Filicinae), gymnosperms, and angiosperms. As noted before, the angiosperms are the flowering plants and this group consists of the two large classes: Monocotyledoneae (monocotyledons) and Dicotyledoneae (dicotyledons).

By changing their shape, the guard cells control the size of the stomatal aperture. The aperture leads into a substomatal intercellular space, the substomatal chamber, which is continuous with the intercellular spaces in the mesophyll. In many plants, two or more cells adjacent to the guard cells appear to be associated functionally with them and are morphologically distinct from the other epidermal cells. Such cells are called *subsidiary*, or *accessory*, cells.

The stomata are most common on green aerial parts of plants, particularly the leaves. They can also occur on stems, but less commonly than on leaves. The aerial parts of some chlorophyll-free land plants (*Monotropa*, *Neottia*) and roots have no stomata as a rule, but rhizomes have such structures. Stomata occur on some submerged aquatic plants and not on others. The variously colored petals of flowers often have stomata, sometimes nonfunctional. Fruits also can have stomata. Stomata are found on stamens and gynoecia (M.B. Kirkham, 2014).

Stomata are pore-like structures located on the leaf surfaces of virtually all vascular, terrestrial plant leaves and are responsible for the uptake of photosynthetic CO_2, as well as for the potentially detrimental water loss (transpiration) from inside the leaf (MacDonald 2002). Thus, stomata play a primary role in regulating carbon uptake for growth and the prevention of plant desiccation (Apple et al. 2000; Croxdale 2000). Moreover, the frequency of these structures on leaf surfaces can dictate the degree of gas exchange potential for both photosynthetic CO2 uptake and transpiration water loss. In response to favorable environmental signals, stomata may open to facilitate carbon uptake, or close to prevent tissue drying and the maintenance of higher

water use efficiency at different times of the growth season or given time of day (Croxdale 2000). Physical obstruction of these structures by a water film can strongly inhibit gas exchange (Brewer et al. 1991; Brewer and Smith 1997) and significantly decrease photosynthetic carbon gain by the plant (Brewer and Smith 1995). Water film coverage of stomata could be ever-present issue in the mountaintop spruce-fir forests of the Appalachian Mountains in the eastern United States, a region characterized by frequent cloud immersion and high humidity that would enhance water condensation on leaf surfaces

3.2.2 Structure of Stomata:

All green plants have certain primary parts, which are essential and play a critical role in different life processes. Stomata are one of the essential attributes that is used for gaseous exchange. It functions as the mouth of a plant and is also called a stoma, or stomas. Stomata are the minute openings, generally found in the epidermis of leaves. They are typically found in the leaves and can also be found in stems and other plant organs. It plays an important role by permitting the movement of gases such as oxygen, carbon dioxide, and water vapour to diffuse between the interior and outer surface of the plant tissues. There are different types of stomata and are mainly classified based on their number and characteristics of the surrounding subsidiary cells. Listed below are the different types of stomata fig. (3.1).

1. **Epidermal Cell:** It is the outermost layer of plants. These specialized cells originate from the dermal tissues of the plant. Epidermal cells are irregularly shaped cells, which functions by providing mechanical support to the plant.
2. **Subsidiary Cell:** These cells are located adjacent to the guard cell in the stoma of a leaf. It functions by providing support in the movement of guard cells. These cells are formed from the nearby mother cells and in some rare cases, they are developed independently.
3. **Stomatal Pore:** They are minute pores or the opening found on the under-surface of plant leaves. These stomatal Pores play a significant role in gaseous exchange.
4. **Guard cells:** They are the kidney-shaped or dumbbell-shaped cell, which functions by controlling the mechanism (opening and closing) of stomata.

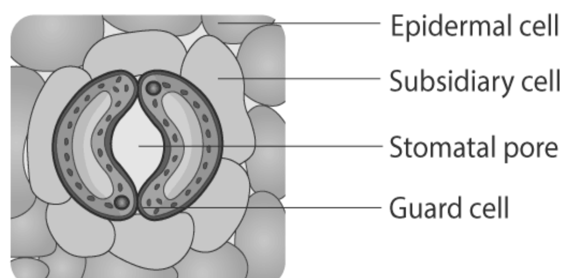

Fig. 3.1. Structure of Stomata

Stomata are small pores present in the epidermis of leaves. They regulate the process of transpiration and gaseous exchange. The stomatal pore is enclosed between two bean-shaped

guard cells. The inner walls of guard cells are thick, while the outer walls are thin. The guard cells are surrounded by subsidiary cells. These are the specialized epidermal cells present around the guard cells. The pores, the guard cells, and the subsidiary cells together constitute the stomatal apparatus.

3.2.3 The mechanism of stomatal opening and closing

The opening and closing of stomata depend on the turgor pressure, caused by the osmotic flow of water in the guard cells. When the guard cells are turgid, they expand resulting in the opening of stomata. When the guard cells lose water, they become flaccid leading to stomatal closure. Stomata normally open when the light strikes the leaf and close during the night (fig**. 3.2).**

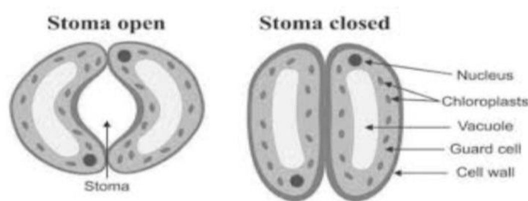

Fig. 3.2 Opening and closing of stomata

3.2.4 Functions of Stomata: The main functions of stomata are:

1. Gaseous exchange- Stomatal opening and closing help in the gaseous exchange between the plant and surrounding.
2. It helps in transpiration and removal of excess water in the form of water vapour.
3. Stomatal closure at night prevents water from escaping through pores.
4. It maintains the moisture balance according to weather by opening and closing.
5. Stomata facilitate carbon dioxide uptake and release of oxygen during the process of photosynthesis.

3.2.5 Factors influence the opening and closing of stomata

There are many factors which lead to stomata opening and closing.

1. There is an endogenous rhythm (a biological clock). Stomata open during the day and close during the night. (Though certain succulents which are native to hot, dry conditions have a reversed rhythm to enable them to economise on water loss.) However, stomata continue to open and close on an approximately 24 hour clock (circadian = about a day) even when switched to continuous light. The phase of this opening and closure can be shifted (made to occur at other times of the day) by control of the end of the dark period.
2. The water balance of a plant affects stomatal aperture. Wilting plants close their stomata. The plant growth regulator abscisic acid (ABA) seems to act as a mediator under these conditions. Water stress in the roots can transmit (in xylem?) its influence to stomata in leaves by the signal of ABA.

3. Low concentrations of CO_2 cause stomata to open. If CO_2-free air is blown across stomata in darkness, their stomates open. High CO_2 causes stomates to close.
4. Light causes stomates to open. The minimum light level for opening of stomates in most plants is 1/1000 to 1/30 of full sunlight, just enough to cause some net photosynthesis. Blue light (430-460nm) is nearly 10 times as effective as red light (630-680nm). The wavelengths that are effective in the red part of the spectrum are the same as those that are effective in photosynthesis ie is absorbed by chlorophyll. However, the blue light effect is quite independent of photosynthesis. Photosynthesis will change intercellular CO_2 concentrations and may have its effect through number iii) above.

3.3 FIND OUT THE STOMATAL FREQUENCY ON THE ADAXIAL AND ABAXIAL SURFACES OF THE LEAVES.

3.3.1 Instrument and material required:

Material required

Tradescantia, broad bean leaves, forceps, Petridish, watch glass, beaker, brush, needle/blade, saffranine, glycerin/canada balsum, fast green, microscope (simple & light microscope) etc.

3.3.2 Procedure

- Take leaf and peel off a small piece of the lower and upper epidermis (adaxial & abaxial surface) and put it in water in watch glass.
- Take out the peel from the water and place it on a slide and add 2-3 drops of saffranine to stain it.
- Remove the excess stain with the help of a filter paper and put a drop of glycerine on the peel.
- Gently place a cover slip on the peel and examine under microscope (high power).
- Count the number of stomata per focus. Repeat viewing in different fields and obtain a mean value.
- Find the area of the field of the microscope by measuring the diameter with the calibrated slide or a transparent ruler.
- The required area is equal to πr^2 (where r is the radius of the field of view and π=3.142).
- The number of stomata per square millimeter can then be calculated.
- Similarly, from the other side of the leaf, the stomatal count can be made.

3.3.3 Inference

In the dorsiventral leaf, the no. of stomata is more in the lower epidermis, and only few stomata are present in the upper epidermis. However, the isobilateral leaf has almost equal number of stomata in both the epidermis.

Precautions:

1. The curling of the peel should be avoided.

2. Always use brush to transfer the peel from watch glass to the slide.

3. Excess of glycerin should be removed by using blotting paper.

3.4 ADAXIAL AND ABAXIAL SURFACE OF LEAVES

Light capturing is the specialization of the adaxial or upper surface and the abaxial or lower surface is specialized for gas exchange (FigThis division into adaxial and abaxial domains is also key for the outgrowth of the leaf blade or lamina, which occurs along the boundary between the upper and lower sides (Fig. 3.3).

Adaxial= Upper surface of leaves; **Abaxial=** Lower surface of leaves.

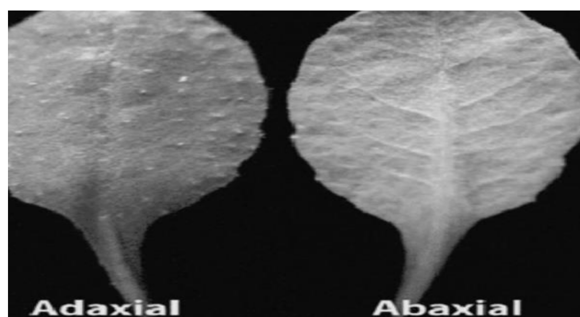

Fig. 3.3. Adaxial and abaxial surface of leaves.

Table 3.1: Example: Stomatal frequency and stomatal index of abaxial & adaxial surface of leaves:

Name of Plant species (Eg.)	Portion of leaf	(Mean) no. of stomata (S) on adaxial & abaxial side of leaves	Mean number of epidermal cell (E)	Area of microscopic field (mm^2)	Stomatal frequency (no. of stomata/(mm^2)	Stomatal index (S*100/E +S)
Persicaria chinensis	**Apex**	29.00	152.33	0.18	161.11	15.99%
	middle	34.33	153.67	0.18	190.72	18.26%
	Base	35.67	148.33	0.18	198.16	19.38%
-----------	**Apex**	25.67	147.00	0.18	142.61	14.87%
	middle	23.00	137.00	0.18	127.77	14.38%
	Base	27.33	141.00	0.18	151.83	16.24%
-----------	**Apex**	31.33	162.67	0.18	174.05	16.15%
	middle	39.67	179.00	0.18	220.38	18.14%
	Base	45.67	191.33	0.18	253.72	19.27%

3.5 STOMATAL FREQUENCY (NUMBER OF STOMATA)

Stomatal frequency can be defined as the number of stomata present per unit area of a leaf. Determination of stomatal frequency and the total area of stomata covered in a leaf are the essential prerequisite to assess the rate of water loss through stomata. Several environmental and genetically factors affect stomatal frequency. The number of stomata in a definite area of leaf varies from plant to plant. Xerophytes possess larger number of stomata than mesophytes. Number of stomata/sq cm. is 1000- 60,000 in different plant species. The number of stomata per unit area of leaf is called Stomatal Frequency. Stomata frequency of trees and shrubs is higher than herbs. Stomata nearly occupy one to two percent of total leaf area when fully open. In isobilateral leaves (in monocots) approximately the same number of stomata is found on upper surface (adaxial) and lower (abaxial) surface.

But in dorsiventral leaves (in dicots) the number of stomata on the upper surface is much less in comparison to those found on the lower surface. The following environmental factors change the stomatal frequency- water availability, carbon dioxide concentration, temperature and light intensity. Water stress results in a greater stomatal frequency. Plants growing in wet soil with high humidity have lower frequency than the plants growing in dry soil with low humidity. Stomatal frequency is reduced in polluted atmosphere, e.g. *Trifolium partense.*

Light intensity has marked effect on stomatal frequency. In low light intensity frequency reduces. Stomatal frequency becomes higher when a plant grows in full sunlight. In an experiment it is observed that tomato plants grown in controlled condition have hypostomatic leaf and when grown at high light intensity the leaves are amphistomatous. The degree of ploidy also affects stomatal frequency. Polyploid plants have less stomatal frequency and larger stomata. Generally stomatal frequency decreases where the stomata are large.

3.5.1 Formula: Stomatal frequency (No. of stomata/ (mm^2)

Stomatal frequency is not constant within a plant. Highest frequencies are often found in those leaves that occur on the top. Even within a leaf there exists variation in frequency. As for example the grass leaf has lowest frequency at the tip. The highest frequency is observed at the point of insertion of the leaf where the cells are still developing and smallest. Because the stomatal frequencies often vary Salisbury (1928) proposed the term ‘stomatal index’. Stomatal index relates the number of stomata to number of epidermal cells.

Stomatal index is calculated in the following way:

$$\text{Stomatal index} = \frac{\text{Number of stomata present per unit area of leaf}}{\text{No. of stomata} + \text{no. of epidermal cells within a unit area of leaf}} \times 100$$

This value is found to be reasonably constant for any particular species.

Stomatal frequency is the frequency of occurrence of stomata i.e., number of stomata per unit area of leaf surface. This number is related to the rate of transpiration, which in turn will affect

the productivity of the plant or Stomatal frequency is determined by counting the number of stomata in the microscope field of view (after we calculate the area of view).

Table 3.2: Example: Stomatal frequencies on the adaxial and lower abaxial epidermis of leaves.

Name of plant species	Name of stomata mm^2	
Monocotyledons	**Upper surface (Adaxial)**	**Lower surface (Abaxial)**
Allium (Onion)	75	80
Hrdeum (Barley)	65	75
Triticum (Wheat)	80	90
Dicotyledons		
Helianthus (Sunflower)	123	145
Quercus (Oak)	125	150
Geranium	29	179

Stomatal Frequency: The stomatal frequency was calculated by following the formula of Ghos & Davis (1973).

Stomatal Frequency (S.F.) =S/A

Where, S = Number of stomata per field

A = Area of the field

3.6 MICROSCOPIC METHOD OF ANALYSIS

3.6.1 Determination of stomatal index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

$$\text{Stomatal index} = \frac{S \times 100}{E + S}$$

Where S = the number of stomata in a given area of leaf; and

E = the number of epidermal cells (including trichomes) in the same area of leaf

For each sample of leaf make not fewer than ten determinations and calculate the average index.

3.7 Distribution and Types of Stomata

Depending upon the distribution and arrangement of stomata in the leaves five categories of stomatal distribution have been recognized in plants (Fig. 3.4).

1. Apple or mulberry (hypostomatic) type: Stomata are found distributed only on the lower surface of leaves, e.g., apple, peach, mulberry, walnut, etc.

2. Potato type: Stomata are found distributed more on the lower surface and less on its upper surface, e.g., potato, cabbage, bean, tomato, pea, etc.

3. Oat (amphistomatic) type: Stomata are found distributed equally upon the two surfaces, e.g. maize, oats, grasses, etc.

4. Water lily (epistomatic) type: Stomata are found distributed only on the upper surface of leaf, e.g., water lily, Nymphaea and many aquatic plants.

5. Potamogeton (astomatic) type: Stomata are altogether absent or if present they are vestigial. e.g., Potamogeton and submerged aquatics.

Metacalf and Chalk recognized four types of stomata on the basis of their structure:

a. Anomocytic type: In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells, e.g., families *Ranunculaceae, Cucurbitaceae, Papaveraceae* and *Malvaceae.*

b. Anisocytic type: In these stomata the guard cells are surrounded by three accessory cells. Of these two are larger whereas one is smaller in size, family *Brassicaceae.*

c. Diacytic type: In these stomata the guard cells are surrounded by two accessory cells. Their common walls are at right angle to the walls of guard cells, families Caryophyllaceae, Acanthaceae.

d. Paracytic type: In these stomata the guard cells are also surrounded by two accessory cells, but their common walls are parallel to guard cells, e.g., families *Rubiaceae, Fabaceae* etc.

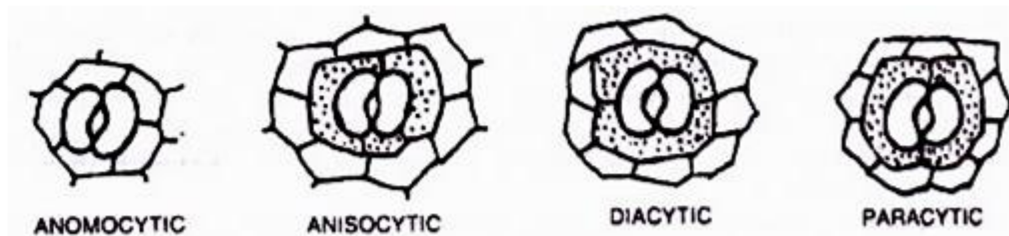

Fig. 3.4 Types of stomata.

On the basis of development (Pant, 1965): There are three types of stomata:

Mesogynous type: In this type of stomata guard cells as well as subsidiary or Accessory cells both are developed from one mother cell. e.g. Rubiaceace &. *Brassicaceae family.*

Perigynous type: In this type guard cells are formed from mother cell while subsidiary cells from nearby mother cells, eg.: Cucurbitaceae family.

Mesoperigynous type: In this type guard cells & one subsidiary cells is formed from mother cell while other subsidiary cells develop independently. e.g.: *Ranunculaceae, Caryophyllaceae* family.

3.8 SUMMARY:

Stomata are composed of a pair of specialized epidermal cells referred to as guard cells. Stomata regulate the gas exchange between the plant, environment & control of water loss by changing the size of the stomatal pore. Stomata have two main functions, such as they allow for gas exchange acting as and entryway for carbon dioxide (CO_2) and releasing the oxygen (O_2) that we breath. The other main function is regulating water movement through transpiration.

Pathogen entry into host tissue is a critical first step in causing infection. For foliar bacterial plant pathogens, natural surface openings such as stomata are important entry sites. Historically, these surface openings have been considered as passive portals of entry for the plant pathogenic bacteria. However, recent studies have shown that stomata can play an active role in limiting bacterial invasion as part of the plant innate immune system. As counter-defense, the plant pathogen *Pseudomonas syringae* PV. *tomato* DC3000 uses the virulence factor coronatine to actively open stomata. In nature, many foliar bacterial disease outbreaks require high humidity, rain or storms, which could promote stomatal opening and/or bypass stomatal defense by creating wounds as alternative entry sites. Further studies on microbial and environmental regulation of stomatal closure and opening could fill gaps in our understanding of bacterial pathogenesis, disease epidemiology and microbiology of the phyllosphere. Plants may have a simple structure externally, consisting mainly of the stem, leaves, flowers and the roots. However, internally there is a whole world of complex mechanisms working together to carry out the different physiological activities. The main force driving plant growth is water. It is the main component of the plant, constituting up to 95% of its structure in some species. How can water be responsible for plant growth? Simply put, it is the medium in which all the complex chemical reactions occur, also being in charge of nutrient absorption and translocation. Water enters the plant mainly through the roots and is then transported to the different organs, distributing nutrients and hormones throughout the plant. Since we have established the importance of water for plant growth, it is important to state the importance of stomata, the pores that drive plant growth. So, what are stomata? Simply put, they are openings in the surface of plants, found mainly on the leaves, but also on stems and other organs. They are pores surrounded by specialized parenchymatic cells, called guard cells. Stomata have two main functions, namely they allow for gas exchange acting as an entryway for carbon dioxide (CO2) and releasing the Oxygen (O2) that we breath.

The other main function is regulating water movement through transpiration. Stomata vary in shape and size, being able to change to adapt to the different environmental factors, thus ensuring optimum conditions for photosynthesis. In summary, stomata play a vital role in plant development, by regulating gas exchange with the atmosphere and controlling transpiration.

Different factors can affect its shape and size, effectively regulating water uptake, transport and the distribution of nutrients and hormonal signals in the different organs of plants, thus controlling growth. Maintaining the plant stress-free is essential for avoiding production losses, which could be a direct effect of stomata condition. Stomatal frequency, also called as stomatal density, refers to the number of stomata per unit area of the leaf. Stomata help in the gaseous exchange. It is generally present at the dorsal surface of the leaf. The greater stomatal frequency increases the rate of transpiration. The distribution of the stomata determines the rate of transpiration. The term stomatal index expresses the stomatal frequency independent to the size of the epidermal cells. The stomatal index is a useful taxonomic character in leaf area studies.

Stomata regulate transpiration and CO_2 intake by changing its size depending on the environmental signals. In optimum conditions, stomata are wide open, allowing gaseous exchange with the atmosphere. **Guard cells are responsible for changing pore size**; they do so by expanding or contracting themselves effectively opening and closing stomata. For stomata opening, water is rushed into the guard cells due to osmosis, which is dependent on potassium concentration in the cells. Potassium enters and leaves the cells through active transport, depending on environmental triggers. Such triggers include ion exchange, temperature, light, hormone signaling and CO_2 concentration etc.

For **stomata to open**, **potassium** is actively **transported to the vacuoles**, which increases its concentration in the cells, thus **driving water entry due to osmosis**, **increasing cell turgency** and size and **exposing the pores**. The **opposite occurs** for **stomata closure, potassium is transported out the cells**, which **attracts the water out** to the exterior, **collapsing** the cells **on the pore**, effectively **closing it**. **Stress is the main reason for stomata closure**, as plant produces abscisic acid (ABA), a plant hormone well known to regulate many key processes involved in plant development and adaptation to biotic and abiotic stresses.

This way the plant can conserve water, avoiding any unnecessary losses, until the stress signal is reduced, therefore lowering the concentration of ABA and its effect on stomata closure. Similarly, it has been observed that **the plant can produce ABA as a response to pathogen attack such as** *Pseudomonas syringae*, which can enter the plant through stomata. The plant synthesizes **ABA** which **induces stomatal closure, avoiding any further pathogen invasion.**

Stress negatively affects growth through **stomata closure**, which in turn **disrupts photosynthesis as well as water and hormonal movement within the plant**, bringing on a **hormonal imbalance** which **will lead to stunted growth**. This is widely observed in the field, causing immense agronomical losses, both in yields and fruit quality. Therefore, controlling stress at a physiological level is important to avoid stomata closure and the subsequent production losses.

Environmental and climatic factors were found to influence the stomatal frequency in plant species. Stomatal frequency response to these factors was aimed at minimizing transpiration losses, while maximizing photosynthetic output, hence allowing the plant to remain competitive across the temperature transects. Carbon dioxide is the major greenhouse gas contributing to

global climate change and atmospheric levels of CO_2 will continue to rise into the future. Thus, the Earth may experience an increase in mean global temperatures not seen since the mid-Pliocene and the impact of climate change upon ecosystem composition and distribution can only be postulated.

Global warming and associated precipitation changes will negatively impact on many agricultural ecosystems. Major food production areas are expected to experience reduced water availability and increased frequency of drought over the coming decades. In affected areas, this is expected to reduce the production of important food crops including wheat, rice and maize. The development of crop varieties able to sustain or improve yields with less water input is therefore, a priority for crop research.

Almost all water used for plant growth is lost to the atmosphere by transpiration through stomatal pores on the leaf epidermis. By altering stomatal pore apertures, plants are able to optimize their CO_2 uptake for photosynthesis, while minimizing water loss. Over longer periods, stomatal development may also be adjusted, with stomatal size and density being adapted to suit the prevailing conditions. Several approaches to improve drought tolerance and water-use efficiency through the modification of stomatal traits have been tested in the model plant *Arabidopsis thaliana*. However, there is surprisingly little known about the stomata of crop species. The current understanding of how stomatal number and morphology are involved in regulating water-use efficiency.

3.9 GLOSSARY

1. **Stomata: Stomata**, also called **stoma**, plural **stomata** or **stomas**, any of the microscopic openings or pores in the epidermis of leaves and young stems. Stomata are generally more numerous on the underside of leaves. They provide for the exchange of gases between the outside air and the branched system of interconnecting air canals within the leaf.
2. **Monocotyledons:** A flowering plant with an embryo that bears a single cotyledon (seed leaf). Monocotyledons constitute the smaller of the two great divisions of flowering plants, and typically have elongated stalk less leaves with parallel veins (e.g. grasses, lilies, palms).
3. **Dicotyledons:** Any of a class or subclass (Magnoliopsida or Dicotyledoneae) of angiospermous plants that produce an embryo with two cotyledons and usually have floral organs arranged in cycles of four or five and leave with reticulate venation called also dicot.
4. **Guard cells:** Guard cells are specialized plant cells in the epidermis of leaves, stems and other organs that are used to control gas exchange. They are produced in pairs with a gap between them that forms a stomatal pore. Oxygen (O_2), produced as a byproduct of photosynthesis, exits the plant via the stomata.
5. **Stomatal frequency:** Stomatal frequency can be defined as the number of stomata present per unit area of a leaf. Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell.

6. **Stomatal index:** In paleobotany, the ratio of the number of epidermal cells to the number of **stomata** in a given area of a leaf, times 100.
7. **Stomatal density:** (SD) is a function of both the number of stomata plus the size of the epidermal cells. Thus, SD is affected both by the initiation of stomata and the expansion of epidermal cells.
8. **Abaxial and Adaxial surface:** The adaxial and abaxial sides correspond to the upper and lower sides of the leaf and to the inner and outer sides of the floral organs, respectively. Lateral organs have two cell populations that have different histological features on the adaxial and abaxial sides.
9. **Transpiration: Transpiration** is the loss of water from a plant in the form of water vapor. Water is absorbed by roots from the soil and transported as a liquid to the leaves via xylem. In the leaves, small pores allow water to escape as a vapor.
10. **Subsidiary cells:** A plant epidermal cell that is located next to a guard cell in the stoma of a leaf and differs in structure from other epidermal cells. Also called accessory cell.
11. **Epidermal Cell:** It is the outermost layer of plants. These specialized cells originate from the dermal tissues of the plant. Epidermal cells are irregularly shaped cells, which functions by providing mechanical support to the plant.
12. **Subsidiary Cell:** These cells are located adjacent to the guard cell in the stoma of a leaf. It functions by providing support in the movement of guard cells. These cells are formed from the nearby mother cells and in some rare cases, they are developed independently.
13. **Stomatal Pore:** They are minute pores or the opening found on the under-surface of plant leaves. These stomatal Pores play a significant role in gaseous exchange.
14. **Abscisic acid (ABA):** The plant growth regulator abscisic acid (ABA) seems to act as a mediator under these conditions. Water stress in the roots can transmit (in xylem?) its influence to stomata in leaves by the signal of ABA.
15. **Hypostomatic type:** Stomata are found distributed only on the lower surface of leaves.
16. **Aamphistomatic type:** Stomata are found distributed equally upon the two surfaces.
17. **Epistomatic type:** Stomata are found distributed only on the upper surface of leaf.
18. **Astomatic type:** Stomata are altogether absent or if present they are vestigial.
19. **Anomocytic type:** In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells.
20. **Anisocytic type:** In these stomata the guard cells are surrounded by three accessory cells. Of these two are larger whereas one is smaller in size.
21. **Diacytic type:** In these stomata the guard cells are surrounded by two accessory cells. Their common walls are at right angle to the walls of guard cells.

22. **Paracytic type:** In these stomata the guard cells are also surrounded by two accessory cells, but their common walls are parallel to guard cells.
23. **Mesogynous type:** In this type of stomata guard cells as well as subsidiary or Accessory cells both are developed from one mother cell.
24. **Perigynous type:** In this type guard cells are formed from mother cell while subsidiary cells from nearby mother cells.
25. **Mesoperigynous type:** In this type guard cells & one subsidiary cells is formed from mother cell, while other subsidiary cells develop independently.

3.10 SELF-ASSESSMENT QUESTIONS

3.10.1 Multiple Choice Questions

1. Stomata are present on underside of:

a) Flower b) Fruit

c) Seed d) leaf

2. The singular word for stomata is:

a) Stomata b) Stomata's

c) Stoma d) Stomas

3. The guard cells flanking stomata control the oxygen and carbon dioxide entering the leaf by opening and closing the:

a) Leaf pores b) Stomata

c) Palisade d) None

4. The main task of stomata is to:

a) Intake of carbon dioxide and the release of oxygen

b) Intake of carbon dioxide

c) Release of oxygen

d) Release of carbon dioxide

5. The inner side of guard cells is:

a) Rough b) Straight

c) Concave d) Convex

6. The outer side of guard cells is:

a) Concave b) Convex

c) Rough d) None of these

7. The ions that flow back to guard cells, from epidermal cells at the end of the day are:

a) Potassium ions b) Magnesium

c) Phosphorus ions d) Sulphur ions

8. Stomatal frequency indicates:

a) Number of stomata in per unit area b) Rate of water loss

c) Rate of gaseous exchange d) Width of stomatal aperture

9. Stomata acts as turgor-operated valves?

a) True b) False

c) Both d) None of these.

10. How much percentage of total transpiration is done by stomata?

a) 5-10 b) 90

c) 1-5 d) 60

11. Mark the one, which is NOT the characteristic of the guard cell?

a) Thickened inner wall b) Connected end to end

c) Does not have chloroplast d) Radial micellation.

12. Which light modulates osmoregulation of guard cell?

a) Green b) Orange

c) Red d) Blue

3.10.2 Answer keys: 1-d, 2-c, 3-b, 4-a, 5-c, 6-b, 7-a, 8-a, 9-a, 10-b, 11-c, 12-d.

3.11 REFERENCES OR SUGGESTED READINGS

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3.12 TERMINAL QUESTIONS

1. What are stomata?

2. Where are stomata found in the plant cells?

3. What is stomatal apparatus? Explain the structure of stomata with a labeled diagram.

4. What are the functions of stomata?

5. How do you calculate stomatal frequency?

6. What is the stomatal index?

7. How do you find the number of stomata per mm2?

8. What are the types of stomata?

9. How do you determine the number of stomata?

10. How can you tell how many stomata a leaf has?

11. What is stomata density?

12. What is Abaxial and Adaxial surface of leaf?

13. What is the role of stomata present on the surface of leaf?

14. What is stomatal frequency?

15. What is the importance and functions of stomata?

UNIT-4: SET UP WILMOTT'S BUBBLER AND STUDY THE EFFECT OF VARYING CO_2 CONCENTRATION AND DIFFERENT WAVELENGTHS OF LIGHT ON THE RATE OF PHOTOSYNTHESIS

4.1 Objectives

4.2 Introduction

4.3 Instrument and Material Required

4.4 Effect of varying CO_2 concentration on rate of photosynthesis

4.5 Effect of different wavelengths (Red, Green and blue) on rate of photosynthesis

4.6 Summary

4.7 Glossary

4.8 Self Assessment Question

4.9 References

4.10 Suggested Readings

4.11 Terminal Questions

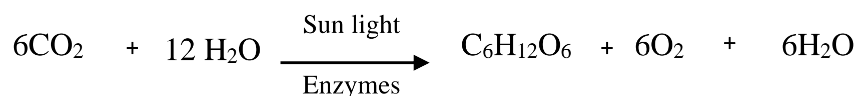
4.1 OBJECTIVES

After reading this unit, students will be able -

- To learn how to set up Wilmott's bubbler and to study the effect of varying CO_2 concentration on rate of photosynthesis
- To study the effect of different wavelengths on rate of photosynthesis using different color cellophane papers.

4.2 INTRODUCTION

On this planet (Earth), Plants are called autotrophs because they are the only living beings can synthesize their own food (carbohydrate) using sun light, water and carbon dioxide gas (Co_2). However not only plants but some bacteria also do photosynthesis and make their food. Photosynthesis takes place in chloroplasts in two steps (light reaction and dark reaction respectively). In light reaction, light quanta are absorbed by the chlorophyll molecules on thylakoid (membranous fold present in stroma and arranged in stacks called grana) and converting this light-quanta energy into energy rich compounds such as ATP and $NaDPH_2$. In dark reaction, that is take place inside the stroma of chloroplast, these energy rich compounds used in fixation of carbon dioxide to form the food (carbohydrate). The overall photosynthetic reaction is as follows:



$$6CO_2 + 12\,H_2O \xrightarrow[\text{Enzymes}]{\text{Sun light}} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

The rate of photosynthesis governed by intrinsic as well as extrinsic factors, major intrinsic factors include; the chloroplast amount and their distribution inside the cell, stomata level and their opening and closing time, transportation mechanism of gases (CO_2 and O_2), different enzyme levels and different CO_2 saturation levels. If we exclude the intrinsic nature of plant, the rate of photosynthesis affected by light intensity, temperature, amount of CO_2, water and the color of light (wavelength) in general.

It has been known for long time that plants absorb red and blue light to maximum and least amount to yellow to carryout photosynthesis. However the rate of photosynthesis varies among all the absorbed wavelengths. The absorption of light and rate of photosynthesis by major chlorophyll molecule-*a* is shown below in figure 4.1. The rate of photosynthesis is usually maximum in red light in spite of maximum absorption of blue light because blue light is absorbed not only by chlorophyll, but also by carotenoids and some carotenoids are not in the chloroplasts. Carotenoids further constitute to carotenes and xanthophylls. Carotenes do not transfer absorbed energy efficiently to chlorophyll, and thus some part of absorbed blue light is not going to photosynthesis. On the other hand, all of red light is absorbed by chlorophylls and used effectively in photosynthesis. The rate of photosynthesis in yellow light is insignificant

being least absorbance of yellow light. Plants do not absorb green light and most of the green light is reflected back to our eyes, visualizing plants green. However some deep ocean algae also use green light in photosynthesis.

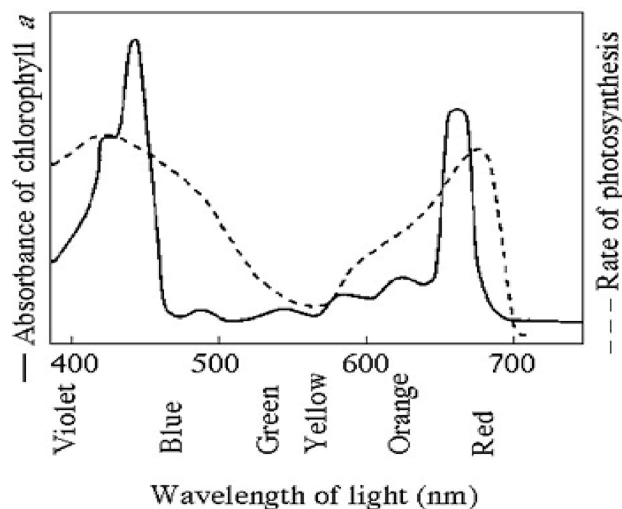


Fig.4.1 Absorption of different wavelengths (solid line) and their respective rate of photosynthesis (dashed line)

The concentration of carbon dioxide (CO_2) with light intensity is limiting factor for photosynthesis. The rate of photosynthesis initially increases with increase in internal CO_2 concentration but after a certain level of CO_2, rate of photosynthesis becomes constant as being saturation of rubisco enzyme. The effect of CO_2 concentration on rate of photosynthesis is shown in fig.4.2.

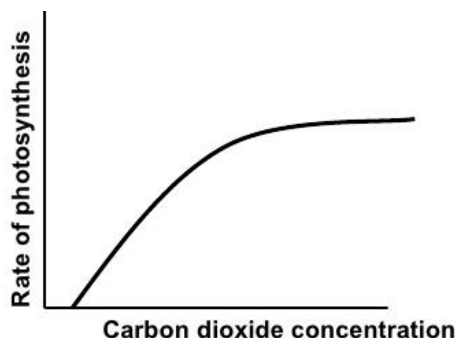


Fig.4.2: Effect of CO_2 level on rate of photosynthesis

4.3 INSTRUMENT AND MATERIAL REQUIRED

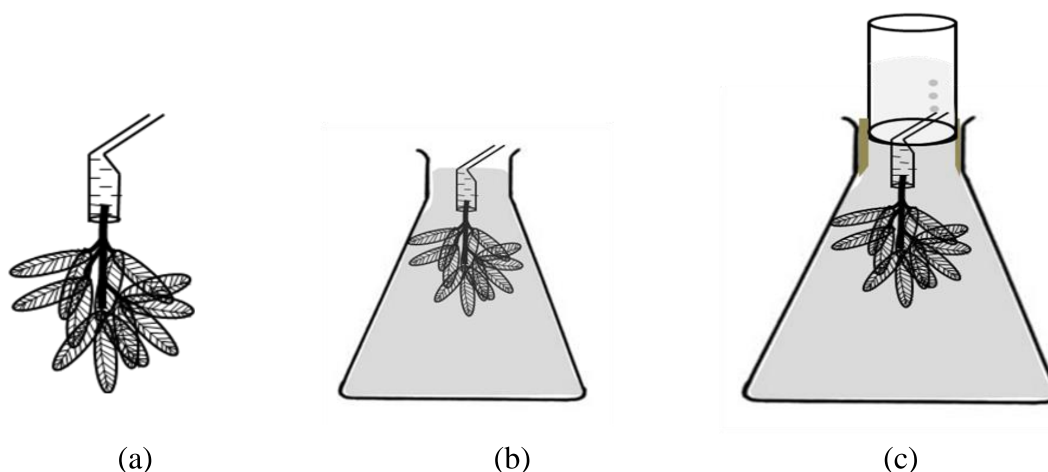
Wilmott's bubbler, Beaker, funnel, test tubes, Cellophane papers of red, blue and green colors, Hydrilla (fresh plants), Vaseline, Sodium bicarbonate, water

4.4 EFFECT OF VARYING CO_2 CONCENTRATION ON RATE OF PHOTOSYNTHESIS

4.4.1 Procedure

1. Take a known amount of actively photosynthesizing Hydrilla plants in a beaker containing 0.05% of sodium bicarbonate solution.
2. The amount of Hydrilla plants taken must be kept constant throughout the experiment.
3. Now insert the hydrilla plant twig on wider side of Wilmott's bubbler in such a way that all the cut ends of the twigs face upwards.
4. Now keep a glass tube in inverted direction over the bubbler like thinner side of bubbler should be inside the glass tube. All the blank space between the flask, bubbler and tube must be sealed with cork or candle wax (as shown in figure 1.)
5. Glass tube must be filled with water up to 2/3 of the length leaving some space empty upside.
6. Keep the experimental set up in light and count the number of air bubbles evolved in each minute for 10 minutes.
7. Record your results in observation table
8. Repeat the above procedure with 0.1%, 0.15%, 0.20% and 0.25% solutions of sodium bicarbonate in the beaker.
9. Count the air bubbles evolved for 0.5%, 0.1%, 0.15%, 0.20% and 0.25% solutions. The volumes of the solutions taken should be same for all the strengths of the solutions.

10. Plot a graph between numbers of O_2 bubbles evolved per minute from Hydrilla plants for different concentrations of CO_2.



(a) (b) (c)

Fig.4.3 Wilmott's bubbler experiment set up

4.4.2 Observation: The rate of evolution of bubbles varies with concentration.

No.	Number of O_2 bubbles evolved per minute at 0.05 %	Number of O_2 bubbles evolved per minute at 0.1 %	Number of O_2 bubbles evolved per minute at 0.1 %	Number of O_2 bubbles evolved per minute at 0.15 %	Number of O_2 bubbles evolved per minute at 0.2 %
1					
2					
3					
Avg.					

4.4.3 Interpretation: With the increase in CO_2 concentration the rate of photosynthesis (no. of bubbles evolved) also increases.

4.4.4 Result: Draw a graph between CO_2 concentration and the number of bubbles release per minute as shown in fig.4.4

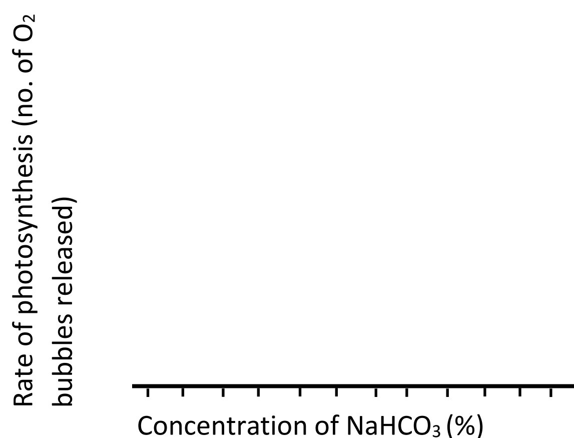


Fig.4.4 Rate of photosynthesis measurement by counting the number of O2 bubbles released

4.5 EFFECT OF DIFFERENT WAVELENGTHS (RED, GREEN AND BLUE) ON RATE OF PHOTOSYNTHESIS

4.5.1 Procedure:

1. Take a known amount of actively photosynthesizing Hydrilla plants in a beaker containing water.

2. The amount of Hydrilla plants taken must be kept constant throughout the experiment.

3. Now insert the hydrilla plant twig on wider side of Wilmott's bubbler in such a way that all the cut ends of the twigs face upwards.

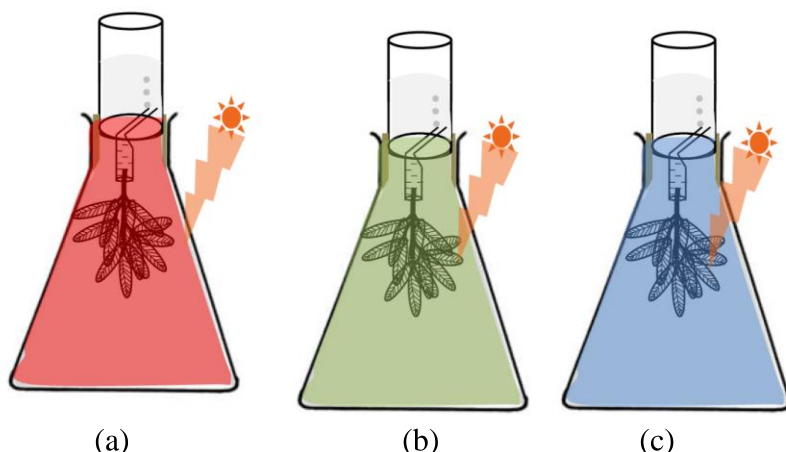
4. Now keep a glass tube in inverted direction over the bubbler in such a way that narrow side of bubbler should be inside the glass tube. All the blank space between the flask, bubbler and tube must be sealed with cork or candle wax (as shown in figure 1.)

5. Glass tube must be filled with water up to 2/3 of the length leaving some space empty upside.

6. Keep the experimental set up in light and count the number of air bubbles evolved in each minute for 10 minutes.

7. Record your results in observation table

8. Repeat the steps XVI and XVII by covering the whole conical flask with red, blue and green cellulose papers one by one and record the results as shown in fig.4.5.



(a) (b) (c)

Fig.4.5 Effect of different wavelength by covering the conical flask with red (a), green (b) and blue (c) color cellophane paper

4.5.2 Observation: Effect of light wavelength on the rate of photosynthesis.

No.	Number of O_2 bubbles evolved per minute in normal light	Number of O_2 bubbles evolved per minute in red light	Number of O_2 bubbles evolved per minute in green light	Number of O_2 bubbles evolved per minute in blue light
1				
2				
3				
Avg.				

4.5.3 Conclusion: Rate of photosynthesis is more in red light and decreases in green light.

4.6 SUMMARY

From the above description, you will understand how to study the rate of photosynthesis using Wilmott's bubbler experiment with different concentration of $NaHCO_3$. Similarly you will know how to study the effect of different wavelength on the rate of photosynthesis using cellophane paper of different colors. This practical exercise will help you to understand how the both limiting factors (light quality and CO_2 concentration) affect to the photosynthesis. The rate of photosynthesis first initially increases exponentially with increase in CO_2 concentration but at high concentration of CO_2, rate becomes constant and further increase in CO_2 concentration the rate of photosynthesis does not increase. In the experiment of light quality effect to the rate of photosynthesis you should know that rate of photosynthesis is maximum in red light because of maximum absorption of red light by chlorophyll molecules and negligible in green light because least absorption of green light by chlorophyll, however plants also absorb blue light to greater extent but maximum part of blue light is absorbed by carotenoids and not by the chlorophyll molecules.

4.7 GLOSSARY

Autotrophs: Living beings on earth those make their food themselves, only plants are capable to do this because the presence of green chlorophyll molecules inside their cells

Chlorophyll molecule-*a:* It is the major chlorophyll molecule present mostly inside the cells of all green plants

Carotenoids: These are accessory pigment molecules also present inside the cells of green plants, their main role to protect the photomachinery from photobleaching.

Carotenes: These are the type of carotenoids and their major function not only to transfer light quanta to chlorophyll molecules and to protect against photobleaching but also to absorb blue light.

Xanthophylls: These are the type of carotenoids

Stomata: These are the natural openings (pores) in plants through which maximum gases transfer take place. Such as O_2, CO_2 and also water vapour.

Thylakoid: these are the membrane like folds in the form of hollow discs and arranged in stacks inside the chloroplast.

Stroma: The plasma or fluid present inside the chloroplast

Light reaction: It is the first reaction of photosynthesis where the light quanta energy is transformed into energy rich compounds such as ATP and $NaDPH_2$.

Dark reaction: It is also called light independent reaction, where fixation of CO_2 takes place with the help of H_2O and energy right compounds such as ATP and $NaDPH_2$ in the presence of Rubisco enzyme.

4.8 SELF ASSESSMENT QUESTIONS

Q.1. What do you understand by light quanta?

Q.2. Write down the name of accessory pigment molecules and their function?

Q.3. Describe why the rate of photosynthesis initially exponentially increases with increase in CO2 concentration but at higher concentration rate becomes constant?

Q.4. Discuss briefly why the rate of photosynthesis maximum in red light and less in blue light?

Q. 5. Why the plants do not show any photosynthesis in green light?

Q. 6. Give the overall general chemical equation of photosynthesis.

Q 7. List the two categories of photosynthetic pigments.

Q 8. Name the type of energy that is used in the process of photosynthesis. In which form does this energy get stored in plant body?

Q 9. Which molecule is the source of evolution of oxygen in photosyntheisis-CO2or H2O?

Q 10. What is the role of $NADPH_2$?

Q. 11. Why is dark reaction called so?

Q 12. Name the two sets of reactions in photosynthesis in which light energy is required.

Q. 13. State the principle of limiting factor.

Q 14. What do you understand by photophosphorylation?

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4.11 TERMINAL QUESTIONS

Q.1. Describe the procedure to study the effect of CO_2 concentration on rate of photosynthesis.

Q.2. Describe the procedure used to measure the effect of light wavelength on rate of photosynthesis.

Q.3. Draw the layout of wilmott’s bubbler set up.

Q.4. Describe rate limiting steps in photosynthesis.

Q.5. Describe the role of accessory pigment molecules in photosynthesis.

UNIT-5: SEPARATE PIGMENTS, VIZ., CHLOROPHYLL ‘a’ AND ‘b’, CAROTENE AND XANTHOPHYLLS FROM GREEN LEAVES BY PAPER CHROMATOGRAPHY AND COLUMN CHROMATOGRAPHY

5.1 Objectives
5.2 Introduction
5.3 Experiment
5.3.1 Materials and reagents
5.3.2 Instrument required
5.3.3 Procedure
5.3.3.1 Extraction of pigments from leaves
5.3.3.2 Separation of pigments from leaves
5.3.3.2 .1 Separation of pigments using Column Chromatography
5.3.3.2 .2 Separation of pigments using Thin-Layer Chromatography (TLC)
5.3.4 Precautions
5.4 Summary
5.5 Glossary
5.6 Self Assessment Question
5.7 References
5.8 Suggested Readings
5.9 Terminal Questions

5.1 OBJECTIVES

The current exercise describing about the extraction and separation of leaf pigments such as Chlorophyll a, b, carotenoids etc. Different leaf pigments can be extracted using organic solvents of different polarities. Here in this exercise, extraction of leaf pigments using Pasteur pipette as a column chromatography explained followed by thin layer chromatography (TLC). By comparing the calculated R_f values from TLC-chromatogram of different sample fractions with standards, one can easily assured about the type of pigment.

5.2 INTRODUCTION

Plant leaves contain a number of important pigments including chlorophylls, carotenes, and xanthophylls. During the spring when leaves contain large amounts of chlorophyll (and are thus green), the presence of the other pigments is not obvious to the eye. During the fall, however, after most of the chlorophyll has been degraded, these other pigments can be more readily observed, and the leaves of many plants take on the variety of beautiful colors that are typical of fall foliage. However this story is not valid for evergreen plants having throughout the year constant content of green chlorophyll pigments with other accessory pigments.

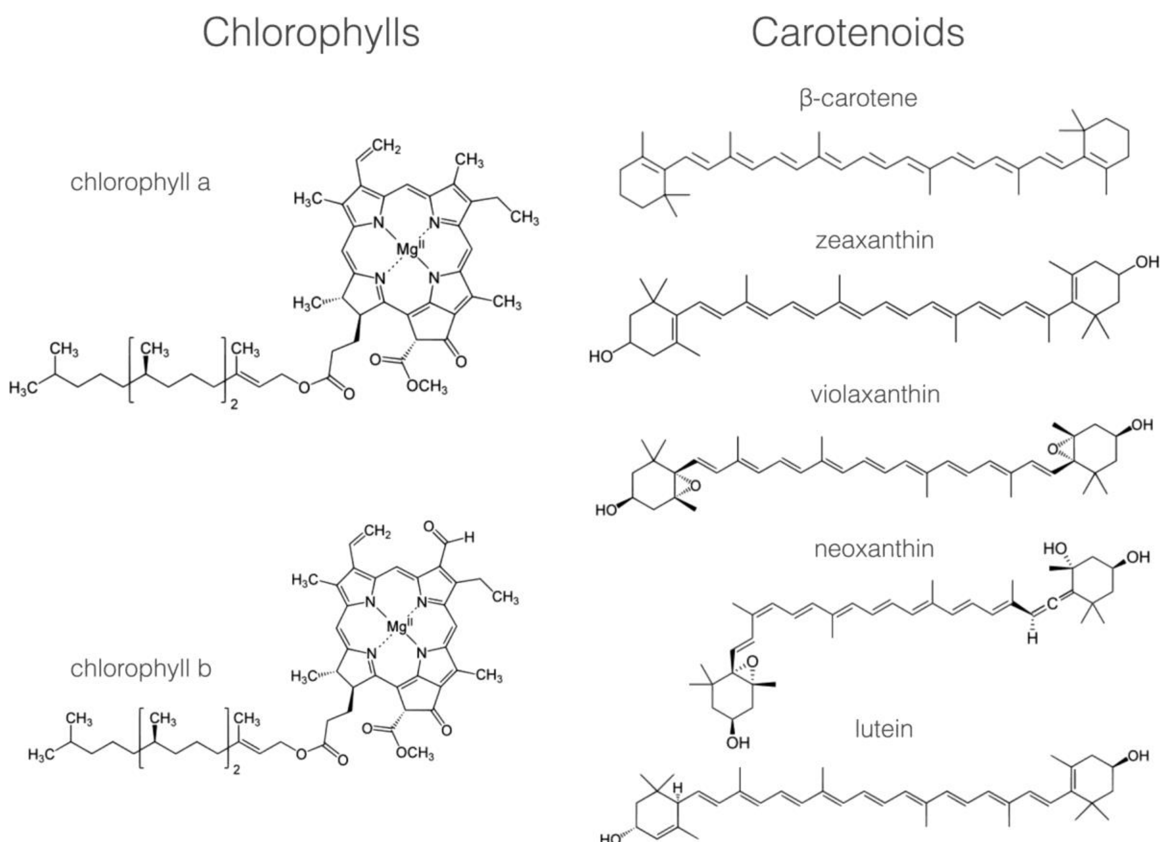


Fig: 5.1 Structure of chlorophyll (*a* & *b*), β-carotene and xanthophyll molecules

The structure of different pigment molecules shown in figure 5.1, and their visible light absorption and reflection characteristics are shown in Table-5.1.

Table 5.1: Chlorophyll and accessory pigment molecules-characteristic absorption and reflection of visible light

Pigment	Absorption of light	Reflection of light
Chlorophyll (membrane-bound)	red and blue light	green
Carotenes (membrane-bound)	violet, blue, and green light	yellow, orange, and red
Xanthophylls (membrane-bound)	violet, blue, and green light	yellow, orange, and red
Anthocyanins (water-soluble)	green, yellow, orange, red	violet, blue, red

However some plant leaves are normally colorful and contain red, yellow, white patches (containing accessory pigment molecules in good quantity with chlorophyll molecules). During this laboratory exercise, you will have a chance to collect leaves of various colors and analyze the different pigments that are present. The whole procedure of leaf pigment analysis is categorized in three parts; isolation of pigment molecules, separation of pigment molecules and characterization of pigment molecules. Once the pigment molecules were isolated using organic extraction method, their separation can be done by column chromatography.

Column chromatography involves the separation of compounds by the same mechanism as other chromatographic techniques, i.e. differences in partitioning between mobile and stationary phases. The stationary phase serves as an adsorbent through which the mobile phase is passed. Many compounds with varying functional groups may be used as the stationary phase and several types of interactions can aid in developing the desired separation (i.e., hydrogen bonding, dipole-dipole interactions, electrostatic interactions, Van-der Waals forces, size exclusion, affinity, etc.). The following sequence illustrates the general affinity of the functional groups towards a polar stationary phase like silica:

ionic > acids/bases > amides > alcohols > ketones > aldehydes > esters > ethers > halides > unsaturated hydrocarbons > saturated hydrocarbons

The major advantages of column chromatography are its ability to handle larger amounts of material (compared to GC, TLC and HPLC) and the ability to change the eluting solvent throughout the course of the separation. This allows one to remove one component while a desired product remains essentially unmoved. A change in the mobile phase moves the desired product through the column, which may include changes in solvent polarity, changes in pH, or changes in ionic strength. The last two are used largely in biological separations (proteins, peptides). By varying the stationary phase and by changing the mobile phase, an efficient separation may be achieved.

In this assignment, alumina serves as the stationary phase, which is considered a polar stationary phase because of the presence of oxygen and hydroxyl groups on the surface. Thus, the more polar components are held to the polar alumina more tightly and, therefore, move through the column more slowly. Increasing the polarity of the solvent moves all components faster but has the largest effect on polar compounds because their solubility increases significantly. Finally, you will characterize pigment fractions using thin layer chromatography, which also separates by polarity.

Thin-layer chromatography (TLC) is a convenient technique for separating and analyzing the different pigments present in a leaf. Different fractions collected from column chromatography are spotted onto a TLC plate at different positions and an organic solvent is allowed to move up the plate, potentially carrying with it the compounds present in the fraction. In this way different components of fractions are separated based on their affinities for the stationary phase (the silica on the TLC plate) and for the mobile phase (the solvent that is moving up the plate). Compounds with more affinity for the silica (i.e. hydrophilic compounds) will not move very far, while compounds with a high affinity for the organic solvent (i.e. hydrophobic compounds) will move much farther.

5.3 EXPERIMENT

5.3.1 Materials and reagents

Green leaves, acetone, anhydrous sodium sulphate (Na_2SO_4), hexane, Chromatograph grade silica powder or alumina, methanol.

Screw cap glass bottles, beakers, centrifuge tubes, spatula, mortar-pestle, Pasteur pipette(to be used as a column in extraction process), pipette holding stand, capillaries, TLC Plates or paper, TLC-Jar, TLC-developing chamber or hood, pencil or marker, scale etc.

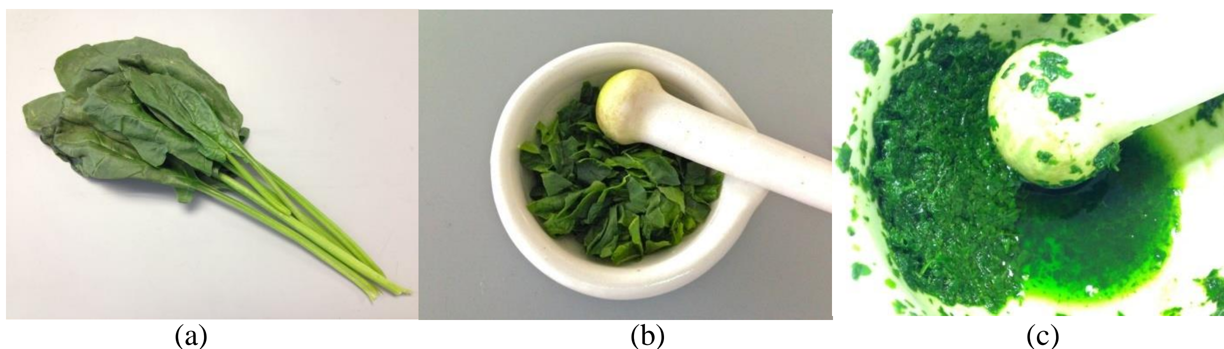
5.3.2 Instrument required

- Centrifuge,
- UV-Visb. spectrophotometer

5.3.3 Procedure

5.3.3.1 Extraction of pigments from leaves

1- Weigh about 1.0 g of fresh green leaves (avoid using stems or thick veins), here as example, fresh green spinach leaves taken. Cut or tear the spinach leaves into small pieces and place them in a mortar along with 0.5 g of anhydrous sodium sulfate (Na_2SO_4) and 2.0 mL of acetone. Grind with a pestle until the spinach leaves have been broken into particles too small to be seen clearly and mix well with Na_2SO_4.



(a) (b) (c)

Fig.5.2: Green spinach leaves (a), small pieces of spinach leaves (b), grinding of leaves with pestle

2- If too much acetone has evaporated, you may need to add an additional portion of acetone (0.5-1.0 mL). Using a Pasteur pipette or spatula, transfer the mixture to a centrifuge tube. Rinse the mortar and pestle with 2.0 mL of cold acetone, and again transfer the remaining mixture to the centrifuge tube. Cap it tightly. Centrifuge the mixture at 2000 rpm for 5 min, collect the supernatant and discard the settled plant tissue debris.

3- Add ~2.0 mL of hexane to the centrifuge tube, cap the tube, and shake the mixture thoroughly. Next, add 2.0 mL of water and shake thoroughly with occasional venting. Centrifuge the mixture at 2000 rpm for 5 min, to break the emulsion, which usually appears as a cloudy green layer in the middle of the mixture. The pigment layer is the top layer, which should be dark green. Most of the acetone will dissolve in the water.

4- Using a dry Pasteur pipette, carefully separate layers and transfer the top organic layer (a dark-green hexane solution of spinach pigments) into a clean test tube. Add another 1.0 mL of hexane to the centrifuge tube that contains the aqueous layer, cap the tube and centrifuge the mixture to break the emulsion. Separate the layers again and add the top layer to the test tube.

5- The dark-green hexane solution of spinach pigments in the test tube may contain traces of water that must be removed before separating the components through chromatography. To dry the solution, add 0.5 g of anhydrous sodium sulfate (Na_2SO_4) to the hexane solution. Cap and gently swirl to allow the sodium sulfate to contact all parts of the hexane. After standing for 5 minutes, use a clean, dry Pasteur pipette to transfer the liquid into another clean test tube. Label this test tube with an "E" for extract so that you do not confuse it with the test tubes you will be working with later in this experiment.

6- Add about 0.5 mL hexane to rinse the hydrated sodium sulfate and transfer this liquid to the same test tube, "E".

5.3.3.2 Separation of pigments from leaves

5.3.3.2.1 Separation of pigments using Column Chromatography

1- Clamp a clean, dry Pasteur pipette vertically and push a very small plug of cotton to the bottom of the pipette. Weigh out about 1.25 g of alumina or silica. Pack the column with alumina

and gently add a little less than one half cm of sea sand to the top of the column. Tap gently to achieve a flat surface.

Note: Once the procedure is started, it should not be stopped: The alumina or silica must be kept wet with solvent all the time. Since the column does not have a stopcock to stop the flow of solvent during the procedure, all the solvents must be at your workspace before starting the process.

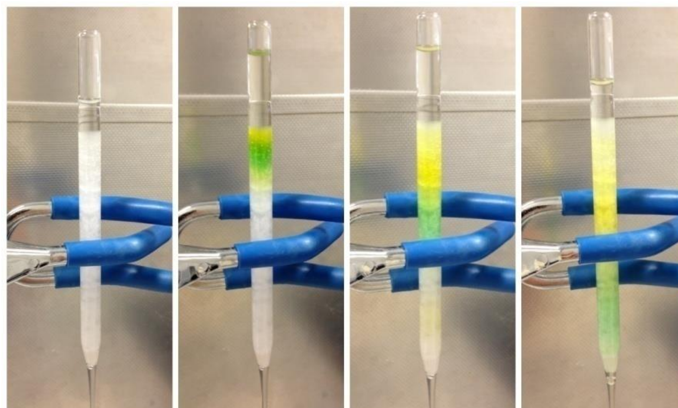


Fig.5.3: Gradual separation of leaves pigments through Pasteur column filled with stationary phase (silica gel or alumina)

2- In separate labeled containers obtain 15 mL hexane, 15 mL of 70 % hexane-30 % acetone solution, 15 mL acetone and 15 mL of 80 % acetone-20 % methanol solution. Also, label six test tubes 1 through 6 and a beaker as "waste solvent."

3- First, transfer about 0.5 mL of your spinach extract (from test tube, *E*) to a small vial for use later in the thin layer chromatography analysis.

4- Then, when you are ready to begin the column separation, place the waste solvent beaker under the column and add about 3.0 mL of hexane to the top of the column. As the alumina is wetted, the hexane will flow into the beaker. When the solvent level drains to the top of the sand add your spinach extract to the top of the column.

5- As the extract drains onto the alumina, the pigments should begin to separate into a yellow carotene band and a green chlorophyll band. If a separation is observed, add an additional 4 mL of hexane and continue collecting solvent in your waste solvent beaker until the yellow band reaches the bottom of the column and the solvent draining out turns yellow. Replace the waste beaker with test tube #1. Continue to add hexane until the yellow band passes through the column switching to test tubes #2, #3, etc as necessary until the yellow eluant becomes clear. Then, replace the test tube with the waste beaker.

6- If the yellow band has not begun to separate from the green band after the initial 4 mL of hexane has drained through the column, add 4 mL of the next more polar solvent (70 % hexane-30 % acetone). When changing solvents, do not add the new solvent until the level of the last

solvent is almost at the top of the alumina. When the appropriate solvent is found add this solvent until the yellow band is completely removed from the column.

7- Once the yellow band has eluted from the column, add several mL of the next more polar solvent when the level of the last solvent is almost at the top of the see sand. If the green band moves down the column, continue to add this solvent until the green band is eluted completely from the column. If the green band does not move, change to the next polar solvent. Collect the green band in a clean test tube. Cover and save these fractions for the next lab period when you will study the visible spectrum (using U.V/Visb. spectrophotometer) and TLC of the green and yellow fractions.

Note: If it is necessary due to time constraints, the vial can be labeled with your name, capped, and stored in freeze at 4 ^{o}C until the next laboratory period.

5.3.3.2.2 Separation of pigments using Thin-Layer Chromatography (TLC)

Note: Before to carry out the thin layer chromatography (TLC) on your fractions and original extract, must save about 3-mL of the chlorophyll and β-carotene fractions for UV-visible spectra.

1- For to start TLC experiment, concentrate the separated fractions. Pass a gentle stream of air over all the solution fractions in a hood to evaporate the solvent until the about 1 mL of yellow and 1 mL of green solution left. If you have several test tubes of the same pigment, combine the contents as you evaporate so that you end up with just one vial of yellow carotenes and one vial of green chlorophylls.

2- Gently draw a horizontal pencil line about 1 cm from the bottom of a thin layer chromatographic paper or plate (10 cm x 4 cm). As shown in the figure.5.4 below, spot your three solutions (*E*, yellow and green) on this line. Use a separate microcapillary tube for each solution. Fill each capillary by dipping it in the solution and gently touch it to the plate to empty it.

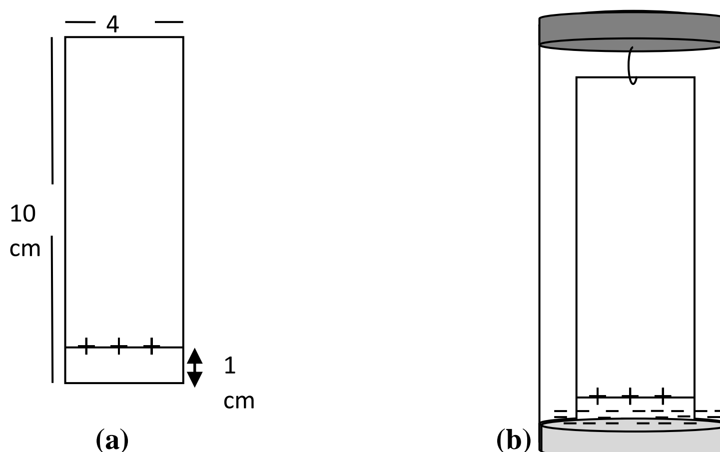


Fig.5.4 Marking on chromatographic paper (a) and running of mobile phase through the chromatographic paper after spotting in chromatographic jar (b).

3- Use several short touches to empty each capillary so that the spots will be small (1-2 mm in diameter). If the spots look light in color, re-spot on top of the original spots until the spots are fairly dark. Allow the spots to dry as shown in fig. 5.5.

4- Obtain a chromatography jar, and to the jar add about 10 mL of developing solvent (70 % hexane-30 % acetone) then place the TLC plate in the developing jar with the spotted end at the bottom of the jar and allow the solvent to rise up the plate undisturbed until about 80 % of the plate is wet (The start line has to be above the solvent level). Remove the plate and quickly draw a pencil line across the plate to mark the farthest reach of the solvent. This is called the solvent front. Allow the wet plate to dry in the hood. Circle the visible spots in pencil since the colors may fade over time. Transfer the diagram into your lab notebook and take a picture with your cell phone as well.

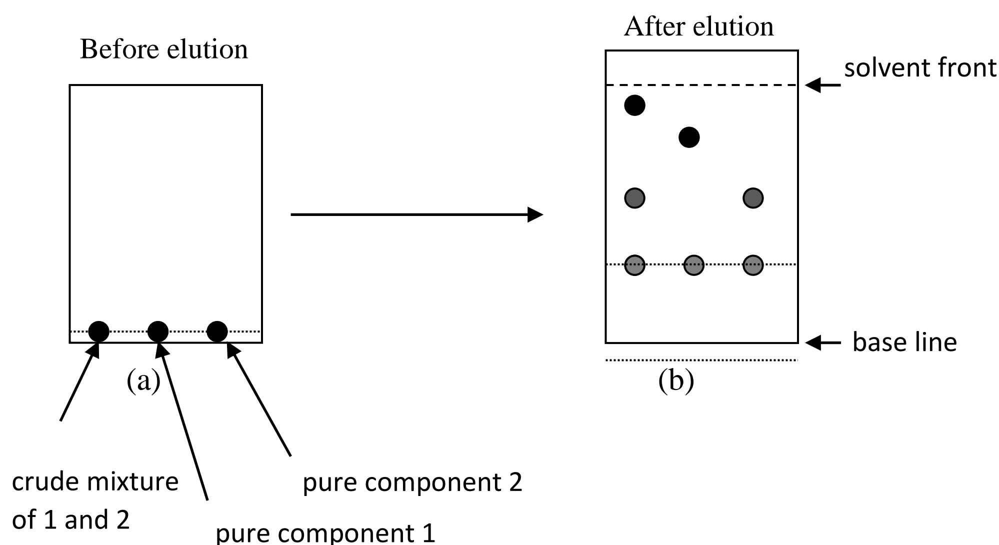


Fig.5.5 Spotting on chromatographic paper (a) before mobile phase running and (b) after running of mobile phase

$$R_f = \frac{distance\ from\ baseline\ to\ spot}{distance\ from\ baseline\ to\ solvent}$$

1- Calculate the R_f-values for each spot on your plate. Spots with the same R_f-values within experimental error and the same appearance are likely to be the same compound.

2- Include a sketch of your TLC plate in your notebook. Your plate, however, may be considerably more complex in appearance than the example above.

3- Depending on the spinach sample, the conditions of the experiment, and how much sample was spotted on the TLC plate, you may observe other pigments. These additional components can result from air oxidation, hydrolysis, or other chemical reactions involving the pigments discussed in this experiment.

4- Be sure to clearly label the lanes as coming from extract, yellow band or green band and label the colors of as many of the separated spots as possible.

5- Using the guide below, identify as many spots in the TLC as you can. Determine which pigments were present in the yellow band and which were present in the green band from your column.

Pigments in spinach in order of decreasing R_f-values:

Carotenes (1 spot) (yellow-orange)
Pheophytin a (gray, may be nearly as intense as chlorophyll **b**)
Pheophytin b (gray, may not be visible)
Chlorophyll a (blue-green, more intense than chlorophyll **b**)
Chlorophyll b (green)
Xanthophylls (possibly three spots: yellow)

6- Record your observation as in table 2.

Table 5.2. Record table of separated leaf pigments

SI. No.	Name of the extract	Colour of the spot	Distance travelled by the components from the reference line in cm	Distance travelled by the solvent from the reference line in cm	R_f values
1.					
2.					
3.					
4.					
5					

5.3.4 Precautions

(a) Use good quality pencil for drawing the reference line so that the mark does not dissolve in the solvent in which TLC is run.

(b) Dip the paper strip in the solvent in such a way that the spot of the mixture is above the solvent level and the movement of the solvent front is not zig-zag.

(c) While spotting the test solution on the paper, do not allow the spots to spread. Use finely drawn capillary to put the spot on the paper.

(d) Ensure that the filter paper strip hangs freely in the jar.

(e) Once the experiment is set, do not disturb the jar as long as the chromatogram is being developed.

(f) Keep the jar covered with the lid when the chromatogram is being developed.

(g) Make the paper strip perfectly dry before developing the spots.

(h) Handle the organic solvent/solvents, with care.

5.4 SUMMARY

This laboratory exercise described about the extraction, isolation and characterization of different pigment molecules present in the specimen (leaf) using solvent extraction, column chromatography and thin-layer chromatography or paper chromatography respectively.

In solvent extraction, pigment molecules were isolated from rest of the components and using column chromatography pigment molecules were separated from each-other followed by characterization using thin-layer chromatography. After running the TLC, interpretation of results done by calculating R_f values of test sample and standard sample and comparing them.

5.5 GLOSSARY

Chlorophyll: Chlorophyll is a green photosynthetic pigment found in plants, algae, and cyanobacteria.

Carotenoids: Carotenoids are yellow, orange, and red pigments synthesized by plants work as a accessory pigment molecules in photosynthesis during light reraction.

Hydrophobic: Nonpolar molecules that repel the water molecules are said to be hydrophobic.

Hydrophilic: Having an affinity for water; able to absorb, or be wetted by water; water-loving.

Mobile Phase: A pure or mixture of solvents or gas passes over stationary phase in chromatography.

Pasteur pipette: Pasteur pipettes, also known as droppers are used to transfer small quantities of liquids.

R_f value: R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

Stationary Phase: The solid or liquid phase of a chromatography system on which the materials to be separated are selectively adsorbed.

TLC-plate: A supporting stationary phase used in thin-layer chromatography.

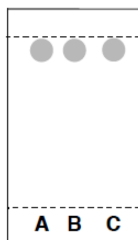
UV/Visib. Spectroscopy: refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region.

5.6 SELF ASSESSMENT QUESTIONS

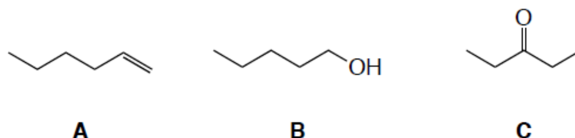
Q.1. What are the characteristic absorption maxima of chlorophyll a, b and carotenoids?

Q.2. How is the Rf value for a spot on a TLC plate calculated? What can the Rf value be used for?

Q.3. A TLC plate is spotted with each of the the three compounds. The plate is developed using hexanes:ethyl acetate (5:95) to give the chromatogram shown below. How could you change the solvent system to give better separation of these three compounds?



Q.4. Consider the following compounds when answering the questions below. Assume these compounds can be separated by TLC.



Q.5. How is the elution of compounds through silica-gel column affected by the change in polarity of eluting solvent?

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5.8 SUGGESTED READINGS

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5.9 TERMINAL QUESTIONS

Q1: What is a Chromatogram? Explain the principle on which the technique of chromatography is based.

Q2: What are the essential characteristics of the substance used as a developer in TLC?

Q3: How is the phenomenon of 'adsorption' applied in the separation of compounds by

chromatography?

Q4: Describe the column chromatography procedure to fractionate the different types of pigment molecules present in the leaf?

Q5: Describe the principles behind the TLC-separation of pigment molecules from leaf?

Q6: Describe the solvent extraction procedure to isolate pigment molecules from leaf?

UNIT-6: MEASURE THE RATE OF PHOTOSYNTHESIS BY WINKLER'S METHOD

6.1 Objectives
6.2 Introduction
6.3 Winkler method
6.4 Summary
6.5 Glossary
6.6 Self Assessment Questions
6.7 References and Suggested Readings
6.8 Terminal Questions

6.1 OBJECTIVES:

After reading this unit students will be able:

- To measure the rate of photosynthesis by Winkler's method.

6.2 INTRODUCTION:

Aquatic plants and animals need dissolved oxygen to live. Fish, invertebrates, plants and aerobic bacteria all require oxygen for respiration. Oxygen dissolves readily into water from the atmosphere until the water is saturated. Once dissolved in the water, the oxygen diffuses very slowly and distribution depends on the movement of the aerated water. Oxygen is also produced by aquatic plants, algae and phytoplankton as a by-product of photosynthesis. This test kit uses the azide modification of the Winkler method for determining the dissolved oxygen. The stream system both produces and consumes oxygen. It gains oxygen from the atmosphere and from plants as a result of photosynthesis. Running water, because of its churning, dissolves more oxygen than still water, such as that in a reservoir behind a dam. Respiration by aquatic animals, decomposition, and various chemical reactions consume oxygen.

Wastewater from the sewage treatment plants often contains organic materials that are decomposed by microorganisms, which use oxygen in the process. (The amount of oxygen consumed by these organisms in breaking down the waste is known as the biochemical oxygen demand or BOD). Other sources of oxygen-consuming waste include storm water runoff from farmland or urban streets, feedlots and failing septic systems. Oxygen is measured in its dissolved form as dissolved oxygen (DO). If more oxygen is consumed than is produced, dissolved oxygen levels decline and some sensitive animals may move away, weaken or die.

DO levels fluctuate seasonally and over a 24-hour time period; they vary with water temperature and altitude. Cold water holds more oxygen than warm water and water holds less oxygen at higher altitudes. Thermal discharges, such as water used to cool machinery in a manufacturing plant or a power plant, raise the temperature of water and lower its oxygen content. Aquatic plants & animals are most vulnerable to lowered DO levels in the early morning on hot summer days when stream flows are low, water temperatures are high and aquatic plants have not been producing oxygen since sunset.

6.2.1 Dissolved oxygen, percent saturation & BOD

Oxygen is critical to the survival of aquatic plants and animals and a shortage of dissolved oxygen is not only a sign of pollution, it is harmful to fish. Some aquatic species are more sensitive to oxygen depletion.

6.2.2 Where does the oxygen come from?

The oxygen found in water comes from many sources, but the largest source is oxygen absorbed from the atmosphere. Wave action and splashing allows more oxygen to be absorbed into the

water. A second major source of oxygen is aquatic plants, including algae; during photosynthesis plants remove carbon dioxide from the water and replace it with oxygen.

6.2.3 Absorption:

Oxygen is continuously moving between the water and surrounding air. The direction and speed of this movement is dependent upon the amount of contact between the air and water. A tumbling mountain stream or windswept, wave-covered lake, where more of the water's surface is exposed to the air, will absorb more oxygen from the atmosphere than a calm, smooth body of water. This is the idea behind aerators: by creating bubbles and waves the surface area is increased and more oxygen can enter the water.

6.2.4 Photosynthesis:

In the leaves of plants, one of the most important chemical processes on Earth is constantly occurring: photosynthesis. During daylight, plants constantly take carbon dioxide from the air and in the presence of water convert it to oxygen and carbohydrates, which are used to produce additional plant material. Since photosynthesis requires light, plants do not photosynthesize at night, so no oxygen is produced. Chemically, the photosynthesis reaction can be written as:

Light + nCO_2 + nH_2O--------------------(C_2HO) n + nO_2

Light + $C0_2$+ Water Carbohydrate + Oxygen Dioxide

6.2.5 Where does the oxygen go?

Once in the water, oxygen is used by the aquatic life. Fish and other aquatic animals need oxygen to breathe or respire. Oxygen is also consumed by bacteria to decay, or decompose, dead plants and animals.

6.2.6 Respiration

All animals, whether on land or underwater, need oxygen to respire, grow and survive. Plants and animals respire throughout the night and day, consuming oxygen and producing carbon dioxide, which is then used by plants during photosynthesis.

6.2.7 Decomposition

All plant and animal waste eventually decomposes, whether it is from living animals or dead plants and animals. In the decomposition process, bacteria use oxygen to oxidize, or chemically alter, the material to break it down to its component parts. Some aquatic systems may undergo extreme amounts of oxidation, leaving no oxygen for the living organisms, which eventually leave or suffocate.

6.2.9 Measuring BOD (biochemical oxygen demand):

Biochemical oxygen demand is determined by measuring the dissolved oxygen concentration in a freshly collected water sample and comparing it to the dissolved oxygen level in a sample that

was collected at the same time, but incubated under specific conditions for a specific length of time. The difference between the two oxygen levels represents the amount of oxygen required for the decomposition of organic material and the oxidation of chemicals in the water during the storage period, a measurement known as the BOD. Unpolluted, natural waters will have a BOD of 5 ppm or less. Raw sewage may have levels of 150 to 300 ppm. Wastewater treatment plants must reduce BOD to levels specified in their discharge permits, usually between 8 and 150 ppm BOD.

6.2.10 Testing dissolved oxygen:

The first step in a DO titration is the addition of Manganous Sulfate Solution (4167) and Alkaline Potassium Iodide Azide Solution (7166). These reagents react to form a white precipitate, or manganous hydroxide, Mn (OH) 2. Chemically, this reaction can be written as:

$$MnSO_4 + 2KOH \text{--------------------} Mn(OH)_2 + K_2SO_4$$

$MnSO_4$ =	Manganous sulfate	$Mn(OH)_2$=	Manganous hydroxide
2KOH =	Potassium hydroxide	$K2SO_4$=	Potassium sulfate

Immediately upon formation of the precipitate, the oxygen in the water oxidizes an equivalent amount of the manganous hydroxide to brown-colored manganic hydroxide. For every molecule of oxygen in the water, four molecules of manganous hydroxide are converted to manganic hydroxide. Chemically, this reaction can be written as:

$$4Mn(OH)_2 + O_2 + 2H_2O \text{----------------------------} 4Mn(OH)_3$$

$4Mn(OH)_2$ =	Manganous hydroxide	$2H_2O$ =	Water
O_2 =	Oxygen	$4Mn(OH)_{34}$=	Manganic Hydroxide

After the brown precipitate is formed, a strong acid, such as Sulfamic Acid Powder (6286) or Sulfuric Acid, 1:1 (6141) is added to the sample. The acid converts the manganic hydroxide to manganic sulfate. At this point the sample is considered fi xed" and concern for additional oxygen being introduced into the sample is reduced. Chemically, this reaction can be written as:

$$2Mn(OH)_3 + 3H_2SO_4 \text{--} Mn_2(SO_4)_3 + 6H_2O$$

$2Mn(OH)_3$ =	Manganic hydroxide	$Mn_2(SO_4)_3$ =	Manganic Sulfate
$3H_2SO_4Mn_2$=	Sulfuric Acid	$6H_2O$ =	Water

Simultaneously, iodine from the potassium iodide in the Alkaline Potassium Iodide Azide Solution is oxidized by manganic sulfate, releasing free iodine into the water. Since the manganic sulfate for this reaction comes from the reaction between the manganous hydroxide and oxygen, the amount of iodine released is directly proportional to the amount of oxygen

present in the original sample. The release of free iodine is indicated by the sample turning a yellow-brown color. Chemically, this reaction can be written as:

$$Mn_2(SO_4)_3 + 2KI \text{ -- } 2MnSO_4 + K_2SO_4 + I_2$$

$Mn_2(SO_4)_3$ =	Manganic sulfate	$2MnSO_4$ =	Manganous Sulfate
2KI =	Potassium Iodide	K_2SO_4 =	Potassium sulfate
		I_2 =	Iodine

The final stage in the Winkler titration is the addition of sodium thiosulfate. The sodium thiosulfate reacts with the free iodine to produce sodium iodide. When all of the iodine has been converted the sample changes from yellow-brown to colorless. Often a starch indicator is added to enhance the final endpoint. Chemically, this reaction can be written as:

$$2Na_2S_2O_3 + I_2 \text{ -- } Na_2S_4O_6 + 2NaI$$

$2Na_2S_2O_3$ =	Sodium Thiosulfate	$Na_2S_4O_6$ =	Sodium Tetrathionate
I_2 =	Iodide	2NaI =	Sodium Iodide

6.2.11 General safety precautions:

- Store the test kit in a cool dry area.
- Read all instructions and note precautions before performing the test procedure.
- Read the labels on all reagent bottles. Note warnings and first aid information. Read all Material Safety Data Sheets.
- Keep all equipment and reagent chemicals out of the reach of young children.
- Avoid contact between reagent chemicals and skin, eyes, nose, and mouth.
- Wear safety glasses when performing test procedures.
- In the event of an accident or suspected poisoning, immediately call the Poison Center phone number in the front of your local telephone directory or call a physician. Additional information for all LaMotte reagents is available in the United States, Canada, Puerto Rico, and the US Virgin Islands from Chem-Tel by calling 1-800-255-3924. For other areas, call 813-248-0585 collect to contact Chem-Tel's International access number. Each reagent can be identified by the four digit number listed on the upper left corner of the reagent label, in the contents list and in the test procedures.

6.3 WINKLER METHOD

The Winkler Method is a technique used to measure dissolved oxygen in freshwater systems. Dissolved oxygen is used as an indicator of the health of a water body, where higher dissolved

oxygen concentrations are correlated with high productivity and little pollution. This test is performed on-site, as delays between sample collections and testing may result in an alteration in oxygen content. It is well-known that, during photosynthetic light reaction, phase hydrolysis of water resulting in the production of O_2 acid-consequently enables to run the photosynthetic electron flow path. In usual cases, the evolution of O_2 is thus measured by volume through water replacement method. But through a suitable titrimetric method dissolved oxygen produced by aquatic plant during photosynthesis could be measured. This is modified version of Winkler's D.O. measuring method.

The test was originally developed by Ludwig Wilhelm Winkler, in later literature referred to as Lajos Winkler, while working at Budapest University on his doctoral dissertation in 1888. The amount of dissolved oxygen is a measure of the biological activity of the water masses. Phytoplankton and macroalgae present in the water mass-produce oxygen by way of photosynthesis. Bacteria and eukaryotic organisms (zooplankton, fish) consume this oxygen through cellular respiration. The result of these two mechanisms determines the concentration of dissolved oxygen, which in turn indicates the production of biomass. The difference between the physical concentration of oxygen in the water (or the theoretical concentration if there were no living organisms) and the actual concentration of oxygen is called the biochemical demand in oxygen. The Winkler test is often controversial as it is not 100% accurate and the oxygen levels may fluctuate from test to test despite using the same constant sample. The most precise determination of dissolved oxygen can be carried out by a iodometric titration according to Winkler in 1888. Although this method has been modified somewhat, the principle is unchanged and it is therefore probably the oldest method for water analysis still employed.

6.3.1 Measurement the rate of photosynthesis by Wrinkler method

6.3.1.1 Requirements

1. Plant materials: Freshly collected Hydrilla plants fig.6.1.

Fig. 6.1 Hydrilla plant

2. Chemicals & Reagents: 40% $MnCl_2$ solution (40 gm. $MnCl_2$, dissolved in 100 ml distilled water); KI and KOH solution mixture (1.75 gm. KOH and 37.5 gm. KI dissolved in 250 ml distilled water) concentrated HC1; 0.009 (N) $Na_2S_2O_3$, solution; 1% starch indicator solution; 0.5% $KHCO_3$ solution; distilled water etc.

3. Glass-wares: Conical flasks, burette, pipette, measuring cylinder, beaker etc.

4. Miscellaneous: Balance with weight box, blotting paper etc.

6.3.1.2 Procedure:

- The conical flasks of almost 250 ml capacity were filled with 200 ml tap water. Then in each flask a pinch of $KHC0_3$ solution was added.
- About 5 gm. of freshly collected Hydrilla plants were then placed in each conical flask and the sets were kept in bright sunlight or under artificial light.
- After an hour, 10 ml of water of each flask were taken separately in 100 ml flask, and then to each flask the following reagents were added: 0.5 ml of 40% $MnCl_2$; 1 ml of KI & KOH solution mixture.
- The flasks were then stoppered quickly and shaken thoroughly. After 2 min 2 ml of conc. HCl was added and stoppered again. The precipitate of $MnCI_2$ was then re-dissolved.
- Dissolved O_2 now liberates free I_2 in the reaction mixture, which is then titrated by sodium thiosulphate solution using starch indicator.
- One control set also maintained for similar titration of dissolved oxygen.

6.3.1.3 Results

The volume of thiosulphate required for titration is tabulated following types.

No of Observations	Control set Thiosulphate vol. (ml)	Mean	Treatment set $Na_2S_2O_3$ sol.	Mn vol.	Dit (n)
1	–		–		
2	–	–	–	–	
3	–		–		

Suppose the excess thiosulphate required for D. O. produced due to photosynthesis was x ml. Thus amount of standard thiosulphate requirement can be computed by the following formula:

$V_1S_1 = V_2S_2$

Where, $V_1 = x$ ml

S_1 = strength $Na_2S_2O_3$ used [0.009 (N)]

V_2= unknown

S_2 = Strength of standard $Na_2S_2O_3$ [0.01 (N)]

It is also known that 0.01 (N) $Na_2S_2O_3$ = 0.08 mg O_2

From this amount, dissolved O_2 thus produced by 5 gm. of plants within 60 min can also be computed.

Finally, rate of increment of D. O. by the photosynthesis can be expressed as μg of O_2 evolved/ gm./ min/ml of water bodies.

6.3.1.4 Applications: Dissolved oxygen analysis can be used to determine:

- The health or cleanliness of a lake or stream.
- The amount and type of biomass a freshwater system can support.
- The amount of decomposition occurring in the lake or stream.

6.3.1.5 Reagent List

1) 2ml Manganese sulfate; **2)** 2ml alkali-iodide-azide; **3)** 2ml concentrated sulfuric acid; **4)** 2ml starch solution; **5)** Sodium thiosulfate.

These reagents are available in dissolved oxygen field kits, such as those made by the Hach Company. Please use caution when using these reagents, as they can be hazardous to one's health.

6.3.1.6 Results Analysis

The total number of milliliters of titrant used in steps 6-8 equals the total dissolved oxygen in the sample in mg/L. Oxygen saturation is temperature dependent - gas is more soluble in cold waters, hence cold waters generally have higher dissolved oxygen concentrations. Dissolved oxygen also depends on salinity and elevation, or partial pressure. Click here to access an online oxygen saturation calculator that takes these parameters into account. Otherwise, use the chart below to find saturation at a given temperature. Dissolved oxygen should be measured as quickly and carefully as possible. Ideally, samples should be measured in the field immediately after collection.

6.4 SUMMARY

The Winkler Method is a technique used to measure dissolved oxygen in freshwater systems. Dissolved oxygen is used as an indicator of the health of a water body, where higher dissolved oxygen concentrations are correlated with high productivity and little pollution. This test is performed on-site, as delays between sample collections and testing may result in an alteration in oxygen content. The Winkler Method is a technique used to measure dissolved oxygen in freshwater systems. The dissolved oxygen in the sample is then "fixed" by adding a series of reagents that form an acid compound that is then titrated with a neutralizing compound that results in a color change. The amount of dissolved oxygen is directly related to the titration, chemical processes, analysis and limitations. The B.O.D. present in the water mass-produces oxygen by way of photosynthesis.

Dissolved oxygen is **used** as an indicator of the health of a water body, where higher dissolved oxygen concentrations are correlated with high productivity and little pollution. While titration is an accurate method of establishing DO, it is really a laboratory tool and will take up valuable time before a result is made, any time wasted could result in major fish kills due to lack of dissolved oxygen in the water system. The uses of DO probes are a necessity for any aqua culturist in maintaining water quality, once calibrated they provide a quick and reliable results that can be acted upon.

Dissolved oxygen (DO) is one of the most important indicators of the water quality. It is essential for the survival of fish and other aquatic organisms. Oxygen dissolves in surface water due to the aerating action of winds. **Oxygen** is also introduced into the water as a byproduct of aquatic plant photosynthesis. The level of oxygen is a much more important measure of water quality. Dissolved oxygen is absolutely essential for the survival of all aquatic organisms (not only fish but also invertebrates such as crabs, clams and zooplankton etc.).Oxygen affects a vast number of other water indicators not only biochemical, but also like the odor, clarity and taste.

As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. The lower the concentration, the greater the stress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills. Total dissolved gas concentrations in water should not exceed 110%. Concentrations above this level can be harmful to aquatic life. Fish in waters containing excessive dissolved gases may suffer from "gas bubble disease"; however, this is a very rare occurrence. External bubbles (emphysema) can also occur and be seen on fins on skin and on other tissue. In a community water supply a high DO level is good, because it makes drinking water taste better. However, high DO levels speed up corrosion in water pipes. For this reason, industries use water with the least possible amount of dissolved oxygen.

6.5 GLOSSARY

1. **Photosynthesis:** Photosynthesis, the process by which green plants and certain other organisms transform light energy into chemical energy. During photosynthesis in green plants, light energy is captured and used to convert water, carbon dioxide, and minerals into oxygen and energy-rich organic compounds.
2. **Biological oxygen demand:** BOD is a measure of the amount of oxygen required to remove waste organic matter from water in the process of decomposition by aerobic bacteria (those bacteria that live only in an environment containing oxygen). BOD is used, often in wastewater-treatment plants, as an index of the degree of organic pollution in water.
3. **Aquatic life:** Living or growing in water: aquatic plant life and animals.
4. **Fresh water: water** that does not contain a large amount of salt. In land **water**, as ponds, lakes, or streams, that is not salt.
5. **Dissolve oxygen (DO):** Dissolved oxygen (DO) is the amount of oxygen that is present in water. Water bodies receive oxygen from the atmosphere and from aquatic plants.

6. **Decomposition:** A **break down** (organic matter) or (of organic matter) to be broken down physically and chemically by bacterial or fungal action; to **break down** or cause to **break down** into simpler chemical compounds. To break up or separate into constituent parts.
7. **Respiration:** The process of breakdown of food in the cell with the release of energy is called cellular **respiration**. Cellular **respiration** takes place in the cells of all organisms. In the cell, the food (glucose) is broken down into carbon dioxide and water using oxygen. Food can also be broken down, without using oxygen.
8. **Reagents:** A reagent is a substance or compound added to a system to cause a chemical reaction, or added to test if a reaction occurs.
9. **Azide-Winkler titration method:** The **Azide**-Winkler titration method is a standard test to determine the concentration of dissolved oxygen in a sample. **Sodium thiosulfate** is used to titrate iodine, which is stochiometrically related to the amount of dissolved oxygen in the sample.
10. **C.O.D:** High COD/BOD levels in storm water runoff are cause by the residual food and beverage waste from cans/bottles, antifreeze, and emulsified oils from industrial food processing and agricultural activities. As most forms of COD is water soluble, this pollutant spreads easily via storm water to waterways.

6.6 SELF ASSESSMENT QUESTIONS

6.6.1 Multiple Choice Questions

1. Photosynthesis occurs in:

a) Chloroplast b) Golgi body

c) Endoplasmic reticulum d) Nucleus

2. Photorespiration occurs in:

a) Four cell organelles b) Two cell organelles

c). One cell organelle d). Three cell organelle

3. Reduction of NADP occurs in

a) Oxidative photophosphorylation

b) Cyclic photophosphorylation

c) Non-cyclic photophosphorylation

d). None of the above

4. Kranz anatomy is found in the leaves of:

a) Wheat b) Mustard

c) Potato d) Sugarcane

5. Photorespiration involves oxidation of:

a) PGA b) RuBP

(c) Chlorophyll-a d) Both a and b

6. C_3 and C_4 plants differ with respect to:

a) Number of ATP molecules consumed

b) First product

c) The substrate which accepts carbon dioxide

d). All

7. Where does the light reaction takes place?

a) Grana b) Stroma

c) Cytoplasm d) Endoplasmic reticulum

8. Electrons from the excited chlorophyll molecules of PS-II are first accepted by:

a) Pheophytin b) Ferredoxin

c) Cytochrome f d) Cytochrome b

9. DCMU inhibits:

a) PS-I b) PS-II

c) Oxidative phosphorylation d) It destroys chloroplast

10. The water-soluble photosynthetic pigment is:

a) Chlorophyll a b) Xanthophyll

c) Anthocyanin d)Chlorophyll b

11. The temperature of sewage increases. What will be the effect on the dissolved oxygen in sewage?

a) Remains constant b) Increase

c) Decrease d) No effect

12. What is the minimum quantity of dissolved oxygen that should be present in the treated sewage?

a) 6ppm b) 4ppm

c) 1ppm d) 10ppm

13._____ is used to find the D.O content of sewage:

a) Winkler method b) Chromatography

c) Kjeldahl method d) Olfactometer

14. The maximum quantity of dissolved oxygen present in wastewater is called:

a) Saturated dissolved oxygen b) Maximum dissolved oxygen

c) Optimal dissolved oxygen d) Peak dissolved oxygen.

15. The Winkler test is used to determine the concentration of dissolved oxygen:

a) In water samples b) Ice samples

c). Both d) None of these

6.6.2 Answer keys: 1-a, 2-d, 3-c, 4-d, 5-b, 6-d, 7-a, 8-a, 9-b, 10-c, 11-c, 12-b, 13-a, 14-a, 15-a.

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6.8 TERMINAL QUESTIONS

Q.N.1. How is dissolved oxygen determined?

Q.N.2. How do you calculate dissolved oxygen using Winkler method?

Q.N.3. What type of chemical reaction is dissolved oxygen titration?

Q.N.4. What is the purpose of adding MnSO4 in Winkler's method?

Q.N.5. How do you make a Winkler solution?

Q.N.6. What is the formula for dissolved oxygen?

Q.N.7. What is the Winkler method used for?

Q.N.8. What do you understand by dissolved oxygen?

Q.N.9. What is the significance of dissolved oxygen determination?

Q.N.10. What is the indicator used for estimation of dissolved oxygen?

Q.N.11. How many ppm is oxygen in water?

Q.N.12. What is 3 factors that influence dissolved oxygen levels?

Q.N.13. What is the definition of dissolved oxygen?

Q.N.14. What happens if dissolved oxygen is too high?

Q.N.15. Why is starch solution an indicator?

UNIT-7: SEPARATION OF AMINO ACIDS BY PAPER CHROMATOGRAPHY

7.1 Objectives

7.2 Introduction

7.3 Experimental

- 7.3.1 Materials and reagents
- 7.3.2 Procedure
- 7.3.3 Ninhydrin reaction with amino acid
- 7.3.4 Precautions

7.4 Summary

7.5- Glossary

7.6 Self Assessment Question

7.7 References

7.8 Suggested Readings

7.9 Terminal Questions

7.1 OBJECTIVES

After reading this unit students will be able-

- To understand about the separation of amino acids by paper chromatography.

The current exercise describing about the separation of given mixture of amino acids (Leu, Ala, Phe, Asp, Ser) using paper chromatography. In this assay, mixture of amino acids is spotted on a piece of filter paper. The rate at which the amino acids separated in paper chromatography depends upon their relative affinity for the paper (which is hydrophilic) and the solvent (which is hydrophobic). Hydrophobic amino acids will move faster because they are more attracted to the hydrophobic solvent (moving up by capillary action) than the hydrophilic paper (stationary phase). The filter paper is composed mostly of cellulose and is very hydrophilic. On the other hand, hydrophilic amino acids will move slower because they are attracted more to the paper than the hydrophobic solvent. The position of the amino acids in the chromatogram can be detected by spraying with ninhydrin, which reacts with amino acids to yield coloured products (purple). By comparing the calculated R_f values from paper chromatogram of different amino acids with reference amino acid, one can easily assured about the type of amino acid.

7.2 INTRODUCTION

Chromatography is an analytical tool for distinguishing different biomolecules based on their chemical properties was discovered in 1903 by Russian-Italian botanist M. S. Tswett. Chromatography is a modern and sensitive technique used for rapid and efficient analysis and separation of components of a mixture and purification of compounds.

The basis of principle of chromatographic technique is based on the differential migration of the individual components of a mixture through a stationary phase under the influence of moving phase. Stationary phase – the phase that is immobilized on the support particles, or on the inner wall of the column tubing. Mobile phase – the phase that moves in a definite direction.

One of the oldest, reliable and most routinely used forms of chromatography is paper chromatography. It is a type of partition chromatography which involves filter paper placed in a jar containing a shallow layer of solvent and sealed. A small dot of sample solution is placed onto a strip of filter paper. As the solvent rises through the paper by capillary action, it meets the sample mixture which starts to travel up the paper with the solvent. Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's performance to be adsorbed onto the paper v/s being carried along with the solvent. This paper is made of cellulose to which polar water molecules are adsorbed while the solvent is less polar usually consisting of a mixture of water and an organic liquid. The compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper quickly and therefore, do not travel so far.

All twenty amino acids share a common structure called the "conserved region" of the amino acid. This conserved region consists of a central carbon called the α-carbon. This α-carbon is linked to a carboxyl group, an amino group and a hydrogen atom. These groups along with the α-carbon make up the "conserved region". All twenty amino acids have this structure. The α-carbon is also attached to a variable structure called the R group. The R group is what differs among the twenty amino acids. General structure of amino acid is given in fig. 7.1.

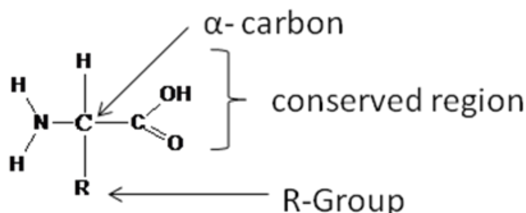


Fig.7.1 General Structure of amino acid

Different amino acids move at differing rates on the paper because of differences in their R groups. The rate of movement of an amino acid during paper chromatography is reported as its relative mobility (R_f). R_F is simply the distance the amino acid moved through the filter paper divided by the distance the solvent moved through the paper.

7.3 EXPERIMENTAL

7.3.1 Materials and reagents

The Following materials should be available at your laboratory:

- Chromatography chamber
- Whatman chromatography paper (21 X 21cm)
- Gloves
- Pencils and Rulers
- 5 known amino acids (2mg/ml in 10% isopropanol: 0.1M HCl) Leu, Ala, Phe, Asp, Ser
- 1 unknown amino acid (2mg/ml in 10% isopropanol: 0.1M HCl)
- Stapler
- p20 with small pipette tips
- Waste beaker

7.3.2 Procedure

1. First placed the chromatography chambers in the hood prior to proceed for further experiment.
2. Wear the gloves and cut a whatman filter paper in rectangle of appropriate size.

Note: The filter paper should never be handled by bare hands since the skin's oils show up on the developed chromatogram

3. Using a pencil and ruler, draw a line approximately 3 cm from the bottom of the paper. Then every 3 cm on the line draw a circle 2 mm in diameter (there should be six circles spread evenly across the line (fig 7.2) Label each circle in pencil (Leu, Ala, Phe, Asp, Ser, Unknown amino acid)

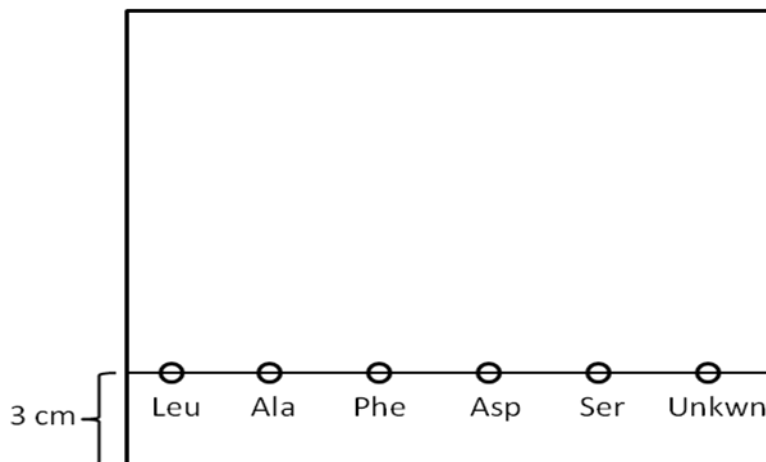


Fig.7.2 Preparation of chromatogram. Make sure the line is 3 cm from the bottom and the sample are at least 1 inch from the edge

4. Start with the 1st amino acid sample. Pipette 2µl on to the appropriate circle, always use a new pipette tip to pipette the 2nd amino acid. Continue for all samples.
5. Then: Let all samples dry.
6. Repeat 4x so that each circle has 10µl of the appropriate sample.
7. When the last sample is dry, roll the paper into a cylinder and staple so that the edges do not touch (fig. 7.3).

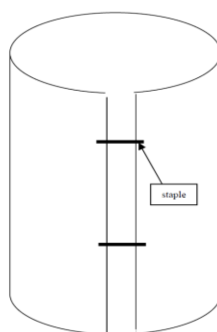


Fig.7.3 Folding and stapling of chromatogram

8. Stand the cylinder in a chromatography chamber (under the hood). Cap the chamber and allow the chromatogram to develop for 60-90 minutes or until the solvent line is within an inch from the top of the paper.

9. Under the hood, remove the paper from the jar and immediately mark the solvent front line in pencil. Allow the paper to thoroughly dry by hanging it on the wire in the hood.
10. Your lab instructor will spray your paper with Ninhydrin developer, which is used to detect the location of amino acids. The paper will then be placed in a drying oven at about 100 C for 3-4 minutes to allow the color to develop.
11. Measure the distance the solvent migrated and the distance each of the amino acids migrated (Fig 7.4). Record the measurements in the table 7.1 (given below). Calculate the relative mobility (R_f) for each amino acid.

$$R_f = \frac{\text{Distance migrated by amino acid}}{\text{distance migrated by solvent}}$$

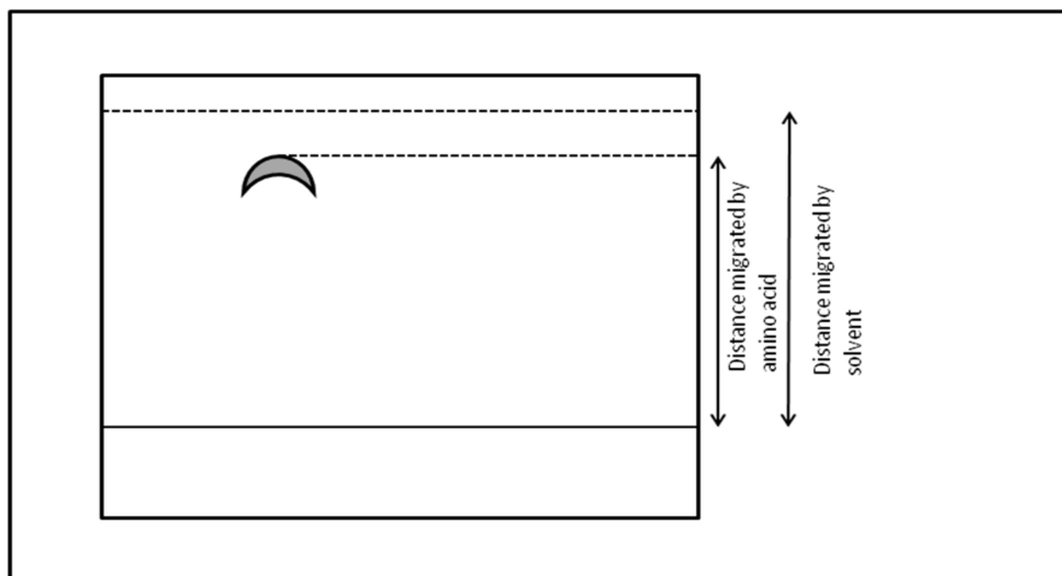


Fig.7.4 A chromatogram showing the measurement of distance migrated by amino acid and solvent to calculate the R_f value.

Table 7.1 Data recording table

Amino acid	**Distance migrated**	**R_f value**
Leucine		
Alanine		
Phenylalanine		
Aspartic acid		
Serine		
Unknown Amino acid		

7.3.3 Ninhydrin reaction with amino acid

Two equivalent of ninhydrin react with one equivalent of amino acid as shown in Fig.7.5below.

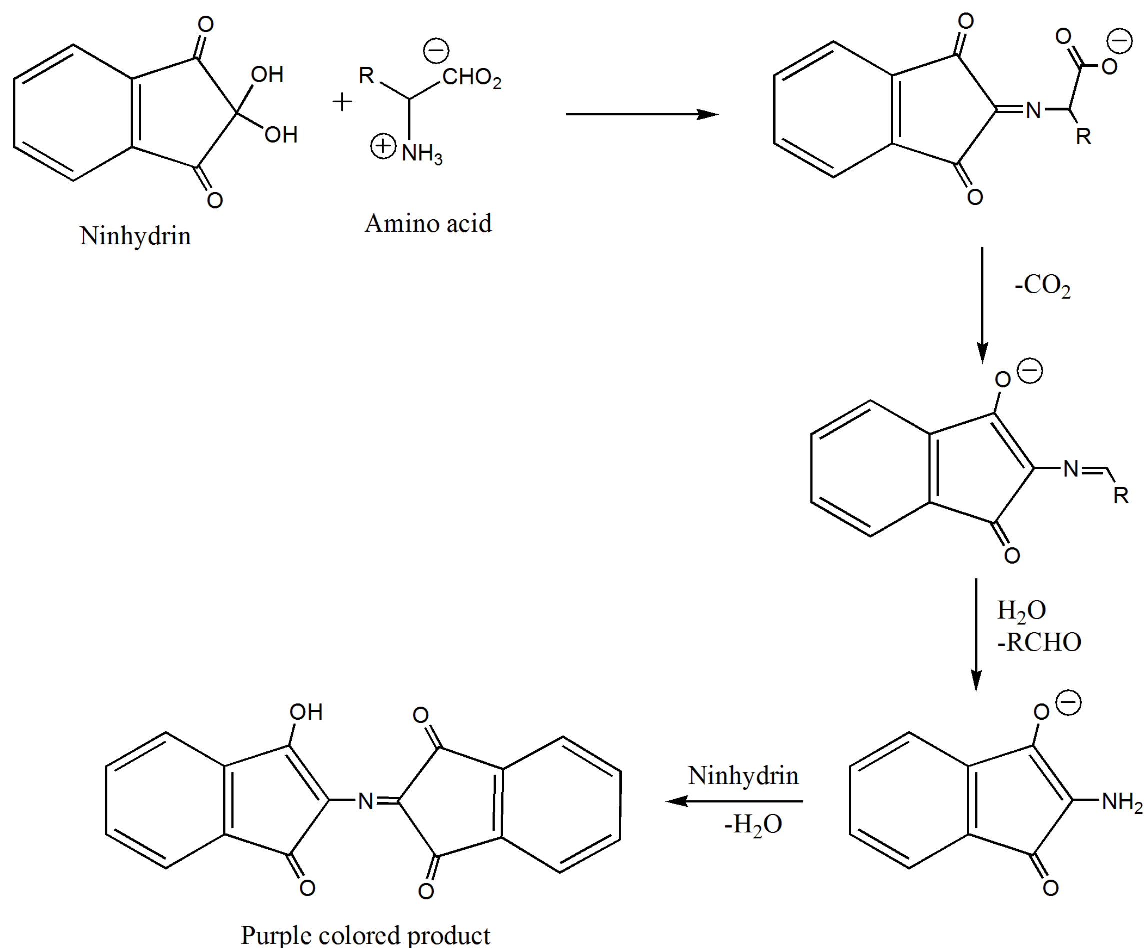


Fig.7.5 Reaction mechanism of ninhydrin with amino acid

7.3.4 Precautions

a) Always wear gloves while handling solvents and during operating procedure.
b) Use good quality pencil for drawing the reference line so that the mark does not dissolve in the solvent.
c) Dip the paper strip in the solvent in such a way that the spot of the mixture is above the solvent level and the movement of the solvent front is not zig-zag.
d) While spotting the test solution on the paper, do not allow the spots to spread.
e) Use finely drawn capillary to put the spot on the paper.
f) Once the experiment is set, do not disturb the jar as long as the chromatogram is being developed.

g) Keep the jar covered with the lid when the chromatogram is being developed.
h) Make the paper strip perfectly dry before developing the spots.
i) Handle the organic solvent/solvents, with care.

7.4 SUMMARY

This laboratory exercise describes about the separation of different amino acids using paper chromatography. Different amino acids have different hydrophilicity or hydrophobicity. Greater the hydrophobicity of amino acid, greater the distance travel by it and vice-vera. Stationary phase is hydrophilic therefore hydrophilic amino acids interact strongly with stationary phase and travel shorter distance compared to hydrophobic amino acid. By comparing the R_f values of known amino acid with unknown sample, we can interpret the hydrophilicity or hydrophobicity of unknown sample.

7.5 GLOSSARY

Amino acid: These are building blocks of protein biomolecules, each amino acid have one carboxyl group and one amino group and one side chain. Side chain of each amino acid determines its hydrophilictiy or hydrophobicity.

Hydrophobic: Nonpolar molecules that repel the water molecules are said to be hydrophobic. Nonpolar side chain of each amino acid imparts hydrophobicity.

Hydrophilic: Having an affinity for water; able to absorb, or be wetted by water; water-loving.

Mobile Phase: A pure or mixture of solvents or gas passes over stationary phase in chromatography.

Pasteur pipette: Pasteur pipettes, also known as droppers are used to transfer small quantities of liquids.

R_f value: R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

Stationary Phase: The solid or liquid phase of a chromatography system on which the materials to be separated are selectively adsorbed.

Whatman filter paper: A supporting stationary phase used in paper chromatography.

Ninhydrin: A chemical that interacts with primary and secondary amines and produces a purple color product. Each amino acid has primary amino group.

7.6 SELF ASSESSMENT QUESTIONS

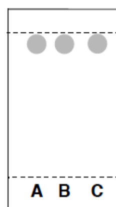
1. Based on the chemical structure arrange the following amino acids in increasing order of hydrophilicty.

(i) Leucine (ii) Glycine (iii) Lysine (iv) Arginine (v) Glutamic acid

2. Describe the procedure to calculate the R_f value for different spots observed in paper chromatography?

3. Compare the R_f values of Leucine and Ser. Is this consistent with their chemical structures? Explain.

4. A TLC plate is spotted with each of the three compounds. The plate is developed using hexanes:ethyl acetate (5:95) to give the chromatogram shown below. How could you change the solvent system to give better separation of these three compounds?



5. Describe the effect of different solution pH on the Lysine, arginine, glutamic amino acids given below.

(i) pH 4 (ii) pH 7 (iii) pH 10

6. Draw the final structure of adduct product formed by the reaction of amino acid with ninhydrin.

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7.9 TERMINAL QUESTIONS

1. Describe the composition of mobile phase used to separate the different types of amino acids

2. What is a Chromatogram? Explain the principle on which the technique of chromatography is based.

3. Describe the procedure used to stain the amino acid with ninhydrin.

4. Describe the procedure used to measure the R_f value

5. Describe the procedure of paper chromatography.

BLOCK-2- PLANT ECOLOGY

UNIT-8: DETERMINATION OF MINIMUM SIZE AND MINIMUM NUMBER OF QUADRAT

8.1 Objectives

8.2 Introduction

8.3 Minimum Size and Minimum Number of Quadrate

- 8.3.1 Shape and Size of quadrats
- 8.3.2 Collection of data
- 8.3.3 Placement of quadrats in the study area
- 8.3.4 Safety measures
- 8.3.5 Exercise-1
- 8.3.6 Exercise-2

8.4 Summary

8.5 Glossary

8.6 Self assessment question

8.7 References

8.8 Suggested Readings

8.9 Terminal Questions

8.1 OBJECTIVES

After reading this unit students will be able-

- To understand the minimum size of quadrat by species area curve method.
- To determine the minimum number of quadrats to be laid down for community structure analysis for the given unit of the area.
- To sample in the field for community structure analysis.

8.2 INTRODUCTION

As we know a community is broadly defined as the natural assemblage of different living populations in a unit area and have three essentials in common i.e., they occupy a definable area (forest community, marine community, grassland community); composed of population; and have typical environment. The assemblage of the community changes with the change in its environment. Smith (1986) defines community as the naturally occurring, mutually sustaining assemblage or mixture of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner. Further, such group of organism (plants) occurring in one area or in particular locality form a stand, which further recognized by its homogeneous physiognomy. Plant community in entire cannot be measured even if it were small. Therefore, we have to depend on samples drawn from the community to approximate the structure of the community. Clement early plant ecologist was a major advocate of quadrat method for study community structure. "Quadrat at present used as a basic sampling unit, in general it is a tool in the hands of the investigator (researcher) used to collect the data for the vegetational analysis. The basic step before sampling in the fields or stands is two workouts the minimum size and number of quadrat required to estimate the vegetation of that area. Species-area and species-quadrat number curves were used to determine the minimum sampling size and the minimum quadrat number for reliable estimate of biomass in any ecosystem (grass land, forest, weed etc.). In both cases, characteristic curves that first increased abruptly and then leveled off as fewer species were added with increased sampling size of quadrat number were obtained.

8.3 MINIMUM SIZE AND MINIMUM NUMBER OF QUADRATE

8.3.1 Shape and Size of quadrats

As we know, quadrat is treated as basic sampling unit but they are often of different sizes and shapes such as square, circular or rectangular areas and quadrat of appropriate sizes are placed at random in the study area. Presence or absence of species, numbers of organisms, or the percentage cover of each species is generally calculated within the quadrat. Square quadrats often preferred over the other two. The size of quadrat varies in accordance with the size of plants to be sampled. The minimum size for each quadrat can be determined using the species area- curve method. This involves beginning with a small quadrat and counting the number of different species within the quadrat and then gradually increasing the size of quadrat. At present

we generally follows the working methodology of Oosting 1956, to record data from the give forest stand i.e., 10×10 m size quadrate for tree layer, 5×5 m size quadrat for woody under growth (Shrubs) and 1×1 m for herb vegetation. According to the purpose of investigation quadrat may be of following types: (i) List (for listing of all species present), (ii) Count (for determining the individuals of species), (iii) Cover (for determining basal area or canopy of species), (iv) Chart (for mapping the plants within the quadrat), (v) Clip or Harvest (for determining biomass or the weight of plants) and, (vi) Denuded quadrat (for determining the sequence or development of vegetation overtime, following a treatment (Singh *et al.* 2006).

8.2.2 Placement of quadrats in the study area

It is important to select any one of the sampling methods before starting our sampling i.e., *completely random* (both selection of stand and placement of quadrat within them is completely random), *stratified random* (community stand is first subdivided into a number of homogenous stands and then quadrats are placed within them randomly), *completely systematic* (selection of stand and placement of quadrat within them are placed at a fixed distance example, selection of stand could be after every 200 m and placement of quadrate within these stands after every 10 m), *random systematic* (in this technique sampling is conducted in two ways i.e., in first case, stand are selected in systematic way and quadrat are placed within them in complete random. In second case stands are selected randomly but quadrats are placed within them in systematic way). In general we select complete random approach, in this approach during sampling quadrats are positioned at repeated and simple randomly in the study area so that every potential point in the study area has an equal chance of being selected for the sample. This can be achieved by following ways i.e., randomly throw the quadrat into the sample area, however, this may involve some bias depending on who is throwing the quadrat, and the size and density of the vegetation at the site or by place a tape measure along the length and breadth of the study area and then made random samples in the study area.

8.2.3 Collection of data

During the sampling for vegetational data following data should be collected for each quadrat where possible:

- Aspect of stand i.e., North/South/East/West;
- Slope of the stand i.e., steep, moderate or flat.
- Elevation or altitude of the stand.
- Position of the stand i.e., latitude and longitude
- Type of soil and its characteristics
- Disturbance at the site i.e., natural (Forest fire, Erosion etc.) and anthropogenic causes.

8.2.4. Safety Measures

During fieldwork it is highly recommended to consider about the following Safety precautions, hazards and behavior at the site of study:

- Wear correct footwear and cloths so that to avoid slip during data collection.
- Proper care should be given to herbal vegetation during yours sampling.
- Be aware and care full regarding trip fall injuries.
- Do not pollute the site by throwing empty water bottles or other plastics.
- Be aware about the wild animals and specially snakes.
- Prevent yourself from sun burn and water hazards.

8.2.5 Exercise-01

Object: To determine the minimum size of the quadrat to be required for reliable estimation of vegetation by species area-curve method.

Requirements: Nails, cord, meter scale, hammer, pencil, note book, polythene bags, news paper.

Theory: Quadrat is basic sampling unit, used to measure the vegetation of a unit area. The shape of quadrat is variable but square quadrat is generally used. The size of quadrat which accommodates maximum species diversity is known as the minimum size of the quadrate to be required to sample that particular community.

Procedure:

1. Fix three nail at right angle to each other in given unit area, tie a thread around them so that to prepare 'L' shaped structure i.e., 1×1 meter sixe in case of grass land.
2. Fix the 4th nail in such a way so that by joining these nails, about 10 cm^2 area is cut off. Note the number of plant species in this area.
3. Then increase the area to 20×20 cm and note the additional plant species.
4. Keep on the increasing this area by 10 cm^2 until there is no increase in the number of new species.
5. Plot a graph between area of the quadrat and number of species observed, using the recorded data.

Observation:

Table 8.1 Gathered tabulated data showing relationship between size of quadrat and number of species

S. No	Area of Quadrat	Total No. of Species
1	10×10 cm	5
2	20×20 cm	7
3	30×30 cm	11
4	40×40 cm	13
5	50×50 cm	15
6	60×60 cm	16
7	70×70 cm	16
8	80×80 cm	16
9	90×90 cm	16
10	100×100 cm	16

Result: A curve is obtained which initially showed increasing abruptly which after the point at 60×60 cm of the graph starts flattening, which showed that after 60×60 cm area of quadrat there is no increase in the number of new species. Thus, minimum size of the quadrat of the given community type is 60×60 cm.

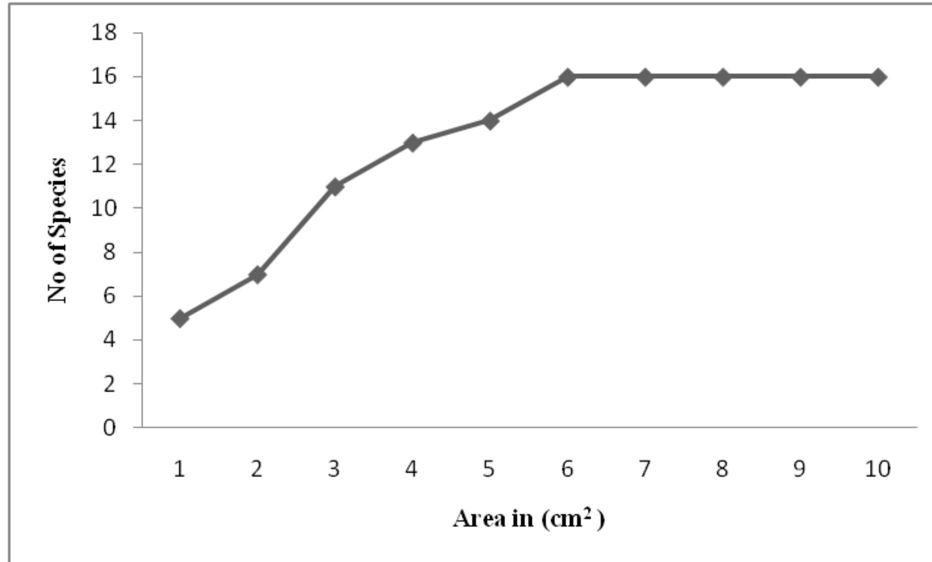

Fig. 8.1 Species area curve to determine the minimum size of the quadrat

8.2.6 Exercise-2

Object: To determine the minimum number of the quadrats required for reliable estimate number of plant species in grass land community.

Requirements: Nails, cord, meter scale, hammer, pencil, note book, polythene bags, news paper.

Theory: After ascertaining the minimum size of the quadrat, it is necessary to find out the minimum number of the quadrat to be used of estimate properly the vegetation of the particular area. Quadrat of minimum size, say 60×60 cm, is laid randomly in the field and the number of species occurring in each quadrat is recorded. By increasing the number of quadrats, the effect on number of species is noticed. It is very essential to find out the minimum number of quadrat to be required to analysis the vegetation before study in detail about the community.

Procedure

1. Determine the minimum size of quadrat by species- area curve method.
2. Laid down randomly about 10-20 quadrats of minimum size i.e., say 60×60 cm. At different site along the entire unit area of grassland.
3. Note down the different species of plant in the each quadrat.
4. Find out accumulating total of the number of species for each quadrat and study the effect of increased number of quadrat on number of species encountered.
5. Plot a graph showing relationship between quadrat and encountered plant species.

Observation:

Table 8.2 Tabulated data showing the relationship between number of quadrat and no. of species

Species	Quadrats									
	1	2	3	4	5	6	7	8	9	10
A	+	-	-	-	+	-	-	+	-	+
B	+	+	+	+	+	+	+	+	+	+
C		+	-	+	-	+	+	-	+	+
D					-	-	-	-	+	+
E					+	-	+	-	-	-
F						+	-	+	-	-
G							-	+	+	+
H								+	+	-
I								+	+	+
Accumulating total number of species	4	7	8	11	12	14	16	19	19	19

Table 8.3 Number of Species present per Quadrat

Number of Quadrat	Number of Species
1	4
2	**7**
3	**8**
4	**11**
5	**12**
6	**14**
7	**16**
8	**19**
9	**19**
10	**19**

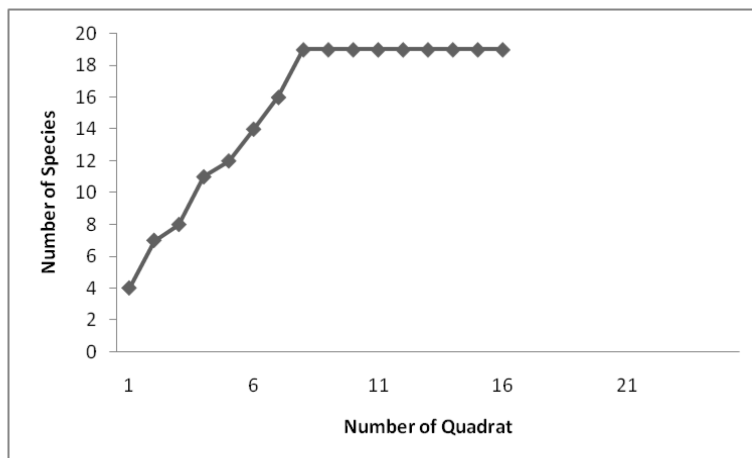

Fig. 8.2 A graph to determine the minimum number of quadrat required.

Result: The "Minimum number of quadrat" is similarly defines as the number of quadrats in which maximum number of diversity of species recorded. A curve was obtained which initially showed increasing curve abruptly which after the point at the 17th quadrat starts flattening. Thus, total 17 quadrats were to be laid down for the present case (grassland community).

8.5 SUMMARY

In this unit we have discuss about the sampling approaches and basic fundamentals for study the vegetation of any community stand. Community is an assemblage of living organism in unit area. Further, it is very difficult to estimate the community structure even if the community is very small. It needs careful observation with proper methodology to accurately studying the vegetation for different quantitative parameters (frequency, density, abundance, canopy cover etc). To study any community stand, the minimum size and the minimum number of quadrats required to approximate the community structure of the given area is pre-request before one plane to work on community structure. The minimum size is calculated by placing quadrat of small size and then increasing the size of the quadrat equally until constant number of species are recorded, further for minimum number of quadrat number of quadrat is placed randomly until maximum number of diversity of species recorded. This gives us idea about the minimum size and minimum quadrate required to analysis the particular community. In this chapter, we have worked out one example for each i.e., on minimum size and number of quadrat and it was found that in both cases the plotted graph shows increase in the graph heap which becomes constant at some point.

8.6 GLOSSARY

Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.

Quadrat: A basic sampling unit of definite shape and size.

Stand: The vegetation of a plot or group of organism occurring in a particular locality.

8.7 SELF ASSESSMENT QUESTIONS

Q.1. What are the different types of quadrat?

Q.2. What do you mean by species area curve?

Q.3. What are different approaches for sampling.

Q.4. What you mean by community stand?

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8.10 TERMINAL QUESTIONS

Q.1. Discuss in brief the procedure of deciding the minimum size of quadrat and its application in the field of ecology.

Q.2. Define the community. Explain in brief the minimum number of quadrat required to estimate the vegetation analysis of community.

Q.3. Discuss the effect of size and number of quadrat on the quantitative characteristics of a community.

UNIT-9: TO DETERMINE FREQUENCY, DENSITY AND ABUNDANCE IN COMMUNITY

9.1 Objectives

9.2 Introduction

9.3 Methodology

9.4 Determination of Frequency

  9.4.1 Exercise-1

9.5 Determination of Density

  9.5.1 Exercise-2

9.6 Determination of Abundance

  9.6.1 Exercise-3

9.7 Summary

9.8 Glossary

9.9 Self Assessment Questions

9.10 References

9.11 Suggested Readings

9.12 Terminal Questions

9.1 OBJECTIVES

After reading this unit students will be able –

- To study the concept of Phytosociology and different characters of plant community.
- To study the community structure.
- To determine the quantitative character of the community i.e., frequency, density and abundance in a community stand.
- To compare the Raunkiaer's normal frequency diagram with the computed frequency diagram of the stand.

9.2 INTRODUCTION

As we know the term Phytosociology literally means plant sociology and frequently used term for the study of the plant community structure and the interrelationship among the different plants of a community. A community is basically composition of various organisms belonging to different taxonomic group occupying a unit area under a particular environment condition. Further, a group of organism occurring in a particular locality in a community is called community-stand or a stand. Thus, in a forest ecosystem the organization of flora (tree, shrubs, herbs, climber, epiphyte etc) and fauna (insects, earthworm, birds, animals etc.) constitute a community. The nature and magnitude of biotic community is very wide varies from the small community to large community. The assemblage of biota in microhabitat is called as small community (community of litter, fallen log etc). Besides, community of forest and grassland are some example of large community. Further, the community is regarded as open community, if provides enough space for the growth and development of migrants and a community leave no space for growth and development of migrants called as close community.

Plant community in entire cannot be measured even if it were small. Therefore, we have to depend on samples drawn from the community to approximate the structure of the community, in order to understand the structure, composition, and tropic organization. During any Phytosociology analysis we study two different aspect of vegetational analysis (analytic and synthetic) which are taken into account at the same time.

Analytic character includes all such features of a stand which are directly observed or measured in the field. They are further of two type i.e., qualitative and quantitative. Qualitative character are those which are not measured but usually described such as physiognomy (the general appearance of vegetation or gross structure of the community); stratification (distribution of organism in the vertical space for the best utilization of the habitat resources); phenology (in the life cycle of plants they exhibits different phases such as, germination, vegetative growth, flowering, fruiting and seeds these phases provide different structure to the community and each phase is called as phenophase, study of the phenophase with respect to time is known as phenology); life form (the sum of the adaptations of a plant to climate); vitality (plant capacity to complete its life cycle under the existing environment condition) etc. Whereas, quantitative

character are those which are measure quantitatively in the field, such as frequency (distribution of plant species in a community); density (numeric strength of the specie in the community); abundance (number of individual per sampling unit of occurrence); canopy cover (ground area cover by the canopy of the plant when projected vertically to the ground); basal area (ground area covered by the stem or the area actually occupied by the species).

Synthetic characters are those characters which are generally described on the bases of data recorded for the analytic character.

9.3 METHOD

As we has been discussed in the previous chapter, that before vegetation analysis (community) of any area we have to select any one approach and one method of sampling, which serve as the mother source of data collection from which the inference to be extracted. In this chapter, we have discussed Phytosociology and its various attributes in order to understand the structure, composition and trophic organization of the community. Of the all attributes, you have to work out only three analytical attributes i.e., frequency, density and abundance.

As we knows in order to gather data from any stand or community stand, we have to first determine the minimum size and minimum quadrat numbers respectively as described or explained in the previous chapter. We generally use quadrate method as a sampling method, in this method we generally placed a quadrat to 1×1 m for herbaceous vegetation, 5 ×5 m for shrub and 10 ×10 m or some time 100×100 m for tree species. The quadrats are laid randomly in the different stand and data gathered accordingly. To study frequency of the different species, the presence (+) and absence (-) of the species is recorded in each quadrat, but to study the density and abundance the total number of individual species in a quadrat is counted. The data so collected arranged and put in a tabular form for analyzing the results.

9.4 FREQUENCY

Frequency is generally expressed as the dispersion of species in the stand or in the community. It denotes the homogeneity of distribution of the various species in an ecosystem. The degree of distribution of the plant species in an unit area is usually expressed in term of percentage occurrence. It expressed as the percentage of sampling unit in which species is present, if individual of a species is present in 6 sampling unit and total 10 sampling units were laid down then the frequency of the individual species comes to be 60 percent. Further, if a species is well distributed in a community stand to be studied, chances to be recorded in all sampling units are high and the frequency may come to 100 percent, while a species having individuals concentrated in a patch or to certain area, chances of encountering in sampling unit is less or may encountered in only few sampling units and will therefore have low frequency value. High value of frequency denotes a greater uniformity of its occurrence or dispersion. To find out the frequency of the stand the area is sampled by any one method (as describe in unit eight) and then

tabulated it as (Table 9.1), from the tabulated data, percentage frequency of the each species is calculated by using following formula:

$$\text{Frequency (\%)} = \frac{\text{No. of quadrats of in which of a species occurs}}{\text{Total number of quadrats sampled}} \times 100$$

The frequency of the community stand or unit area sampled show variation in the frequency value among species. Among the early plant ecologists, C. Raunkiaer (1934) grouped the species into five frequency classes based on frequency value (20% interval) after studying more than 8,000 frequency percentages and found that class 'A' included 53% of the total species, class 'B' class included 14%, class 'C' 9%, class 'D' 8% and class 'E'= 16%. He concluded that the frequency continuously declines in first four classes, and then increases in the terminal class giving reversed 'J' shaped curve Fig. 9.1.

On the bases of these results, Raunkiaer's proposed law of frequency, his law includes five frequency classes based on frequency value as follows:

Frequency class	**Frequency percentage**
Class A	1-20 % frequency value
Class B	21-40 % frequency value
Class C	41-60 % frequency value
Class D	61-80 % frequency value
Class E	81-100 % frequency value

Accordingly, the frequency law suggested:

$$\begin{array}{c} > \\ A>B>C=D<E \\ < \end{array}$$

In general, higher value of class A corresponds to higher number of species in a community with low frequency and higher value of E corresponds to the species that dominate the community. From the law of frequency, Raunkiaer's states that, higher values of classes B, C and D indicate heterogeneity of the stand, and greater value of class E shows homogeneity of the stand. If the value of the ratio (E+D)/(B+C) is < 1.0, the stand is heterogeneous, whereas the stand is homogenous if the value is > 1.0.

After studying about the frequency and Raunkiaer's normal frequency diagram and frequency law, students may assign the exercise for their local community to better understand the concept and compare comparison with Raunkiaer's normal frequency diagram by plotting recorded frequency on the graph sheet.

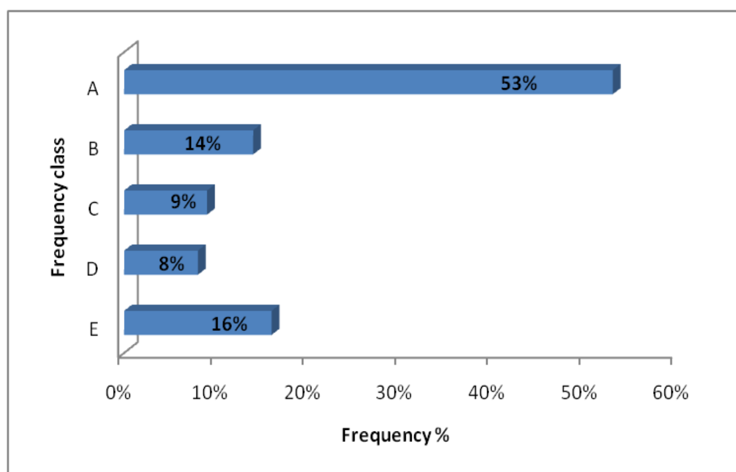

Fig. 9.1 Raunkiaer's normal frequency diagram

9.4.1Exercise: 1

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

Requirements: Quadrates of required size, measuring tape, poly bag, nails, hammer, notebook, pencil, newspaper.

Procedure

1. Lay down 5 quadrat of required size randomly in unit area.
2. Note down the botanical or common name of the species and distinguish them if possible, if not then distinguish them as A, B, C etc and at the same time take the sample specimen in note book for future reference for plant identification.
3. After collecting data from all quadrats, arrange it accordingly.
4. Calculate the frequency % and placed them in respective frequency class.
5. Compare the obtained frequency graph with Raunkiaer's normal frequency diagram.

Observation:

Table 9.1 Frequency (%) of plant species studied in a given stand

S. N.	Name of the species	Quadrat studied					Total no. of quadrats in which species occurred	Total No. of quadrats Studied	Frequency (%)	Frequency class
		1	2	3	4	5				
1	*Cyperus rotundus*	+	-	-	-	+	2	5	40	B
2	*Eclipta alba*	+	+	-	+	+	4	5	80	D
3	*Sida cordifolia*	-	-	+	-	-	1	5	20	A
4	*Euphorbia hirta*	-	+	-	+	+	3	5	60	C
5	*Cynodon dactylon*	+	+	+	+	+	5	5	100	E

Calculation: Calculate the frequency of the species by using following formula

$$\text{Frequency (\%)} = \frac{\text{No. of quadrats of in which of a species occurs}}{\text{Total number of quadrats sampled}} \times 100$$

Thus, frequency % of different species as follow:

Cyperus rotundus = $\frac{2}{5} \times 100 = 40\%$

Eclipta alba = $\frac{4}{5} \times 100 = 80\%$

Sida cordifolia = $\frac{1}{5} \times 100 = 20\%$

Euphorbia hirta = $\frac{3}{5} \times 100 = 60\%$

Cynodon dactylon = $\frac{5}{5} \times 100 = 100\%$

Table 9.2: Distribution according to Raunkiaer frequency classes

Name of plant species	Frequency percentage	Frequency class
Cyperus rotundus	1-20%	A
Eclipta alba	21-40%	B
Sida cordifolia	41-60%	C
Euphorbia hirta	61-80%	D
Cynodon dactylon	81-100%	E

Result

From the above calculation it was concluded that *Cynodon dactlylon* showed maximum frequency percentage (100%) that mean plant is present in all sampling units. Further, *Cyperus rotundus* showed least frequency percentage (20%) that mean present in least number of sampling units.

9.5 DENSITY

Density may be defined as the numerical strength or number of individual of a species in a unit area. It is expressed as number of rooted plants within each sampling unit. Density provides an idea of competition between individuals of the species. Higher the density more will be the competition stress. Density is calculated as follows:

$$\text{Density} = \frac{\text{Total number of individuals of the species recorded in all quadrats studied}}{\text{Total number of quadrats studied}}$$

9.5.1 Exercise: 2

Object: To determine the density of various species occurring in a given unit area.

Requirements: Quadrates of required size, measuring tape, poly bag, nails, hammer, notebook, pencil, newspaper.

Procedure:

1. Lay down 10 quadrat of required size randomly in unit area.
2. Note down the botanical or common name of the species and distinguish them if possible, if not then distinguish them as A, B, C etc and at the same time take the sample specimen in note book for future reference for plant identification.
3. Numbers of individual of each species occurring in each quadrat are recorded.
4. After collecting data from all quadrats, arrange it accordingly.

Observation:

Table 9.3: Density of plant species in a given stand

S. No.	Name of the species	No. of individuals in each sampling units										Total No. of individuals	No. of sampling units studied	Density
		1	2	3	4	5	6	7	8	9	10			
1	A	-	8	-	4	9	-	10	7	-	1	38	10	3.8
2	B	1	-	2	-	-	3	-	-	2	3	11	10	1.1
3	C	6	-	1	4	-	2	-	1	2	1	19	10	1.9
4	D	11	7	2	5	8	-	4	3	-	3	43	10	4.3
5	E	2	-	2	-	1	-	3	-	-	-	08	10	0.8

Calculations:

Density of species A = $\frac{38}{10}$ = 3.8

Density of species B = $\frac{11}{10}$ = 1.1

Density of species C = $\frac{19}{10}$ = 1.9

Density of species D = $\frac{43}{10}$ = 4.3

Density of species E = $\frac{08}{10}$ = 0.8

Result: ………………………………………………………………………..

9.6 ABUNDANCE

Abundance may be defined as 'the number of individual species per sampling unit of occurrence' or number of individual of a species per unit area. In contrast to density, number of individuals of a species is counted and divided with the number of quadrats in which species is present. It also did not provide the numerical strength of species in an area. Abundance is calculated as follows:

$$\text{Abundance} = \frac{\text{Number of individual of the species in all quadrats}}{\text{Total number of quadrats in which the species occurred}}$$

Further, in order to present the distribution pattern of any species in the community the ratio of abundance to frequency is measured. When abundance is divided by frequency the resultant is called Whitfort's index, represent the distribution pattern as follow:

Range of A/F value	Distribution
<0.025	Regular distribution
0.025 to 005	Random distribution
>0.05	Contagious distribution

For this index the abundance must be represent as individual per sampling unit of the size used to calculate frequency. Example, if the size of sampling quadrat is 10×10 cm, then the value of abundance should be as number of individuals per 100 cm^2 .

9.6.1 Exercise: 3

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

Requirements: Quadrates of required size, measuring tape, poly bag, nails, hammer, notebook, pencil, newspaper.

Procedure:

1. Lay down 10 quadrats of required size randomly in unit area.
2. Note down the botanical or common name of the species and distinguish them if possible, if not then distinguish them as A, B, C etc and at the same time take the sample specimen in note book for future reference for plant identification.
3. Count the numbers of individual of each species occurring in each quadrat.
4. After collecting data from all quadrats, arrange it accordingly.
5. After calculating the abundance of each species, calculate A/F ration and placed encountered species accordingly.

Observation:

Table 9.4: Abundance of plant species in a given stand

S. No	Name of the species	No. of individuals in each sampling units										Total No. of individuals	No. of sampling units which species occurred	Abundance
		1	2	3	4	5	6	7	8	9	10			
1	A	-	8	-	4	9	-	10	7	-	1	38	6	6.33
2	B	1	-	2	-	-	3	-	-	2	3	11	5	2.20
3	C	6	-	1	4	-	2	-	1	2	1	19	7	2.71
4	D	11	7	2	5	8	-	4	3	-	3	43	8	5.37
5	E	2	-	2	-	1	-	3	-	-	-	08	4	2.00

Calculations:

Abundance of species A = $\frac{38}{6} = 6.33$

Abundance of species B = $\frac{11}{5} = 2.20$

Abundance of species C = $\frac{19}{7}$ = 2.71

Abundance of species D = $\frac{43}{8}$ = 5.37

Abundance of species E = $\frac{08}{4}$ = 2.00

Result :………………………………………………………..

9.7 SUMMARY

Community is assemblage of large number of living organism in a specific environment. Study of structural attributes of any community is very difficult task, even if the community is small one cannot explain it as it is, which requires some method to study and explain the different attributes of the community stand such as frequently encountered species, rare species, dominant species etc. In previous chapter we have explain number of different technique to study the community of an area or to study stand structure. In sampling approach we have studied about the minimum quadrat size and minimum number of quadrat required to analyze the vegetation of that stand. In this chapter, we have studied different analytical character and their importance in the course of study during the vegetation analysis. In first attribute, we studied about frequency (distribution of species in the community) that how we can compare the calculated frequency with the Raunkiaer's normal frequency diagram, and what Raunkiaer's law of frequency suggest about the different classes of frequency. In second and third attributes, we discussed about the density and abundance and their role in classify the community, explain the pattern of distribution of species in the unit area or sampling unit or in community stand.

9.8 GLOSSARY

Abundance: Number of individual per sampling unit of occurrence

Analytic character: All such features of a stand which are directly observed or measured in the field**.**

Association: It is a product of artificial synthesis of stands and an abstract unit of vegetation.

Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.

Canopy cover: Ground area cover by the canopy of the plant when projected vertically to the ground.

Density: Numeric strength of the specie in the community.

Frequency: Distribution of plant species in a community**.**

Stand: The vegetation of a plot of suitable size is a stand.

Stratification: Distribution of organism in the vertical space for the best utilization of the habitat resources

Synthetic character: Those characters which are generally described on the bases of data recorded for the analytic character

Quadrate: Basic sampling unit for definite shape and size.

Vitality: Plant capacity to complete its life cycle under the existing environment condition.

9.9 SELF ASSESSMENT QUESTIONS

Q1. Explain in brief different analytical and synthetic character of the community.

Q2. To study forest community and determine and explain either the forest is homozygous or heterozygous.

Q3. Explain the term Phytosociology. Describe various quantitative character of the community.

9.10 REFERENCE

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9.12 TERMINAL QUESTIONS

Q1.To prepare frequency diagram and compare it with Raunkiaer's normal frequency diagram of your campus.

Q2.To study different communities by quadrat method and calculate its frequency, density and abundance.

Q3. Explain any one local forest by using the above parameter and compare your results.

Q4. Explain distribution pattern of the species in the stand.

Q5. Define community and its magnitude. Why density of plant is called as numerical strength of the community stand?

UNIT-10: CALCULATION OF RELATIVE FREQUENCY, RELATIVE DENSITY AND RELATIVE ABUNDANCE AND COMPUTE IVI (IMPORTANT VALUE INDEX)

10.1 Objectives
10.2 Introduction
10.3 Methodology
10.4 Determine the mean basal area (cover) and total basal area (cover).
10.5 Exercise-01
10.6 Summary
10.7 Glossary
10.8 Self Assessment Questions
10.9 References
10.10 Suggested Readings
10.11 Terminal Questions

10.1 OBJECTIVES

After reading this unit students will be able-

- To study the concept of IVI and calculate its value for different forest/ grassland community.
- To calculate the relative values of frequency, density, basal cover.
- To determine the mean basal area (cover) and total basal area (cover).
- To determine the dominant tree species of the community.

10.2 INTRODUCTION

As an adjunct to the floristic studies, a study of phytosciology of an area is imperative to understand the structure of the vegetation and classify it meaningfully. Phytosociology literally means plant sociology and frequently used term for the study of the plant community structure and the interrelationship among the different plants of a community. The general structure of a community is determined by the dominating plants and not by any other characteristic. The vegetational data are quantitatively analyzed for each species for, density, frequency and abundance (Curtis and McIntosh, 1950). The relative values of frequency, density and dominance are determined following Phillips (1959). These values are summed to represent Importance Value Index (IVI) of individual species (Curtis 1959).

This index proposed by Curtis and McIntosh to express the dominance and ecological successes of any species. It considers the relative values of density, frequency and basal area of every species in a study area. The importance value index (IVI) is a statistical quantity, which gives an overall picture of the importance of the species in the vegetative community obtained by summing up the percentage values of Relative Frequency, Relative Density and Relative Dominance.

Importance Value Index (Out of 300) =Relative Frequency +Relative Density

IVI = Relative density + Relative frequency + Relative basal area.

Relative values are very essential while comparing the ecological performance of the plants amongst the different species. Relative values are all expressed in terms of percentage composition.

10.3 METHODOLOGY

As we has been discussed in the previous two chapters regarding the data collection and its interpretation for studying the phytosciology of an area. In this chapter, we have extended our interpretation to Important Value Index for the determination of the dominance and ecological successes of any species, by calculating the relative values of, frequency, density, basal area and then summing up the three.

Frequency is generally expressed as the dispersion of species in the stand or in the community and relative frequency is expressed as the proportion of frequency of a species in the sampled area. Relative frequency is denoted by (RF) and calculated by using the following formula:

$$RF = \frac{\text{Frequency of occurrence of the species}}{\text{Total frequency of all the species}} X100$$

Density may be defined as the numerical strength or number of individual of a species in a unit area. It is expressed as number of rooted plants within each sampling unit. Relative density is the proportional representation of a species in a sample. Relative density is denoted by (RD) and calculated by using the following formula:

$$RD = \frac{\text{Density of the species}}{\text{Total density of all species}} X\ 100$$

Relative basal area (RBA) is the proportion of basal area of a species in the total area.

$$RBA = \frac{\text{Sum of basal area of all individuals of a species in the sample}}{\text{Total basal area of all the species in the sample}} X100$$

Calculation of total basal cover = (Density of all species) x (Average Basal area of per tree).

RBA is the area occupied by the base of a species. It is considered as a good indicator of the size, volume or weight of a species. The GBH (Girth at breast height) or DBH (Diameter at breast height) measures are used to calculate the basal area of free species. Girth at Breast Height of Tree is the girth (circumference) of a tree at a height of 1.37m from the ground (Standard breast height), may used to understand another important measure, the standing biomass of the trees. Height of Tree provides information on the vegetation, growth rate and length of (main stem, trunk).

A species which attains the highest value of IVI within the community is considered as dominant species of that community.

10.4 DETERMINE THE MEAN BASAL AREA (COVER) AND TOTAL BASAL AREA (COVER)

The area of the ground actually occupied by the species is known as basal area of that species or basal cover. Compared to the density values, basal cover is of greater ecological significance as it provides a better estimate of plant biomass. The basal cover or basal area is regarded as an index of dominance of a species. Thus, a higher basal area is an expression of dominance of a species.

To estimate the basal area of herbaceous species, basal area is measured few cm (2.5 cm) above ground by clipping by sharp scissors, the diameter of the each individual at its base is measured with the help of Vernier caliper or scale and then the average diameter of individual species can be obtained. The procedure is repeated for each species found in the sampling per unit area.

By using the following formula, the diametric value recorded during the study is converted into its basal area.

$$\text{Basal area or cover} = \pi r^2;$$

where we know the value of π (3.17) and

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

Thus, basal area = $\frac{3.14 \text{ x (Average diameter)}^2}{4}$

Total basal cover = (Density of all species) x (Average Basal area of per herb).

Since the density of each species per square meter is determined and multiplied by its basal area, the results are expressed in (sq cm) per square meter.

In case of tree clipping is not possible, thus the basal area of woody species is estimated by measuring the circumference at breast height, i.e., 1.37 m by using measuring tape.

And then the circumference values are converted into basal area by using the following formula:

$$\text{Basal area} = \pi r^2;$$

$$\text{Where, } r^2 = \frac{\text{Circumference}^2}{4\pi}$$

Total basal cover = (Density of all species) x (Average Basal area of per tree).

Basal area is of great ecological importance, thus its value regarded as an index of dominance of a species. Which further, indicates its importance in the community?

10.5 EXERCISE-1

Aim: To calculate the IVI for a temperate forest on the basis of relative frequency, density and relative abundance.

Requirements: Quadrates of required size, measuring tape, poly bag, nails, hammer, notebook, pencil, newspaper.

Procedure

- Lay down quadrat of required size randomly in unit area.
- Note down the botanical or common name of the species and distinguish them if possible, if not then distinguish them as A, B, C etc and at the same time take the sample specimen in note book for future reference for plant identification.
- After collecting data from all quadrats, arrange it accordingly.
- Calculate the relative values for frequency, density, dominance.

- Sum all the relative values to calculate the IVI of individual species, and arranged in order of decreasing importance.

Observation and Calculations

Calculate the relative values for frequency, density and dominance of the species by using following formulas**:**

1. Relative Frequency = $\frac{\text{Frequency of occurrence of the species}}{\text{Total frequency of all the species}}$ X100

   Relative frequency of different species is as follows:

   Pinus roxburghii = $\frac{70}{370} \times 100$ =18.91

   Cupressus torulosa = $\frac{60}{370} \times 100$ = 16.21

   Quercus lecuotrichophora = $\frac{100}{370} \times 100$ = 25.02

   Myrica esculenta = $\frac{90}{370} \times 100$ = 24.32

   Rhododendron arboreum = $\frac{50}{370} \times 100$ = 13.51

2. Relative Density = $\frac{\text{Density of the species}}{\text{Total density of all species}}$ X 100

   Pinus roxburghii = $\frac{2.20}{7.70} \times 100$ = 28.57

   Cupressus torulosa = $\frac{1.70}{7.70} \times 100$ = 22.07

   Quercus lecuotrichophora = $\frac{1.40}{7.70} \times 100$ = 18.18

   Myrica esculenta = $\frac{1.50}{7.70} \times 100$ = 19.48

   Rhododendron arboreum = $\frac{0.90}{7.70} \times 100$ = 11.68

3. Relative basal area or relative dominance is calculated in two steps

In first step, total basal area is calculated from the mean basal area of the all species encountered during analysis by using following formula:

Total basal area = Mean basal area × Density

Pinus roxburghii = 686.28 × 2.20 =1509.81.

Cupressus torulosa = 496.92 × 1.70 = 844.78.

Quercus lecuotrichophora = 411.25 × 1.40 = 575.61.

Myrica esculenta = 271.72 × 1.50 = 407.58.

Rhododendron arboreum = 304.19 × 0.90 = 273.70.

In second step, relative dominance is calculated by using the following formula:

$$\text{Relative Basal Area} = \frac{\text{Sum of basal area of all individuals of a species in the sample}}{\text{Total basal area of all the species in the sample}} \text{X100}$$

Pinus roxburghii = $\frac{1509.81}{3611.48} \times 100 = 41.80$.

Cupressus torulosa = $\frac{844.78}{3611.48} \times 100 = 23.39$.

Quercus lecuotrichophora = $\frac{575.61}{3611.48} \times 100 = 15.93$.

Myrica esculenta = $\frac{407.58}{3611.48} \times 100 = 11.28$.

Rhododendron arboreum = $\frac{273.70}{3611.48} \times 100 = 7.57$.

4. Important Value Index (IVI) = Relative density + Relative frequency + Relative basal area.

Result

A species which attains highest value of IVI within community is considered as dominant species of that community. Thus in present community stands *Pinus roxburghii* is the dominant tree species.

S. No	Name of the species	Frequency (%)	Density (100^{-2})	Mean basal area (Cm^2 Pl^{-1})	Total basal cover (Cm^2 100 m^{-2})	Relative Frequency (%)	Relative Density (%)	Relative dominance (%)	Important value index (IVI)
1	*Pinus roxburghii*	70	2.20	686.28	1509.81	18.91	28.57	41.80	89.28
2	*Cupressus torulosa*	60	1.70	496.92	844.78	16.21	22.07	23.39	61.67
3	*Quercus leucotrichophor*	100	1.40	411.15	575.61	25.02	18.18	15.93	61.13
4	*Myrica esculenta*	90	1.50	271.72	407.58	24.32	19.48	11.28	53.08
5	*Rhododendron arboreum*	50	0.90	304.19	273.70	13.51	11.68	7.57	32.76
Total		**ΣF= 370**	**ΣD= 7.70**		**ΣTBC= 3611.48**				**299.92**

10.6 SUMMARY

Community its own is group of assemblage of different species and among the different species few are well adopted then the other, with the well dispersion within the community. There are different attributes to study the community structure i.e., frequency, density, abundance etc. the quantitative values of each having its own importance. But the total picture of ecological importance cannot be obtained by any one of these. In this chapter, we worked out the methods to calculate the relative values of frequency, density, basal area and then the value of three summed up to generate the IVI value of the present species. IVI provides us with the overall image of that community with the dominant and least dominant species of the community based on their IVI values. The values of relative frequency, relative density are directly calculated from their frequency and density value but relative basal area value are calculated by first calculating the mean basal area for a species and then total basal area per species. The area occupied by the stem or shoot of an individual species on the ground of a stand is the basal area (cover).

10.7 GLOSSARY

Breast height: Height at breast level of a normal human. It is generally considered as 1.37 m above the flat ground.

Canopy cover: Ground area cover by the canopy of the plant when projected vertically to the ground.

Community: A naturally occurring, mutually sustaining, and interacting assemblage of plants and animals living in the same environment and fixing, utilizing and transferring energy in some manner.

Density: Numeric strength of the specie in the community.

Frequency: Distribution of plant species in a community**.**

Important Value Index: Index for the determination of the dominance and ecological successes of any species, by computing and then adding the relative values of, frequency, density, basal area.

Stand: The vegetation of a plot of suitable size is a stand.

10.8 SELF ASSESSMENT QUESTIONS

1. What do you mean by basal area?

2. What do you mean by relative frequency?

3. What is canopy cover?

4. Expand IVI and how it is calculated.

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10.11 TERMINAL QUESTIONS

1. Why do we study IVI? Explain its importance in regard to phytosciology of an area.
2. What is relative basal cover and how it is computed.
3. What is basal cover and how it is different from the canopy cover.

BLOCK-3- PLANT RESOURCE UTILIZATION AND CONSERVATION

UNIT-11: STUDY OF FOOD CROPS, VEGETABLES AND FRUITS OF THE LOCALITY

11.1 Objectives
11.2 Introduction
11.3 Study of food crops
11.4 Vegetables
11.5 Fruits of the locality
11.6 Summary
11.7 Glossary
11.8 Self Assessment Questions
11.8.1 Multiple Choice Questions
11.8.2 Fill in the blanks
11.9 References
11.10 Suggested Readings
11.11 Terminal Questions

11.1 OBJECTIVES

The main objective of this unit is to study about the food crops, vegetables and fruits growing in the local area. After reading of this unit learner will be able to:

- Study about common food crops, vegetables and fruits grown in our locality
- Their importance and uses.

11.2 INTRODUCTION

Food is the most essential need of human beings, which mainly comes from plants in different forms such as cereals, pulses, vegetables and fruits. The most important source of food for humans are cereals, and pulses which belong to the family Gramineae and Fabaceae respectively. Rice, wheat, maize, barley, oats and rye are main cereals having a high amount of carbohydrates. However, millets are also grown in India from pre-historic times. The common millets in India are Sorghum, Finger Millet, Ragi etc. Pulses are one of the most important sources of protein in our diet. The main pulses grown are peas, chick pea, lentil, soyabean etc.

Vegetables are defined as edible plants which store up reserve food in their body parts such as stem leaves and roots. The vegetables are either eaten as cooked or in raw form as salads. They rank second important source of carbohydrates in food and also contains important minerals, salts and vitamins. Therefore, they have high nutritive value.

Fruits are also grown in India from ancient times. The fruit is the seed-bearing portion of the plant and consists of ripened ovary and its contents. Fruits are known for high nutritive value.

11.3 STUDY OF FOOD CROPS

Food crops can be broadly divided into cereals and pulses (Fig. 11.1).

11.3.1 Cereals

In this section we shall study about common cereals growing in our locality e.g., Rice, Wheat, Maize, Barley.

i) Rice

Botanical name - *Oryza sativa* Linn.

Family - Gramineae (Poaceae)

Vernacular name - English – Rice

Hindi – Chawal, Dhaan

Rice is a monocot plant belonging to the family Gramineae. It is a staple food in many parts of India especially in eastern and southern parts. It is the main dietary source of carbohydrates many countries.

Uses

1. Rice is one of the most important crops with regard to human nutrition.
2. It is good source of carbohydrates, also contains proteins and fat.
3. Oil is extracted from rice bran. It used for edible and industrial purposes.
4. Rice bran oil is also used in the preparation of cosmetics and soaps.
5. Broken grains of rice from the milling process used as cattle feed and is also used in preparation of alcoholic beverages. It is also used as human food.
6. Rice straw is used for making mats.
7. Rice husk is used as fuel.

Fig. 11.1 Some important food crops (Cereals and Pulses)

ii) Wheat

Botanical name	-	*Triticum aestivum*
Family	-	Gramineae
Vernacular name	-	English – Wheat
		Hindi – Gehu

Wheat is cereal plant of family Gramineae, having higher protein content than rice and maize. Whole grain is a good source of carbohydrates, proteins, minerals and vitamins.

Uses

1. It is a staple food in most parts of world and in northern and western India. It grounded and used to produce atta, maida and suji.
2. The flour is used for making bread, chapatis, biscuits, cakes, noodles, pasta etc.
3. Broken wheat (dalia) is used to make porridge.
4. Fermentation is done to make biofuels, beer and other alcoholic beverages.

5. Straw is used in the production of furfuryl alcohol.
6. Wheat is also used as fodder crop for livestock.
7. Wheat straw is used as fodder. It is also used for making paper pulp.
8. Wheat bran is used for making animal feed.

iii) Maize

Botanical name	-	*Zea mays* Linn.
Family	-	Gramineae
Vernacular Name	-	English – Maize,Corn
		Hindi –Makka

Maize is widely grown cereal crop belonging to family Gramineae. It is the third most important crop after wheat and rice.

Uses

1. Maize is used as food for human and livestock in many parts of world. The seed is rich in carbohydrates and also contains protein and fat.
2. The immature cobs (Bhutta) are eaten after roasting as a traditional snack
3. It is processed to make cornflakes which are popular breakfast for kids. It is also used to make popcorn which is a popular snack.
4. Maize grains are rich in protein *Zein, thus used to make artificial fibres.
5. Maize is also used in production of industrial alcohol.
6. The corn oil is uses in preparation of soap and also for edible purposes.

iv) Barley

Botanical Name	-	*Hordeum vulgare*
Family	-	Gramineae
Vernacular name	-	English – Barley
		Hindi - Jau

Barley is herb plant of family Gramineae and is cultivated as food crop.

Uses

1. Barley flour is used for making chapatis.
2. The six rowed type of barley has high protein content and used for food purposes.
3. The two rowed type of barley is widely used for making malt which is fermented to make beer from which whiskey and other alcoholic beverages are distilled.
4. Barley straw is used as fodder for livestock.
5. Barley grains are used to make sattu, which is used to make cooling drinks.

11.3.2 Pulses

In this section we shall study about common pulses grown in our locality.

Pulses are dry and edible seeds of plants belonging to family Leguminosae. They are very important source of food for human beings, as they are very rich in protein. Beside protein they also contain carbohydrate, fat, vitamins and minerals.

In most of the legumes root contains nodules in which nitrogen fixing bacteria are present. Pulses grow in a pod. The most important legumes include beans, peas, lentil, soyabeans, chickpeas etc. They are cultivated all over the world because of their high nutrition content.

The common pulses grown in our region are:

i) Chick Pea (Gram)

Botanical name - *Cicer arietinum* Linn.

Family - Leguminosae

Vernacular name - Hindi – Kabuli chana, Chana

English – Chick Pea, Gram

Chick pea is an important pulse crop containing protein, carbohydrate, fat, vitamins and minerals. India is ranked first in area of cultivation and production of Chick pea in the world. It is grown at the end of rainy season as a rabi crop and harvested from February to April.

Uses

1. It is consumed as dal in most of country.
2. Unripe seeds are eaten raw as a snack.
3. Tender leaves are used to make vegetable. They contain citric and mallic acid.
4. The flour is called as besan and used in preparation of many dishes like pakora, halwa, laddo, barfi, dhokla, namkeen etc. It is integral part of Indian cuisine.

ii) Soyabean

Botanical name - *Glycine max*

Family - Leguminosae

Vernacular name - Hindi (Pahari) – Bhat

English – Soyabean

Soybean is commonly known as bhat in Kumaon hills, it is a native of South-east Asia. It is a valuable source of both protein and oil. It contributes to 25% of global edible oil and is an important ingredient in formulated feeds of fish and poultry.

Uses

1. Soyabean contains many nutraceutical compounds such as lecithin, isoflavones, oil and protein. It has potential to remove protein malnutrition in poor sections of society.
2. It is rich in Potassium, Magnesium, Iron and vitamins.

3. Defatted oil cakes as high-quality cattle and poultry feed.
4. Soyabean flour mixed with wheat for improving protein content and used to make biscuits, snacks etc.
5. Oil is used for edible purposes and also for making soaps, varnishes and paints.
6. Soya milk and curd (tofu) are high nutrition foods.

iii) Lentil

Botanical name - *Lens culinaris*

Family - Leguminosae

Vernacular name - Hindi – Masur, Malka

English – Lentil

Lentil is a branched, annual twining bushy herb. The seeds are lens shaped, small in size and enclosed inside broad and short pods (usually two seeds in each).

Uses

1. Lentil is rich in carbohydrate, dietary fibre, fat, protein, vitamins (Thiamine, Riboflavin, Niacin, Vitamin D, E & K) and minerals (calcium, iron, magnesium, phosphorus, magnesium, zinc etc.).
2. Used to cook lentil curry (dal) in India and many parts of world.
3. Also used as ingredient in many dishes, like soup, parathas, sweets, khichdi, papadum etc.
4. Unripe pods are used as vegetable.
5. The seeds can be eaten soaked, fried, germinated, baked or boiled,

iv) Kidney Bean

Botanical name - *Phaseolus vulgaris*

Family - Leguminosae

Vernacular name - Hindi – Rajmah

English – Kidney Bean

Kidney bean is named for its kidney shaped structure. It is an annual herbaceous plant cultivated throughout India. The seeds are enclosed in slender pods.

Uses

1. The dried ripe seeds are eaten in many cuisines in India, mostly as dal.
2. The seeds are good source of protein.
3. The plant straw is used as animal feed.

v) Pea

Botanical name - *Pisum sativum*

Family - Papilionaceae, Fabaceae

Vernacular name - English – Pea

Hindi – Matar

Pisum sativum is an annual plant having pod shaped vegetable. Pea pods are fruits as they contain seeds and develop from ovary of pea flower.

Uses

1. Dried and spilt pea is used as a pulse.
2. They are rich source of fibre protein, Vitamin A, B6, C & K, zinc, phosphorus and iron.

11.4 VEGETABLES

In this unit we shall study about common vegetables grown in our locality.

A. Root vegetables

i) Carrot:

Botanical name - *Daucus carota*

Family - Umbelliferae

Vernacular name - English – Carrot

Hindi – Gajar

Uses

1. The carrot root contains high amount of alfa and beta carotene and are good source vitamin K and vitamin B6.
2. The roots are used in cooked form as vegetable and eaten raw as salad.
3. The carrot juice is widely used as a health drink.
4. The carrot seeds yield an essential oil known as carrot seed oil.
5. The seeds are carminative and useful in dropsy.
6. Seeds are also used in treatment of kidney diseases.

ii) Radish

Botanical name - *Raphanus sativus*

Family - Cruciferae

Vernacular name - English – Radish

Hindi – Muli

Radish is an edible root vegetable grown in most parts of world.

Uses

1. The roots are either eaten raw or cooked as vegetable.

2. The tender leaves are used as green leafy vegetable.
3. Fresh leaf juice is a laxative and diuretic.
4. Roots are also used in treatment of piles, gastrodynia and urinary problems.

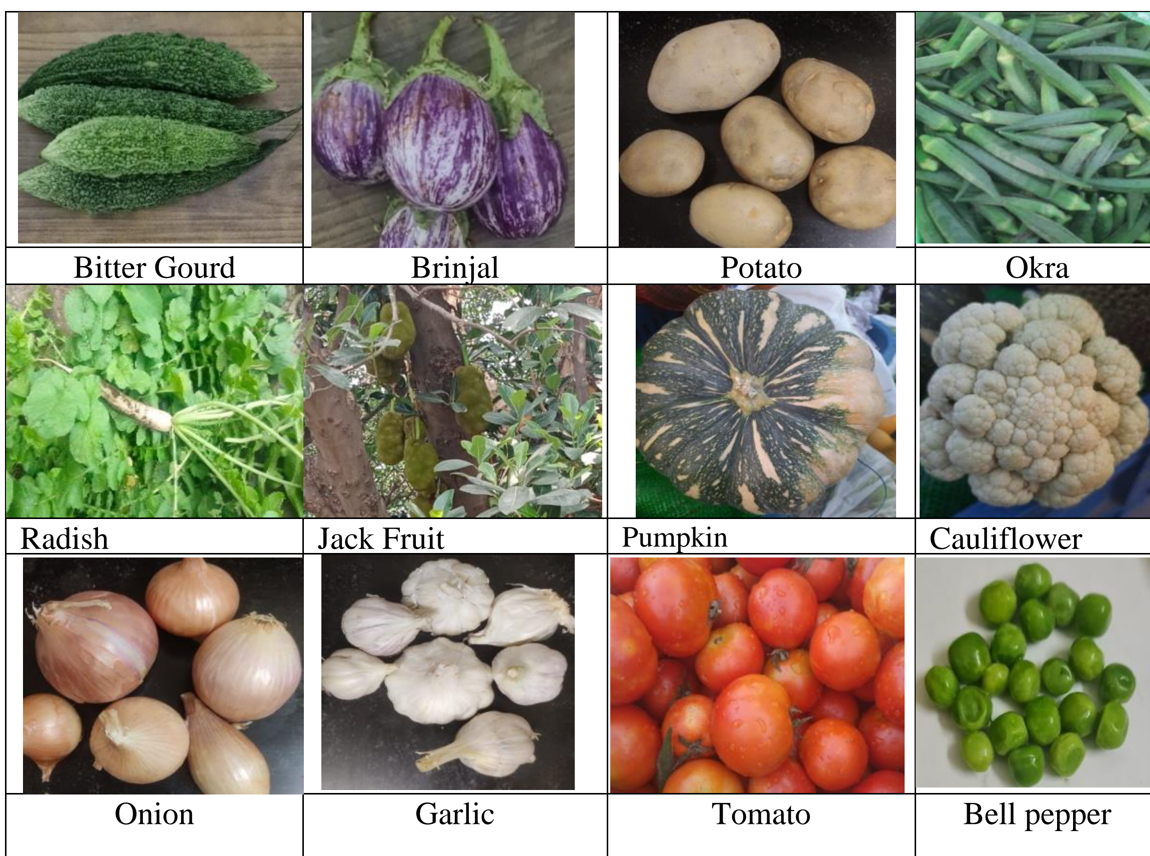

Fig. 11.2 Some common vegetables grown in our locality

iii) Turnip

Botanical name - *Brassica rapa*

Family - Cruciferae

Vernacular name - English – Turnip

Hindi – Shalgam

Turnip is a root vegetable, popular for both human consumption and livestock feed.

Uses

1. Turnip is a rich source of minerals, fibre, vitamins, omega 3 fatty acids and protein.
2. The roots are either eaten raw as salad or cooked to make vegetable.
3. The leaves are used as fodder.
4. Turnip seeds yield oil which is used in treatment of Dengue fever.
5. The high fibre content id helpful in constipation.

6. The seed oil is also used for making soap.

B. Stem vegetables

i) Potato

Botanical name - *Solanum tuberosum*

Family - Solanaceae

Vernacular name - English – Potato

Hindi - Alu

Potato is an important starchy, tuberous crop of family Solanaceae grown all over the world.

Uses

1. Potato is cash crop of great importance. Tubers are used as vegetable, for making potato chips, French fries and fermentation products.
2. Small tubers are utilized for production of industrial alcohol and as livestock feed.
3. Tubers are highly nutritious and contain potassium and good amount of carbohydrate as well as protein, vitamins and minerals.
4. Tubers are used as a raw material for the manufacture of starch.
5. Raw potato can be used as medicine to be applied on affected areas for boils, burns and infections.
6. Raw potatoes are rich in minerals like sulphur, phosphorus and potassium and these can be used in skincare.

ii) Onion

Botanical name - *Allium cepa*

Family - Amaryllidaceae

Vernacular name - English – Onion

Hindi – Pyaz

Onion is a bulbous plant of family Amaryllidaceae.

Uses

1. The bulbs can be used in almost all the dishes we eat.
2. Also popularly eaten raw as salads.
3. Onions are healthy whether eaten cooked or raw.
4. Onions contain vitamins, minerals and antioxidants.

iii) Garlic

Botanical name - *Allium sativum*

Family - Amaryllidaceae

Vernacular name - English – Garlic

Hindi – Lehsun

Garlic is a bulbous plant of family Amaryllidaceae.

Uses

1. Garlic bulbs and oil are used for flavouring food.
2. Garlic paste is used as a condiment and possesses antibacterial property.
3. Garlic juice used as ear drops and also used in skin problems.
4. It is used as a traditional medicine for common cold, hypertension, hypercholesterolemia, and many other diseases.
5. Garlic oil is used in toothache and for preventing hair fall.

C. Herbage vegetables

Herbage vegetables are rich in protein, vitamins and minerals. We shall study common herbage vegetables.

i) Cabbage

Botanical name - *Brassica oleracea*

Family - Cruciferae

Vernacular name - English – Cabbage

Hindi – Bandgobhi, Pattagobhi

Cabbage consists of large, thick, overlapping leaves forming a head like structure and short stem. It contains large amount of water, starch, protein and few sugars.

Uses

1. Cabbage is either cooked for making vegetable or eaten raw as salad.
2. It is also used to treat morning sickness, osteoporosis etc.
3. Its fibre content is high.
4. It also contains antioxidants, such as polyphenols and sulphur compounds.

ii) Cauliflower

Botanical name - *Brassica oleracea* Linn.

Family - Cruciferae

Vernacular name - English – Cauliflower

Hindi – Phulgobhi

Cauliflower is a vegetable of family Cruciferae having rich amounts of fibres, nutrients and antioxidants. It consists of short stem and large head having condensed inflorescence.

Uses

1. It is cooked and eaten as a vegetable.

2. As it is a fibre rich vegetable it helps in treatment of heart disease, obesity prevention and may reduce risk of many chronic diseases.
3. It is a good source of antioxidants.
4. Stem and leaves are used as livestock feed.

iii) Spinach

Botanical name - *Spinacia oleracea*

Family - Chenopodiaceae

Vernacular name - English – Spinach

Hindi – Palak

Spinach is an herbaceous vegetable with green leaves, having high nutrient value. It provides vitamins, proteins and minerals especially iron.

Uses

1. The leaves are cooked for making vegetable soup and added as an ingredient to many dishes.
2. It is one of the best sources of dietary magnesium.
3. The leaves are a good source of beta carotene, phosphorus and vitamin K.

iv) Pigweed

Botanical name - *Chenopodium album* Linn.

Family - Chenopdiaceae, Amaranthaceae

Vernacular name - English – Pigweed

Hindi – Bathua

The plant is a weedy annual herb, however cultivated in certain regions.

Uses

1. The leaves and young shoots of Pigweed are cooked for making vegetable.
2. The seeds are rich in protein, calcium, phosphorus and vitamins.
3. The leaves are used in making many dishes like paranthas, soup and raita.
4. The leaves and seed are also used as cattle feed.

v) Amaranth

Botanical name - *Amaranthus blitum*Linn. Var. *Oleracea* Hook

Family - Amaranthaceae

Vernacular name - English – Amaranth

Hindi – Chaulai

Amaranth is an annual herbaceous weed. It is also cultivated and eaten as vegetable in many parts of world.

Uses

1. The leaves, tender shoots and flower buds are cooked and eaten as vegetable.
2. The seeds are used to make sweet snacks.

D. Fruit vegetables

Fruit vegetables are pulpy, seed rich and high in calories. The common fruit vegetables are.

i) Tomato

Botanical name - *Lycopersicon esculentum*

Family - Solanaceae

Vernacular name - English – Tomato

Hindi – Tamatar

Tomato is bright red coloured berry, edible fruit of family Solanaceae. Plants are vines having weak stem growing about 1 to 3 feet in height. It is very popular vegetable used all over world.

Uses

1. Fresh ripe fruits are consumed raw as salads.
2. Tomato is cooked and used in many dishes.
3. It is also used in making soups, ketchup, juice, sauces etc.
4. It is rich in antioxidant lycopene, vitamin C, potassium and vitamin K.

ii) Brinjal

Botanical name - *Solanum melongena*

Family - Solanaceae

Vernacular name - English – Brinjal, Egg plant

Hindi – Baigun

Brinjal is an erect annual small shrub of family Solanaceae. It is grown widely for its edible fruit which is used in many cuisines. Fruit is a berry.

Uses

1. Brinjal is usually consumed as cooked vegetables, sometimes also eaten raw.
2. The fruit is able to absorb oils and flavours into its flesh during cooking hence used in culinary arts.
3. Fruit is rich in iodine and vitamins.

iii) Jackfruit

Botanical name - *Artocarpus heterophyllus*

Family - Moraceae

Vernacular name - English – Jackfruit

Hindi – Kathal

Jackfruit is a tree of about 60 – 70 feet in height, having large fruits borne on tree trunk and branches. The fruits are large with many large seeds.

Uses

1. The seeds are boiled or baked or roasted and used as snacks.
2. Ripe jackfruit is very sweet, and its flavour is very delicious.
3. In many countries' unripe jackfruit are boiled and used in curries.
4. Seeds are rich source of starch.
5. The flesh is a source of dietary fibre.
6. The wood of the tree is used in the manufacture of furniture, doors and windows. Also used in manufacture of musical instruments like drums and veena.
7. A yellow dye is obtained from wood.

iv) Okra (Lady's Finger)

Botanical name - *Abelmoschus esculentus* Linn.

Family - Malvaceae

Vernacular name - English – Okra, Lady's finger

Hindi – Bhindi

Okra is a flowering plant of family Malvaceae, having characteristic green seed pods. It is mucilaginous. It is cultivated as annual crop throughout India and often grows around 2 to 2.5 meters tall. The fruit is a capsule.

Uses

1. The pods are cooked for curries or stuffed with condiments.
2. It contains carbohydrates, protein and vitamins and minerals.
3. It is a rich source of dietary fibre.

v) Cucurbits

a) Red Pumpkin

Botanical name - *Cucurbita maxima*

Family - Cucurbitaceae

Vernacular name - English – Red pumpkin

Hindi – Kaddu

Red pumpkin is an annual vine having yellow-coloured flowers. All parts of plant are useful in various ways.

Uses

1. Fruits, tender leaves and flowers are cooked to make vegetable.
2. Pumpkin is cooked better, sugar and spices to make sweet dish called Kaddu ka halwa.
3. Tender leaves are used for making soup.
4. Pumpkin seeds are nutrient rich and used as snack. They also yield oil.
5. Pumpkin has high dietary fibre.

b) Bottle Gourd

Botanical name - *Lagenaria siceraria*

Family - Cucurbitaceae

Vernacular name - English – Bottle gourd

Hindi – Lauki

Bottle gourd is cultivated throughout India all year round. Plant is a running or climbing vine having white coloured flowers. Plant grows very rapidly and stem needs support.

Uses

1. The fruit of bottle gourd is cooked and eaten as a vegetable.
2. Fruit is rich in vitamin B.
3. The skin of fruit is used to make chutney.
4. Fruit is also used to make sweet dishes.
5. Fruit juice is considered as a health drink.

c) Bitter Gourd

Botanical name - *Momordica charantia*Linn.

Family - Cucurbitaceae

Vernacular name - English – Bitter gourd

Hindi – Karela

Bitter gourd is cultivated throughout India. Plant is a trailing vine of family Cucurbitaceae. Its fruits are widely used in cuisines all over the world.

Uses

1. The fruits are known for their bitter taste and eaten as vegetable. It is cooked either as a curry or stuffed with spices and fried.
2. Leaves are also used as vegetable, and are source of ascorbic acid, carotene and sodium.
3. Seeds are used as condiment.

4. Bitter gourd is used as herbal medicine for treatment of diabetes, cough, skin disease, wounds, gout and respiratory diseases.

d) Cucumber

Botanical name - *Cucumis sativus*

Family - Cucurbitaceae

Vernacular name - English – Cucumber

Hindi – Kheera

Cucumber is a creeping vine having yellow flower, cucumiform fruits, belonging to family Cucurbitaceae. Botanically it is pepo type of berry having hard outer rind.

Uses

1. The fruits are eaten raw as salad.
2. Fruits contain large amount of water with protein and potassium.
3. It is high in beneficial micronutrients.
4. Fruits are used in preparation of sandwiches, soup and raita.
5. Fruit is also used in skincare.

E. Legumes

The family Fabaceae is commonly known as legume family is a group of economically important plants. The plants are recognised by their fruits (legume). The important vegetables of this family are.

i) Peas

Botanical name - *Pisum sativum*

Family - Papilionaceae, Fabaceae

Vernacular name - English – Pea

Hindi – Matar

Pisum sativum is an annual plant having pod shaped vegetable. Pea pods are fruits as they contain seeds and develop from ovary of pea flower.

Uses

1. Fresh pea seeds are used in various dishes such as making curry, matar-paneer, matar-mushroom etc.
2. Peas can also be consumed raw.
3. Pea soup is commonly consumed across country.
4. Dried and spilt pea is used as a pulse.
5. They are rich source of fibre protein, Vitamin A, B6, C & K, zinc, phosphorus and iron.

ii) French bean

Botanical name - *Phaseolus vulgaris*

Family - Fabaceae

Vernacular name - English – French bean

Hindi – Bean

French bean is an herbaceous annual plant of family Fabaceae.

Uses

1. The unripe fruit are used for making vegetable.
2. They are rich in protein, vitamins and minerals.
3. Tender leaves are used as green leafy vegetable.
4. Dried seeds are used as pulses. They are rich source of protein.

iii) Cowpea

Botanical name - *Vigna sinensis*

Family - Fabaceae

Vernacular name - English – Cowpea

Hindi – Lobia

Cowpea is an annual herbaceous leguminous plant of family Fabaceae. Plant can either be short and bushy or may be vine needing support to climb. The important feature of cowpea is long peduncles which hols flower and seed pods (4 or more). The pod contains seeds within it.

Uses

1. Unripe pods are cooked and consumed as vegetable.
2. Dried seeds are used as pulses.
3. The seeds can be processed into flour.
4. Seeds are rich source of protein, minerals and vitamins especially folic acid.

11.5 FRUITS OF THE LOCALITY

Fruits are essential part of balanced diet of humans and essential for maintaining good health.

Fruit is defined as the seed-bearing structure of plant which consists of ripened ovary and its contents. Fruits are rich in digestible and indigestible carbohydrates such as sugars, starch, and cellulose and pectin material. Fruits may be simple, developed from a simple ovary. They may be aggregate or composite fruit, developing from multiple ovaries, from complete inflorescence.

On the basis of climactic adaptability fruits may classified as tropical fruits – growing in tropical climate (e.g. Mango, Pineapple) or sub-tropical climate (e.g. Litchi, Grapes, Guava)or temperate fruits – growing in colder climates (e.g. Apple, Pear, peach).

In this unit we shall learn fruits grown in local area. The commonly occurring local fruits are as following.

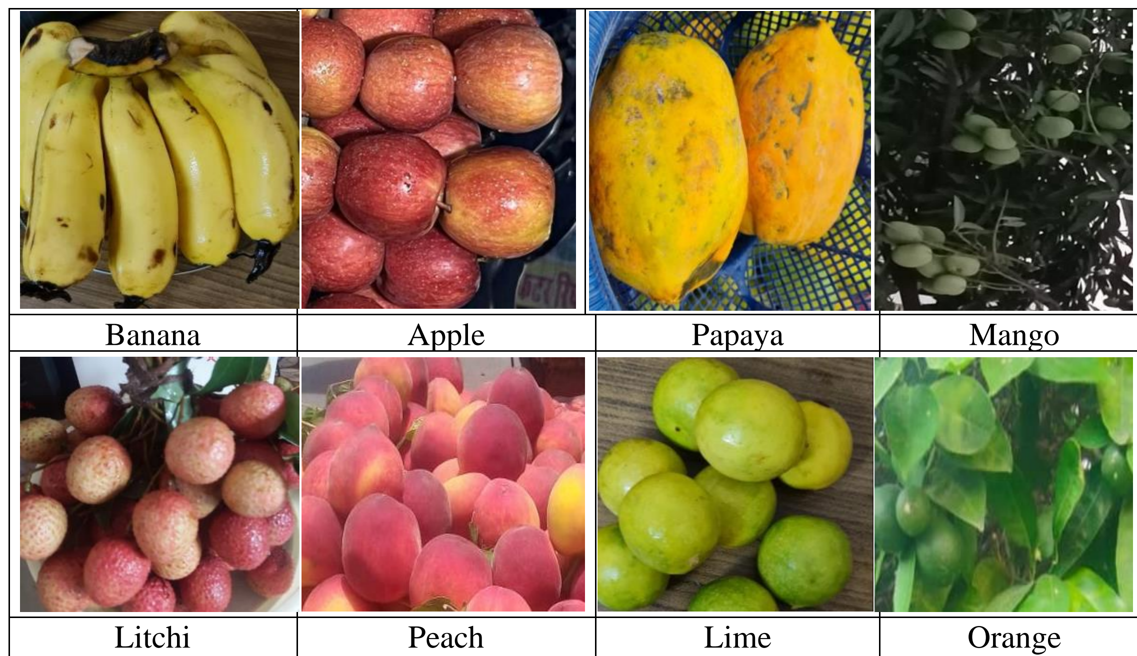

Fig. 11.3 Fruits of the locality

i) Mango

Botanical name - *Mangifera indica*

Family - Anacardiaceae

Vernacular name - English – Mango

Hindi – Aam

Mango is a large evergreen tree about 10 to 25 meters in height and grown in all tropical parts of world especially South Asia. The leaves are dark green, somewhat leathery. The fruit is juicy yellowish green to red and drupe having pericarp differentiated outer epicarp middle fleshy mesocarp and hard stony endocarp. The seed is single and large in size. Mango is national fruit of India it is also known as king of fruits.

Uses

1. The fruit is used in both ripe and unripe forms.
2. Sour unripe mangoes are used to prepare pickle, chutneys and in culinary preparations.
3. Dried unripe mango powder called amchur is used as condiment.
4. Unripe mangoes are used to prepare cooling drink – aam-panna,
5. Ripe mangoes are consumed directly as fruit. They are rich in sugars, vitamin A, carotenes, vitamin C and potassium.

6. Ripe mangoes are used in processed form as jam, jelly, murabba, amras, etc.
7. The leaves are used in treatment of diabetes and burns, bark is used in diarrhoea and many illnesses.
8. The wood is used for furniture and as firewood.

ii) Banana

Botanical name - *Musa paradisiaca* Linn.

Family - Musaceae

Vernacular name - English – Banana

Hindi – Kela

Banana is a perennial herbaceous plant of about 3 to 9 meters in height and has underground rhizomes. At the top of stem, there is a crown of large smooth, light green, glossy leaves are present which may be up to 12 feet in length and up to 2 feet in width. The fruit of banana usually develops parthogenically.

Uses

1. The fruits are edible and are rich in carbohydrate, it is rich in vitamin A and C. They also have magnesium, potassium, iron and calcium.
2. Bananas are also used to make wafers, shakes, juices.
3. Bananas are also used in cooking in sweet dishes.
4. Unripe bananas are cooked to make vegetable.
5. The leaves are used as disposable plates.

iii) Guava

Botanical name - *Psidium guajava*

Family - Myrtaceae

Vernacular name - English – Guava

Hindi – Amrud

Guava is a shrub or small tree of about 5 to 10 meters in height. The leaves are elliptic. Flowers are white in colour. The fruits are oval, rounded berry having variously coloured flesh.

Uses

1. Fruits are eaten raw or used in preparation of jam. jelly, chutneys etc.
2. Fruit is used to make fruit juice.
3. Guava is a rich source of vitamin C, it also contains carbohydrate, minerals and proteins.
4. Leaves contain essential oil.
5. Flowers are used in treatment of bronchitis.
6. Bark is used for dyeing, and decoction is also useful in treatment of diarrhoea.

iv) Litchi

Botanical name - *Litchi chinensis*

Family - Sapindaceae

Vernacular name - English – Litchi

Hindi – Lichi

Litchi belonging to family Sapindaceae is delicious juicy fruit. Its origin is from southern China and India is the largest producer of Litchi in world.

Litchi tree reaches height of 7.5 to 10.5 meters and is propagated by seed. The tree produces fruit after 3 to 5 years of age. the fruits are oval to round having brittle outer coveringwhich encloses a white fleshy translucent aril with one seed. Fruits are harvested in May-June in most parts of northern India.

Uses

1. Fruits are consumed raw and they are delicious and juicy.
2. Fruits are also canned or dried.
3. Litchi juice is processed from fruit.
4. The fruit contains good amount of Vitamin C, B complex and phytonutrient flavonoids. It is also rich in magnesium, copper, iron etc.

v) Papaya

Botanical name - *Carica papaya* Linn.

Family - Caricaceae

Vernacular name - English – Papaya

Hindi – Papita

Papaya belongs to family Caricaceae is a small branched tree up to 20 to 35 feet in height with spirally arranged leaves at the top of trunk. The plant part contains latex. Plant is dioecious. The flowers are wind or insect pollinated. The fruit is a large berry yellow-orange in colour. The plant is native to Mexico and northern South America.

Uses

1. The ripe fruit of papaya is usually eaten raw as an excellent breakfast fruit.
2. The unripe fruit can be consumed raw as salad or cooked as vegetable.
3. Papaya is used in juices and shakes and ice creams and confectionary.
4. The latex is known papin having medicinal value.
5. The young leaves are steamed and eaten as green vegetable.
6. The ripe fruit is a rich source of vitamins and minerals.

vi) Bael

Botanical name - *Aegle marmelos*

Family - Rutaceae

Vernacular name - English – Bael

Hindi – Bel

Bael belonging family Rutaceae is a species of tree native to the South-east Asia and Indian subcontinent. The tree is considered auspicious by Hindus. The tree is a deciduous shrub about abut 10-13 meters tall. The fruit is globose or pear shaped with very hard rind. The fully ripe fruit is yellow and has orange aromatic pulp divided in sections, in each section 6-15 seeds are present. The seeds are hairy and encapsulated in shiny mucilage.

Uses

1. The fruits can be eaten fresh or dried to produce pulp powder.
2. A drink from pulp is made, known as Bel-panna by adding sugar, water and citron juice.
3. The leaves are used as salad greens.
4. Fruit pulp is used prepare juices, murabba, puddings etc.
5. The bark roots and seeds are used in traditional medicine.
6. The tree leaves and fruits are used in worship by Hindus (offering to Lord Shiva).

vii) Citrus fruits

Citrus is an aromatic shrub or small tree of family Rutaceae. Several citrus fruits are described here.

a) Lime

Botanical name - *Citrus aurantiifolia*

Family - Rutaceae

Vernacular name - Hindi – Kagzinimbu

English – Lime

It is a small tree cultivated throughout India. The fruit is spherical, green in colour but becomes yellow after ripening. The fruit is hesperidium a modified berry.

Uses

1. The fruit is used for preparation of lemonade, lime juice, lime pickle, jams, jellies, alcoholic and non-alcoholic drinks.
2. It is a rich source of vitamin C.
3. The leaves are used for preparation of tea and in cooking sea foods.

b) Mandarin

Botanical name - *Citrus reticulata*

Family - Rutaceae

Vernacular name - Hindi – Santara

English – Mandarin, Orange

The plant is a moderate size tree of about 25 feet in height. The fruits are globose, orange-red, orange or orange yellow. They are sweet in taste, delicious and rich in vitamin C. The fruit may be seedless or with a few seeds.

Uses

1. Fruits are eaten fresh after removing peel or used in salads and desserts.
2. The peel is used either fresh or as a spice for cooking, baking, drinks or candy.
3. The juice is either consumed fresh or packed, also used in preparation of many drinks.
4. The peel yields essential oil which is used for flavouring for candies, in ice cream, chewing gums, bakery items and flavouring liqueurs,
5. It the most valued among citrus fruits.

c) Sour Orange

Botanical name - *Citrus aurantium*

Family - Rutaceae

Vernacular name - Hindi – Khatta

English – Sour Orange

Sour orange is a small tree native to South-east Asia and found scattered with other citrus in many parts of the world.

Uses

1. The fruit is a rich source of vitamin C.
2. The leaves are used for their essential oil.
3. The peel is used in the production of bitters.
4. Unripe fruit is used for making pickle.
5. Juice of ripe fruit is used as salad dressing or as marinade, liqueurs and others drink preparations.

d) Lemon

Botanical name - *Citrus limon*

Family - Rutaceae

Vernacular name - Hindi – Nimbu

English – Lemon

It is a small evergreen tree about 10 – 20 feet in height with large white and purple flowers. The fruits are round or oval in shape, yellow in colour with blunt pointed end.

Uses

1. The juice of fruit is used for culinary and cleaning purposes.
2. The juice contains about 5% to 6% citric acid, giving fruit a sour taste, which is a key ingredient in drinks and fruits.
3. The whole lemon is used to make marmalade, lemon liqueur.
4. Lemon slices and rind are used to garnish food and drinks.
5. The grated outer rind is used to flavour rice, puddings, baked goods etc.
6. Juice is used to make soft drinks and cocktails, also used as a short-term preservative on certain foods.
7. Peel is used in manufacture of pectin, which is used as a gelling agent and stabilizer in food and other products.
8. Leaves are used to make tea.
9. Oil from rind is used perfumes and preparation of toilet soaps.

viii) Grapes

Botanical name - *Vitis vinifera* Linn.

Family - Vitaceae

Vernacular name - Hindi – Angur

English –Wine grape

Grapes are most delicious fruits of family Vitaceae, grown in subtropical parts of world. The plants are propagated by cuttings. Plant vine is a woody climber, climbs with help of tendrils. The leaf is large and palmately nerved. Flowers are small, borne in panicles, laterally near the base of leafy branches. The fruit are produced in bunches. The fruit is juicy round or elongated in shape. The fruit is berry.

Uses

1. The fruits are eaten fresh or can be used for making jelly, raisins, vinegar, jam, juice, wine and grape seed oil.
2. Grape leaves and fruits are rich in thiamine and ascorbic acid and used in Aurvedic medicine.
3. Grape seed oil is used for cooking purposes.
4. Grapes are commercially most important for making wines.

ix) Apple

Botanical name - *Malus pumila, Malus domestica*

Family - Rosaceae

Vernacular name - Hindi – Seb

English – Apple

Apple belonging to family Rosaceae is an important fruit of temperate region. The plant is grown in temperate countries of the world. The plant has height of 2 to 5 meters, having

simple, ovate to broadly elliptic, dull green leaves mostly clustered on spurs. Flowers are pinkish white, and fruit is fleshy pome with a depression at each end. Thalamus is the fleshy and edible part of the fruit. In India apple is mainly grown in temperate regions of north India and generally propagated by budding or grafting.

Uses

1. Apple is mostly consumed a fresh fruit.
2. It contains calcium, magnesium, potassium, sodium, phosphorus etc. It is rich in pectin.
3. Fruit is used to make Juice, jams and jellies.
4. Juice is fermented to make wine, cider and vinegar.
5. Apple is also used to make murabba in India.
6. Fruits are used in bakery like apple pie.

x) Peach

Botanical name - *Prunus persica* Linn.

Family - Rosaceae

Vernacular name - Hindi – Aru

English – Peach

Peach is a small tree about 7 -87 meters in height. The leaves are oblong to broad lanceolate. Flowers are solitary and pink in colour. The fruit is round and velvety drupe with deeply pitted stone. Plant propagated by budding on a seedling stock and requires deep loam soil rich in humus for growth.

Uses

1. Fruits are mostly eaten raw but also canned and dried.
2. The fruits are used in making wine and other alcoholic drinks.
3. They are good source of thiamine and ascorbic acid.
4. The seed oil is used for cooking purpose.

11.6 SUMMARY

Plants play an important role in human life. Food,clothing and shelter are 3 primary necessities of human being and the most essential among these three is food.The food comes primarily from the plants in different forms such as cereals,vegetables,pulses,fruits etc.The cereals are mainly cultivated for their seeds.Cereal grain crop provides more food energy than any other crop.

Pulses are part of healthy diet,very high in both soluble and insoluble fibres,vitamins,minerals and constitute main source of protein for most of the population.Their nutritional qualities make them helpful in fighting against many diseases.

More than ten thousand plant species are used as vegetable throughout the world.Vegetables are important source of vitamins and minerals neededfor human health.They are rich source of β-

carotene, thiamine, riboflavin, niacin, pantothenic acid, folic acid, minerals such as iron, zinc, calcium, magnesium, antioxidants and fibres.

Fruits are good source of vitamins and minerals.They play an important role in overall health and maintenance of human body system.They are essential part of balanced diet.

Thus cereals, pulses, vegetables and fruits play an important role in diet and overall health of human being.

11.7 GLOSSARY

Stem: A negatively geotropic structure arises from plumule.

Leaf: Leaf is the lateral appendage of plant stem specialized for photosynthesis.

Root: The organ of plant which provides anchorage and takes in water and nutrients into plant body.

Flower: Flower is the reproductive part of plant consisting of calyx, corolla, androecium and gynoecium.

Fruit: Fruit is the product of ripened ovary and seed-bearing portion of the plant.

Cotyledons: A significant part of the embryo within the seed of a plant.

Vegetables: Vegetables are edible part of plants including flowers, fruits, stem, leaves, roots, and seeds.

Cereals: Any grass grown for the edible grains.

Pulses: The dried seeds of family Fabaceae, rich in protein.

11.8 SELF-ASSESSMENT QUESTIONS

11.8.1Multiple choice questions

1. The botanical name of barley is
   a) *Hordeum vulgare* b) *Avena sativa*
   c) *Triticum aestivum* d) *Secale cereale*
2. Small seeded cereals and forage grasses are known as
   a) Legumes b) Millets
   c) Pulses d) Fruits
3. *Pisum sativum* belongs to family
   a) Poaceae b) Fabaceae
   c) Rosaceae d) Cucurbitaceae
4. Botanical name of Tomato is
   a) *Lycopersicon esculentum* b) *Cucumis sativus*
   c) *Allium cepa* d) *Allium sativum*
5. Potato belongs to family

a) Solanaceae b) Liliaceae
c) Malvaceae d) Apiaceae

6. The edible part of litchi is
   a) Mesocarp b) Thalamus
   c) Aril d) Seed
7. Apple belongs to family
   a) Rosaceae b) Musaceae
   c) Vitaceae d) Rutaceae
8. Grape is a _______ type of fruit
   a) Simple, berry b) Drupe
   c) Nut d) Thalamus
9. Botanical name of apple is
   a) *Pyrus malus* b) *Pyrus communis*
   c) *Pyrus avium* d) *Prunus persica*
10. *Musa paradisiaca* is
    a) Banana b) Apple
    c) Orange d) Grape
11. Botanical name of Palak is
    a) *Spinacia oleracea* b) *Brassica oleracea*
    c) *Solanum tuberousum* d) *Allium cepa*
12. The edible part of Mango is
    a) Mesocarp b) Thalamus
    c) Endocarp d) Pericarp
13. Banana plant is
    a) Tree b) Shrub
    c) Herb d) All of the above
14. Wheat is
    a) Achene b) Caryopsis
    c) Samara d) Sorosis
15. A true fruit is developed from
    a) Thalamus b) Calyx
    c) Ovary d) Receptacle

11.8.2 Fill in the blanks

1. All cereals belong to the family ……… and their characteristic fruit is ………
2. In India wheat is grown as ………crop.
3. Botanical name of rice is………..
4. Wheat is an ………herb.
5. The family of lady finger is……..
6. Peach belongs to family……………..
7. Edible part of mango is its……….

8. Vitis vinifera is commonly called as……

11.8.1 Answer keys: 1-a, 2-b, 3-b, 4-a, 5-a, 6-c, 7-a, 8-a, 9-a, 10-a, 11-a, 12-a, 13-a, 14-b, 15-c.

11.8.2 Answer keys: 1- Poaceae,caryopsis; 2- Rabi; 3- *Oryza sativa*; 4-Annual; 5- Malvaceae; 6- Rosaceae; 7- Mesocarp; 8- Grapes.

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11.11 TERMINAL QUESTIONS

1. All cereals belong to which family? Write Botanical name and uses of any five cereals studied by you?
2. Write Botanical name and uses of five vegetables growing in your locality?
3. Define fruits? Write Botanical names and uses of six fruits growing in your area?
4. Write a short note on fruit vegetables of your locality?
5. Write short note on any two pulses?

UNIT-12: FOOD CROP'S MORPHOLOGY, ANATOMY AND MICRO-CHEMICAL TESTS FOR STORED FOOD MATERIALS

Contents

12.1 Objectives

12.2 Introduction

12.3 Morphological (vegetative and floral) characters of some important plants

12.4 Anatomical investigation of some important plants or plant parts

12.5 Anatomical investigation of anomalous growth in some important plants

12.6 micro-chemical tests for stored food materials

12.7 Summary

12.1 OBJECTIVES

After studying this chapter, you will be able to:

- Identify and distinguish various types of food crops morphologically.
- Identify and distinguish various types of food crops anatomically.
- Discriminate various types of plant cell and tissue system in respect to its adaptation and significance.

12.2 INTRODUCTION

Identifying the food crops by their appearance is a great task among the botanist to systemizeits position in the taxonomic categories. Once the plant is identified, a system can reach further decisions on treatment needed. The plant morphology or phytomorphology is the study of the physical form and external structure of plants. Morphology is the backbone of plant identification and classification since ancient time. Morphology plays an important role in identifying plants it also provides us with range of variations found in different species of plants. Morphology helps us to understand different aspects of plant life-like ecological adaptation and genetically appearance of traits.

Plant Breeding, crop protection, and crop productivity only depend on morphological characteristics like size, shape, thickness and orientation of leaves, number of flowering shoots, etc. Plants that have morphological peculiarities are adopted by horticulture for growth in gardens, park, etc. It also indicates the structural adaptations of plants to their environment like storage, protection, climbing, etc. In this section, we focus on characterization of food crop morphologically.

Plant anatomy is a powerful tool that can be used to help solving the problem, whether in classroom, or national botanical facilities. Anatomy is particularly useful in plant taxonomy identifying disassociated plants parts whether those parts be leaves, roots, stems, fruits or seeds of living or fossil plants. Most of the time anatomy is overlooked by many researchers but it is an essential requirement for clear understanding about the plants.

12.3 MORPHOLOGICAL (VEGETATIVE AND FLORAL) CHARACTERS OF SOME IMPORTANT PLANTS

1. Wheat (*Triticum aestivum* L.)

Vegetative characters

Habit: Annual or perennialherbs.

Root: Adventitious or fibrous root.

Stem: Erect or prostrate, cylindrical, jointed (culm) with hollow internodes (solid throughout in sugarcane, sorghum and maize), herbaceous or woody, supra-nodal intercalary meristem occurs in node many plans, runner, stolons, suckers, rhizomes, and corms present.

Leaves: Cauline and ramal, alternate, sessile (petiolate in bamboos), exstipulate, leaf base sheathing, lamina simple long and narrow with parallel veins, ligulate due to the presence of an outgrowth at the junction of lamina and leaf sheath.

Floral characters

Inflorescence: Spikelet has 1-8 sessile flowers or florets borne alternately on a short axis called rachilla. The spikelet is surrounded at the base by two opposite but alternate scales called glumes. Spikelets are further arranged variously, e.g., spike (wheat), panicle (oat).

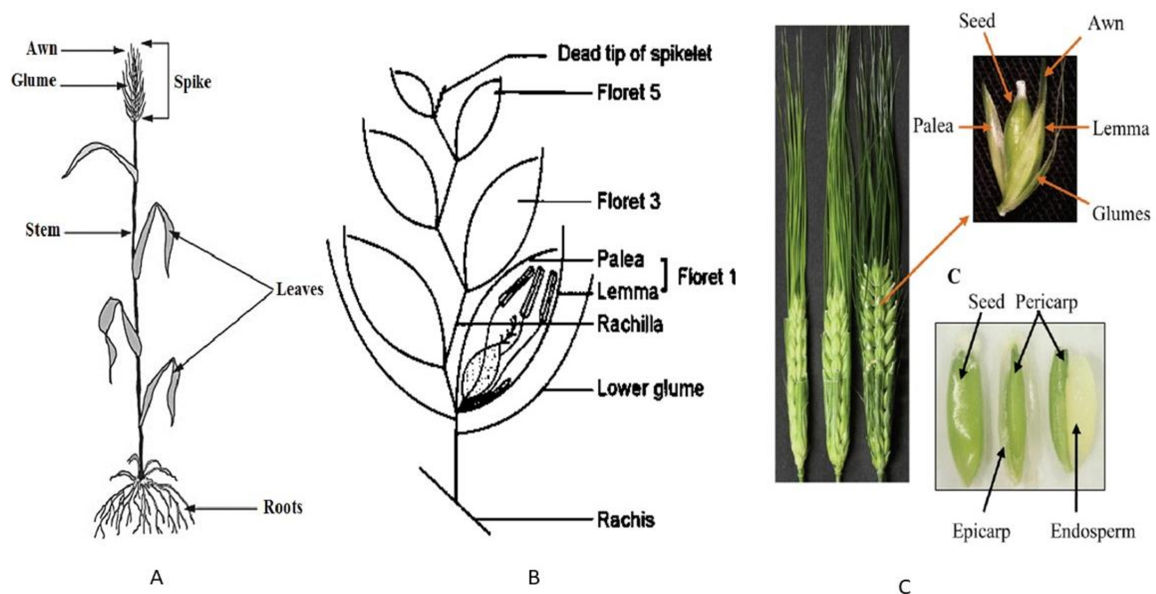


Fig. 12.1 Morphological features of wheat A. Mature plant; B-C. Inflorescence and seed

Flower or floret: Small, sessile surrounded by two scales, lemma (inferior or outer palea) and palea (superior or inner palea). The lemma is considered to represent a bract while the palea is thought to be formed by the fusion of two bracteoles. The lemma may bear a long stiff process called awn or arista which is a continuation of its midrib, irregular, zygomorphic, incomplete, perfect or unisexual (like maize), hypogynous.

Perianth: Represented by 2 or 3, small hyaline or succulent scales called lodicules, inferior. The lodicules are hygroscopic and are supposed to help in the opening of florets.

Androecium: 3 or 6 stamens, free in one two whorls, filaments long and slender, anthers basifixed or versatile, sagittate and divaricate (two anther lobes separated), dehiscence longitudinal, inferior.

Gynoecium: Tricarpellary, syncarpous appearing monocarpellary, ovary superior, unilocular and uniovuled, placentation basal, style generally absent or short (exception maize), two feathery generally arising directly from the wall of the ovary, style represented by a long silken thread in maize.

Fruit: Caryopsis or grain.

2. Maize (*Zea mays* L.)

Vegetative character

Root: Maize plants have three types of roots, i) seminal roots - which develop from radical and persist for long period, ii) adventitious roots,fibrous roots developing from the lower nodes of stem below ground levelwhich are the effective and active roots of plant and iii) brace or prop roots,produced by lower two nodes.

Stem: It is 3-4 cm, the internodes are short and fairly thick at the base of the plant; becomelonger and thicker higher up the stem, and then taper again.

Leaves: The upper leaves in corn are more responsible for lightinterception and are major contributors of photosynthate to grain.

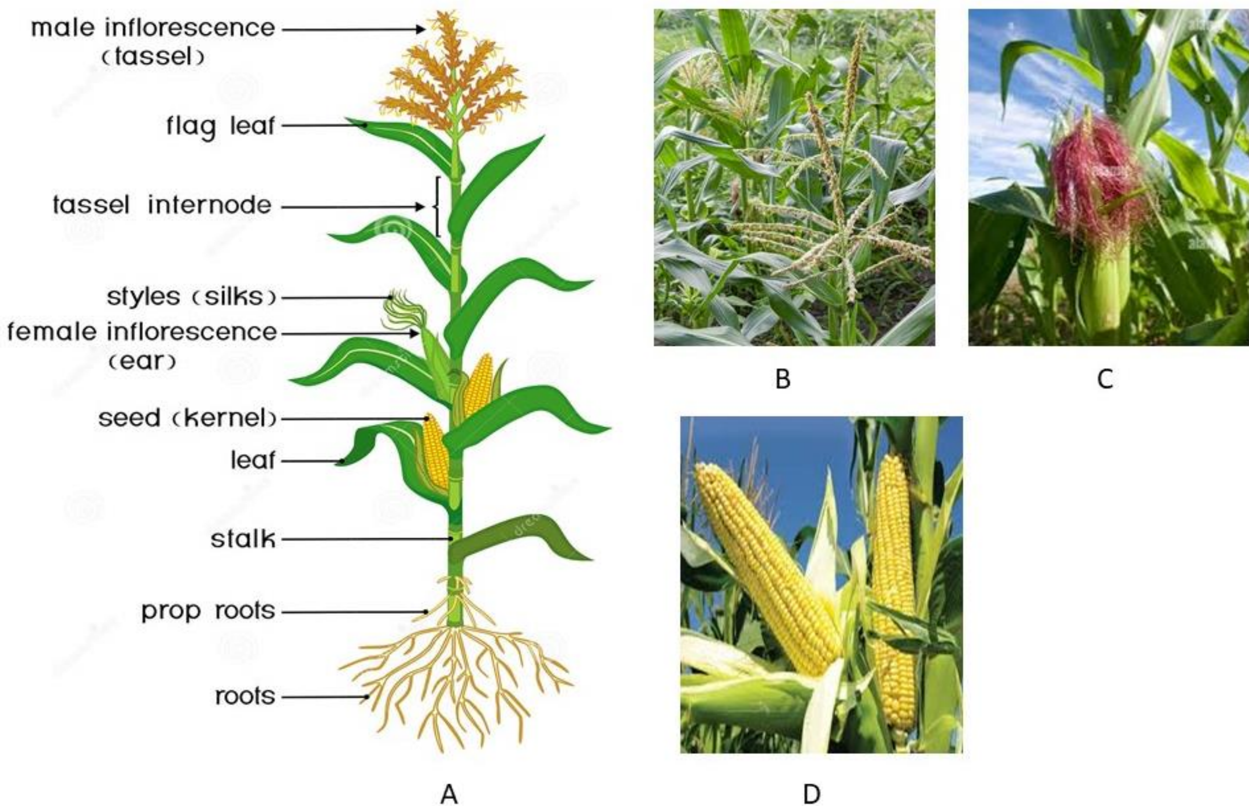


Fig. 12.2 Morphological features of maize A. Mature plant; B. Inflorescence; C-D. Cobs

Floral character

Inflorescence: The apex of the stem ends in the tassel, an inflorescence of maleflowers and the female inflorescences (cobs or ears) are borne at the apexof condensed, lateral branches known as shanks protruding from leaf axils.

Male flower: The male (staminate) inflorescence, a loose panicle, produces pairs of freespikelets each enclosed by a fertile and a sterile floret.

Tassel: The tassel consists ofseveral long, indeterminatebranches bearing shortdeterminate branches (spikeletpairs) that bear two spikelets(compact auxiliary branches ofgrass inflorescence, that inmaize consists of two bractssubtending one reduced maleflower).

Female flower: Pistillateinflorescence, a spike, produces pairs of spikelets on the surface of a highlycondensed rachis (central axis, or “cob”). The female flower is tightly coveredover by several layers of leaves, and so closed in by them to the stem thatthey don’t show themselves easily until emergence of the pale yellow silksfrom the leaf whorl at the end of the ear.As the internodes of the shanks are condensed, the ear remains permanentlyenclosed in a mantle of many husk leaves.

Fruit: Caryopsis (Grain)

3. Mustard (*Brassica campestris* L.)

Vegetative characters

Stem: Herbaceous, aerial, erect, cylindrical, branched, solid, smooth and green.

Leaf: Cauline and ramal, alternate, exstipulate, simple, sessile, lower leaves lyrate with deeply cut margins, acute, glabrous, unicostate, reticulate.

Floral character

Inflorescence: Racemose raceme.

Flower: Ebracteate, pedicellate, complete, actinomorphic, hermaphrodite, tetramerous, hypogynous, cyclic and yellow.

Calyx: Sepals 4 in two whorls of 2 each, polysepalous, slightly petaloid.

Corolla: Petals4, polypetalous, cruciform, each petal is distinguished into a claw and a limb, valvate.

Androecium: Stamen 6 in two whorls (2+4), polyandrous, tetradynamous, 4 inner long and 2 outer short, dithecous, basifixed, and introse, glands are present at the base of 4 longer stamens.

Gynoecium: Bicarpellary, syncarpous, ovary superior, unilocular but becomes bilocular later on due to the development of a false septum (replum), ovules many in each locule, placentation parietal, style short and stigma is bilobed.

Fruit: Siliqua

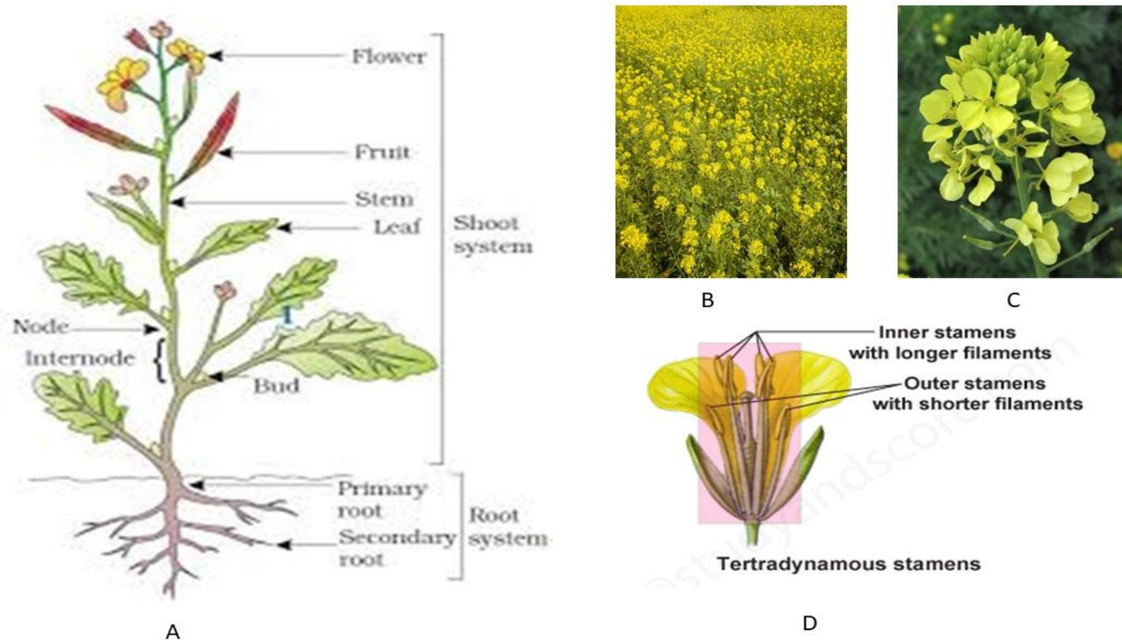


Fig. 12.3 Morphological features of mustard A. Mature plant; B. mustard field; C. Inflorescence; D. Flower

4. Soybean (*Glycine max* L.)

Vegetative character

Root: The nodulated root system consists of a taproot from which emerges a lateral root system. The plants of most cultivars are covered with fine trichomes, but glabrous types also exist.

Leaves: The primary leaves are unifoliate, opposite and ovate, the secondary leaves are trifoliate and alternate, and compound leaves with four or more leaflets are occasionally present.

Floral character

Flower: The papilionaceous flower consists of a tubular calyx of five sepals, a corolla of five petals (one banner, two wings and two keels), one pistil and nine fused stamens with a single separate posterior stamen. The stamens form a ring at the base of the stigma and elongate one day before pollination, at which time the elevated anthers form a ring around the stigma.

Fruit: The pod is straight or slightly curved, varies in length from two to seven centimeters, and consists of two halves of a single carpel which are joined by a dorsal and ventral suture.

Seed: The shape of the seed, usually oval, can vary amongst cultivars from almost spherical to elongate and flattened.

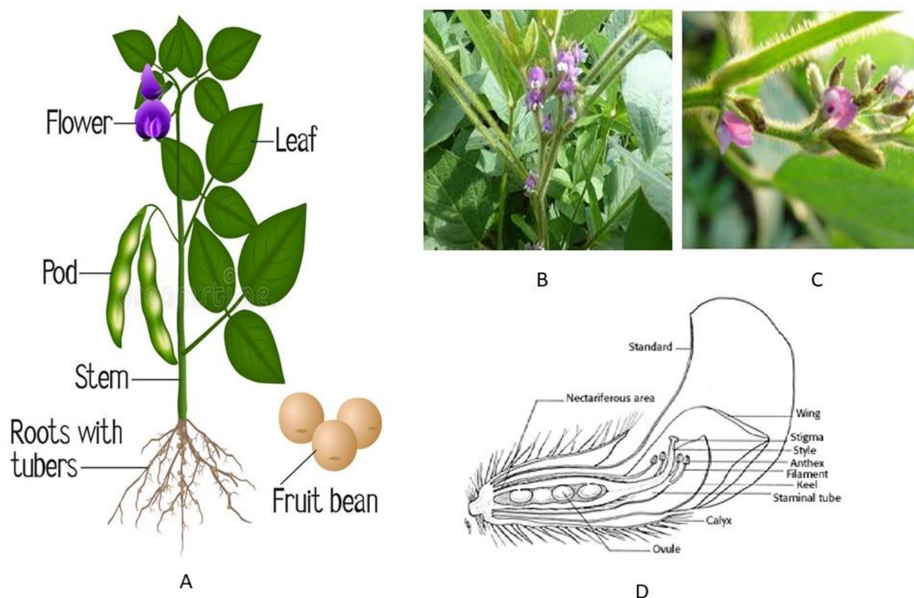


Fig. 12.4 Morphological features of soybean: A. Mature plant; B. Inflorescence; C-D. Flower

12.4 ANATOMICAL INVESTIGATION OF SOME IMPORTANT PLANTS OR PLANT PARTS

The plant consists of three basic structures leaves, stem and roots. All these organs composed of tissue system which is derived from apical meristematic tissue. There are two broad categories of meristems can be studied. These are shoot apical meristem and root apical meristem. These Meristem contribute to the formation of different parts of plant body. The details of root apex and shoot apex given as below:

1. L. S. of the shoot apex

The shoot apex is hemispherical to slightly flatten in longitudinal lane. It remains protected by the covering of young and developing leaves. The shoot apex shows an apical promeristem, consists of tunica and carpus. Tunica is outermost covering, cells divide anticlinal plane and increase the surface area of shoot apex.Carpus is generally mass of randomly dividing cells just below the tunica. In the center just below the carpus; rim meristemis present. The cells of this region are arranged in regular files and give rise to pith.Near the periphery, surrounding the rim meristem lies the peripheral or flank meristem. This region shows actively dividing cells and lateral organs like leaves and branches arise from these cells (Fig. 12.5).

2. L.S. of the root apex

The longitudinal section of the root apex appears gradually tapering. It is the terminal portion of the root covered by root cap. Calyptrogen follows root cap and lies closer to the root apex. It gives rise to the tissue of the root cap.Inside the calyptrogen the root apex which is subterminal in position. The cells in this region divide actively and produce the tissues of root. Three regions

based on structure and growth of the root apex is recognized. These are dermatogen, periblem andplerome. Dermatogen is single layered. It gives rise to the outermost layer i.e., epiblema. Periblem is singe layered, it forms middle region or the cortex of the root.Plerome gives rise to pericycle, medullaryrays, pith and the vascular bundles. Some procambial strands give rise to bundles of xylem and others to the bundles of phloem in an alternate manner (Fig. 12.6).

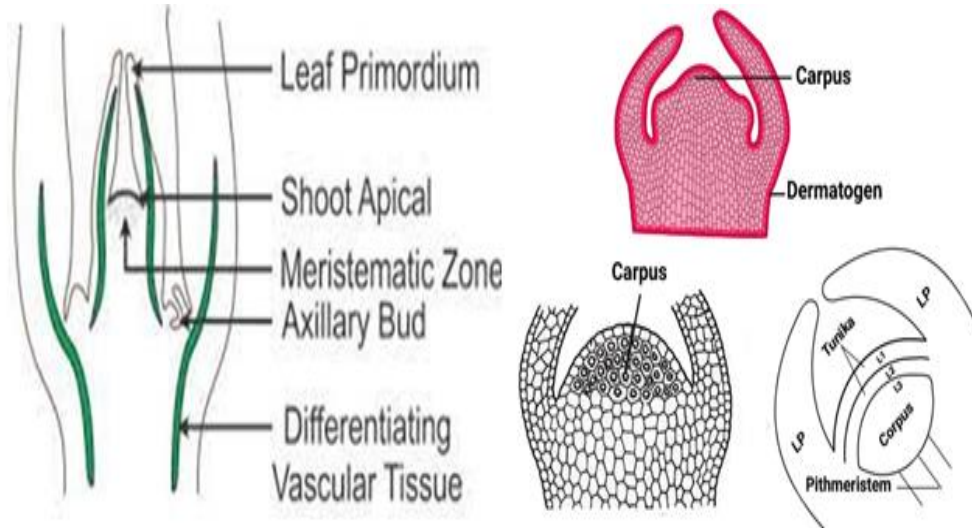


Fig. 12.5 L. S. of the shoot apex. A. Diagrammatic B. Cellular

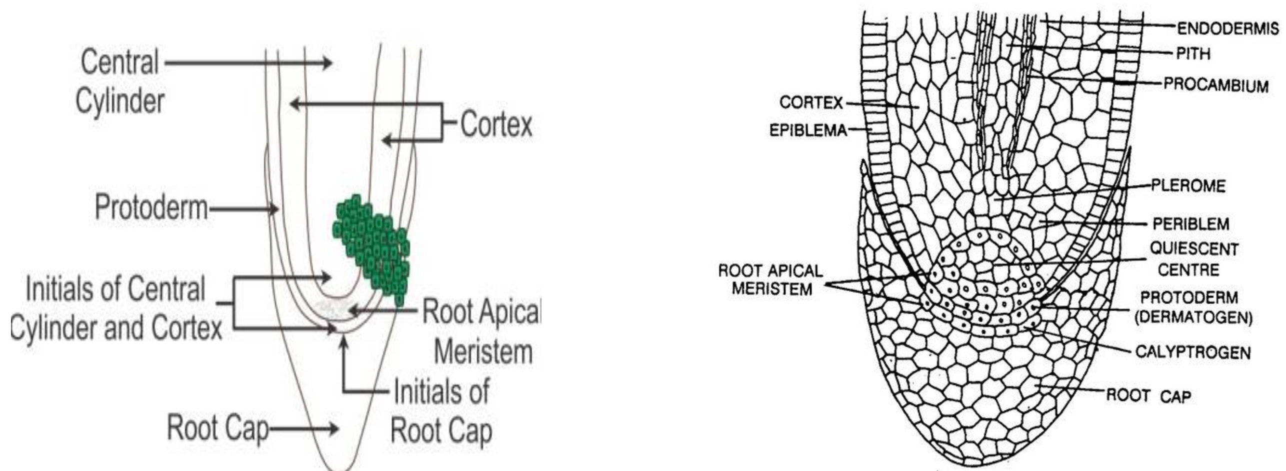


Fig. 12.6 L. S. of the root apex A. Diagrammatic B. Cellular

Plant organs: Plants contain internal structure, which could be seen as soon as primary structure of an organ is completely formed. The organization of internal structure can be seen in transverse section and organ could be easily identified.

3. To cut and study the transverse section of stem, root and leaves.

Requirements: Razor/Blade, petri dish, watch glass, slide, cover slip, brush, needle, forceps, staining rack with stain and glycerin, distilled water, and microscope.

Procedure

1. Cut a transverse section of the material with a sharp razor/ blade.
2. Select a thin and uniformly cut section.
3. Stain the section either in safranin-fast green combination or crystal violet-erythrosine combination.
4. Mount a properly stained section in glycerin.
5. Observe the section under the microscope and study.
6. Once the observation is completed, a diagrammatic outline of the section and a part of the section drawn to show different types of cells found in the section.
7. Label the diagram neatly.
8. Write down the description, starting from outer to inner side. As follows: (i) Epidermis; (ii) Cortex/ Ground tissue; (iii) Endodermis (iv) Pericycle and (v) Vascular tissues
9. Identify the material
10. Provide description of peculiar or abnormal characters if any.

Key for Identification

1a. Epidermis, cortex and vascular tissues well differentiated; endodermis not well developed; vascular bundlesare arranged in a ring; vascular bundles are conjoint, collateral open and endarch; Pith well developed. **Dicotyledonous stem**

1b. Epidermis differentiates, presence of ground tissue; vascular bundle conjoint collateral and always closed, scattered; pith not well marked………….. **Monocotyledonous stem.**

2. Vascular bundle radial and exarch, endodermis conspicuous with casparian strip.. **Root**.

2a. Protoxylem groups up to six in number; pith small; secondary growth present……………………………………………………..…... **Dicotyledonous root.**

2b. Protoxylem groups more than six in number; pith well developed; secondary growth absent…………………………………………………….. **Monocotyledonous root.**

4. Describe the T.S. of *Luffa* stem

Cut the transverse section of the material, stain in safranin and fast green combination and mount in glycerin.

Observations: The outline of the section shows ridges and furrows; differentiated into epidermis, cortex, vascular bundles and pith (Fig. 12.7).

Epidermis

1. It is the outermost layer of the stem; consist of single-layered with tangentially flattened cells and externally it is surrounded by cuticle.
2. From some of the cells develop multicellular epidermal hairs.
3. The continuity of the epidermal layer is broken by certain stomata.

Cortex

4. It consists of outer few layers of collenchyma, some layers of parenchyma and an innermost layer of collenchyma region is 3 to 6 layered, and the cells are thickened at the corners due to the deposition of pectin and cellulose.
5. Parenchyma is present inner to the collenchyma in the form of few layers
6. The cells are thin walled and the parenchymatous region contains many intercellular spaces.

Endodermis

7. Endodermis is the innermost layer of cortex and consists of barrel shaped cells.
8. Cells of the endodermis contain casparian strips and many starch grains.

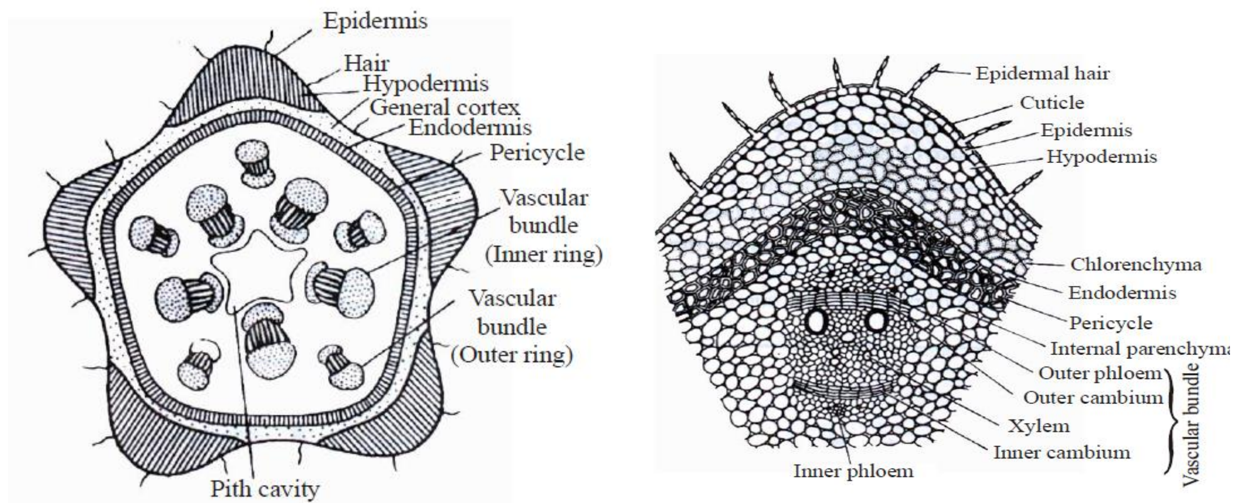


Fig. 12.7 T.S. of stem A. Diagrammatic B. Cellular

Vascular Bundles

9. These are conjoint, collateral, open and endarch.
10. These are arranged in a ring, and each consists of phloem, cambium and xylem.

    Phloem: It consists, of sieve tubes, companion cells and phloem parenchyma. Sieve tubes are narrow and associated with the companion cells.

    Cambium is present in between xylem and phloem of the vascular bundles and consists of thin walled, rectangular cells arranged in radial rows.

Xylem: It consists of vessels, tracheids, wood fibers and woody parenchyma. Big vessels represent metaxylem while the smaller vessels represent the protoxylem.Trachcids are thick walled cells and surround the metaxylem. Wood fibres are thick walled and lignificd.Wood parenchyma is present in the form of thin-walled cells.

11. **Pith:** Central part of the section occupied by well developed parenchymatous cells called pith.

[II] Identification:

1. Presence of vessels in xylem……………………………..………………… **Angiosperms**
2. Cortex well differentiated, vascular bundles are bundles are conjoint, collateral (bicollateral) and open, protoxylem endarch, arranged in a ring, pith well developed……………………………………………………….. **Dicotyledonous stem**

Peculiar feature: Pericycle is present in the form of semilunar patches of sclerenchyma outside the phloem.

5. Describe the T. S. of *Cicer* Root

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

Observations: The outline of the section is almost circular (Fig. 12.8).

Epiblema

1. This is the outermost single row of thin-walled cells.
2. Unicellular root hairs are present.

Cortex

1. It consists of several layered deep undifferentiated parenchymatous cells.
2. Numerous intercellular spaces are present.
3. Epiblema is short lived in a few members and, thereafter, some of the outer layer of cortex becomes cutinized. These layers of the cortex together are known as exodermis.

Endodermis

1. It separates vascular tissue from the cortex.
2. The barrel- shaped cells are closely packed.
3. The radial and radial tangential walls show casparian strips.
4. The cells of endodermis in close approximation with the protoxylem are thin walled, these are called passage cells.

Pericycle

1. It follows the endodermis.
2. The cells are thin walled and compactly arranged.

Vascular tissue system

1. It consists of vascular bundles. The vascular bundles are radial and exarch.
2. The xylem and phloem form equal number of separate bundles with protoxylem towards the periphery (Exarch).
3. The number of the xylem and phloem group is four each.
4. The phloem consists of sieve tube elements, companion cells and phloem parenchyma.
5. The xylem consists of tracheid, vessels and xylem parenchyma.
6. Protoxylem element is smaller and show annular or spiral thickenings.
7. Mature cambium appears as a wavy layer below the phloem and above the protoxylem elements. As a result of secondary growth, primary xylem elements are pushed towards the center, where they larger in size and show reticulate and pitted thickenings.

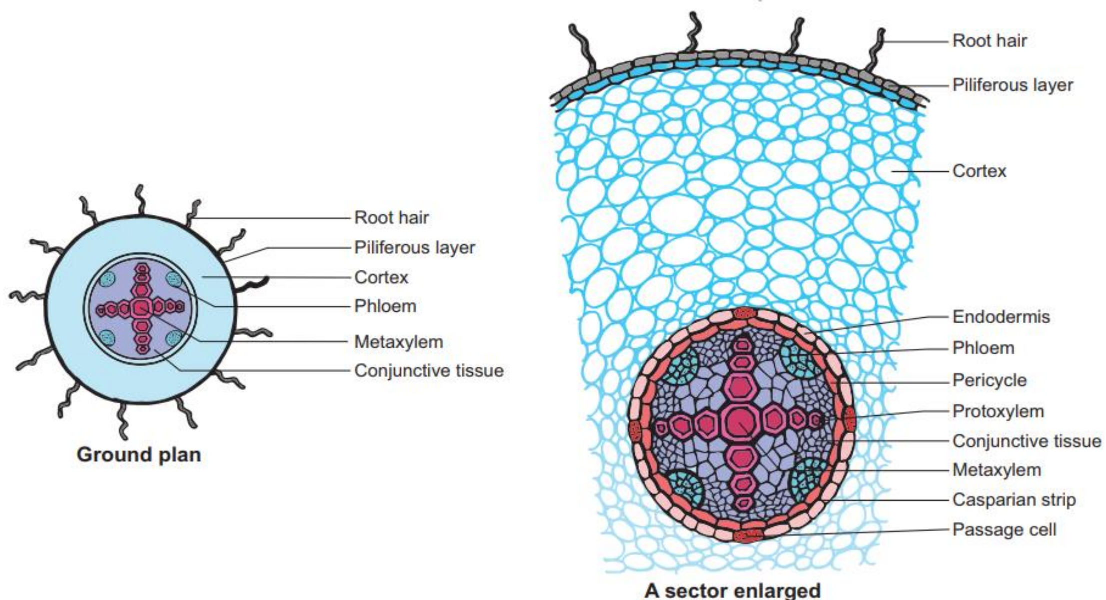


Fig. 12.8 T. S. of Root A. Diagrammatic B. Cellular

Pith

1. It is small and occupies the center of the axis.
2. The cells are parenchymatous.
3. After secondary growth, pith gets completely reduced due to the addition of secondary tissues.

Identification: 1a. Unicellular root hairs; cortex undifferentiated; vascular bundle radial and exarch…...…..... **Roots**

1b. Xylem groups are four showing tetrarch condition; cambium appears as secondary meristem; pith is very small...**Dicotyledonous root**

6. Describe the T.S. leaf of *Mangifera indica*

Cut a transverse section of the material. Stain in safranin and fast green combination and mount in glycerin (Fig. 12.9).

Observations

1. Lower and upper epidermis are single layered.
2. The cells are barred shaped and compactly arranged.
3. Upper epidermis has a thick cuticle and lack stomata.
4. Lower epidermis has thin cuticle and stomata are present.

Mesophyll

1. It is differentiated into palisade and spongy parenchyma.
2. Palisade occurs below upper epidermis in two layers, with parenchyma near the larger vascular bundle. The cells are compactly arranged, long and tubular and chloroplast is present.
3. Spongy parenchyma form rest of tissue. The cells are small varied in shape and size, loosely arranged andenclosed with small air spaces.
4. A few air spaces lead to stomatal openings which forms sub-stomatal cavity. Numerous chloroplasts are present near the walls (Fig. 12.9).

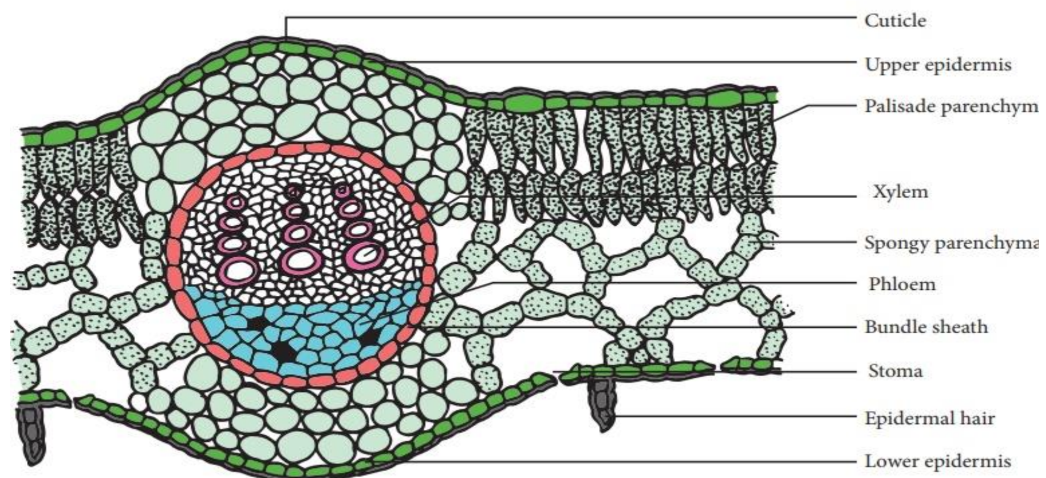


Fig. 12.9 T.S. of a dicot leaf

Vascular tissue

1. It consists of large vascular bundle in the middle and numerous small bundles in the wings.
2. Each bundle is conjoint. Collateral and closed and surrounded by parenchymatous bundle sheath. Large vascular bundle has an extensive bundle sheath that extends both towards lower and upper epidermis.
3. Metaxylem is situated towards the lower epidermis and protoxylem directed towards the upper epidermis.
4. Phloem of the vascular bundle is directed towards the lower epidermis.

Identification

Mesophyll is differentiated into palisade and spongy parenchyma.. **Dorsiventral leaf**

Point of interest

Most of the leaves of dicotyledons are dorsiventral. They grow in horizontal direction with distinct upper and lower surface. Palisade form a few layers near the upper epidermis and spongy parenchyma near the lower epidermis.

7. Describe the T.S. stem of *Zea mays*

Observations: Cut a T.S. of the material, stain in safranin and fast green combination and mount in glycerin. Transverse section is almost circular in outline (Fig. 12.10).

Epidermis

1. Its outermost layer of single row of cells covered by a thin cuticle.
2. Epidermal hairs are absent.

Hypodermis

1. It lies below the epidermis.
2. Hypodermis is two to three layered thick and is made of sclerenchymatous cells.

Ground tissue

1. It extends from hypodermis to the center of the axis.
2. The cells are parenchymatous and numerous large intercellular spaces are present.
3. Cortex, endodermis and pericycle are not differentiated.

Vascular tissue system

1. Numerous vascular bundles are scattered in the ground tissue.
2. The vascular bundle near to the periphery are smaller than the center of the section.

3. Each bundle is conjoint, collateral, endarch and closed.
4. A vascular bundle is almost completely surrounded by parenchymatous or sclerenchymatous bundle sheath. It is prominent towards the upper and the lower margins of the bundle.
5. The xylem is almost Y- shaped and consists of very large and pitted metaxylem elements.
6. The protoxylem is situated near the center of the axis, just below lysigenous cavity.
7. Phloem is consisting of sieve tubes and companion cells only, phloem parenchyma being absent.
8. A small band of obliterated phloem occurs near the periphery of the bundle called protophloem.
9. Metaphloem lies just below protophloem and extends up to Y-shaped xylem, consisting of very prominent sieve tubes and companion cells.

Identification

1. Vascular bundles are conjoint collateral and endarch…….........................… **Stem**
2. Cortex is undifferentiated. ground tissue is present, endodermis and pericycle are absent. Vascular bundle is closed, numerous and scattered, bundle sheath is prominent……………………………………….……… **Monocotyledonous stem**

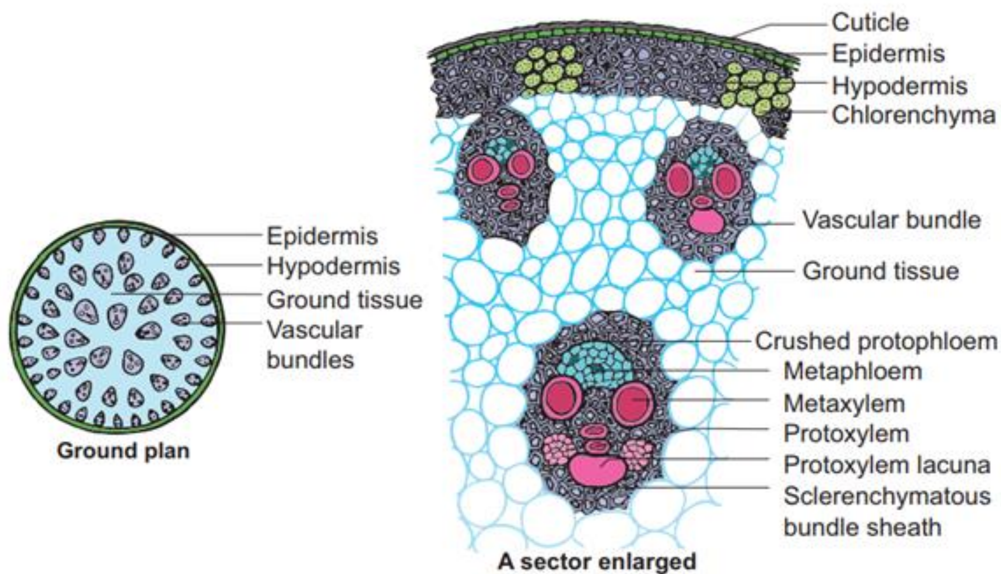


Fig. 12.10: T.S. of stem A. Diagrammatic B. Cellular

8. Describe the T.S. of *Zea mays* root

Cut a T.S. of the material, stain in safranin and fast green combination and mount in glycerin.

Observation: Outline of the section is almost circular (Fig. 12.11).

Epiblema

1. This is the outermost layer, barrel-shaped thin-walled cells.
2. Unicellular hairs arising as outgrowth of this layer are present.

Cortex

1. Several layers deep consists of thin-walled parenchymatous cells.
2. Numerous intercellular spaces are present.
3. In an old root, when epiblema gets disorganized, a few outer layers of the cortex undergo suberisation and thus outer part of the cortex become thick walled (exodermis). This is a protective later which protect delicate internal tissue from the external forces.

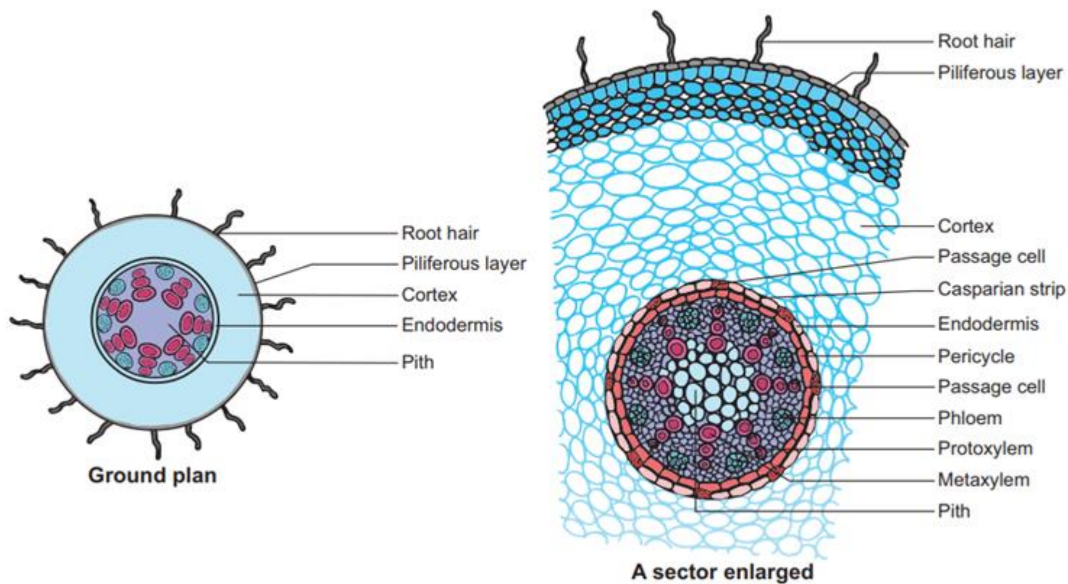


Fig. 12.11 T.S. of root A. Diagrammatic B. Cellular

Endodermis

1. This is the innermost layer of the cortex that separates underlying vascular tissue from the cortex.
2. It forms a definite ring around the stele
3. The cells are the barrel shaped, compactly arranged and casparian strips are present.
4. A few cells opposite the protoxylem elements are thin walled and are call passage cells.

Pericycle

1. The cells are thin-walled forms a complete ring.

Vascular tissue system

2. Vascular tissue system consists of radial and exarch vascular bundle.
3. Many groups of xylem and phloem located on alternate radial .
4. A xylem element consists of tracheid and xylem parenchyma.
5. Protoxylem is exarch being located close to the pericycle.
6. Protoxylem show annular or spiral thickening.
7. Metaxylem show reticulate pitting.
8. Phloem consists of sieve tube and companion cells.
9. Conjunctive tissue occurs in between and around the vascular tissue.

Pith

1. Pith occur in the center of the axis, the cell are parenchymatous sometime become thick walled and lignified.

Identification

1. Vascular bundle is radial exarch, cortex is massive and undifferentiated, hairs are Unicellular………………………………………………………………………. **Root**
2. Polyarch condition of xylem, pith is well differentiated, secondary growth is absent……………………………………………….………… **Monocotyledonous root**

9. Describe the T. S. leaf of *Zea mays*

Observations: Cut a transverse section of the material (if required) use pith. Stain insafranin and fast green combination and mount in glycerin (Fig. 12.12).

Epidermis

1. Leaf is bounded by lower and upper epidermal layers, both layers have thick cuticles.
2. Stomata are present in both epidermal layers.
3. A few large empty colorless, bulliform cells occur in upper epidermis.

Mesophyll

1. It is differentiated not differentiated into palisade and spongy parenchyma. It occurs between upper and lower epidermis.
2. The cells are isodiametric and contain numerous chloroplasts. The cells are compactly arranged and only few intercellular spaces are present.

Vascular tissue

1. There are numerous vascular bundles of variable size arranged in parallel series. Each bundle is collateral and closed. There is a distinct parenchymatous bundle sheath. The cell of the sheath possesses plastid and starch grain.
2. A patch of sclerenchyma is present above and the below larger vascular bundle and extends up to the upper and lower epidermal layer respectively.
3. Large bundle have distinct and more amount of xylem and phloem than smaller one.
4. Bundle possess xylem of the upper site to upper epidermis and phloem to lower site.

Identification

Mesophyll is not differentiated into palisade and spongyparenchyma; stomata are present on both lower and upper epidermal layers……………….... **Isobilateral leaf**

Point of interest

Most of the leaves of monocotyledonous are isobilateral. Thestomata are present on both lower and upper epidermal layers. Mesophyll in this case is undifferentiated into palisade and spongy parenchyma. Buliform cells are present in upper epidermis. There is characteristics of plants growing under dry condition. These motor cells help the leaf to role due to the change in turbidity, here by the reducing the stomatal transpiration under xeric condition. Other xerophytic character includes thick cuticle sclerenchymatous patch and more stomata on lower surface.

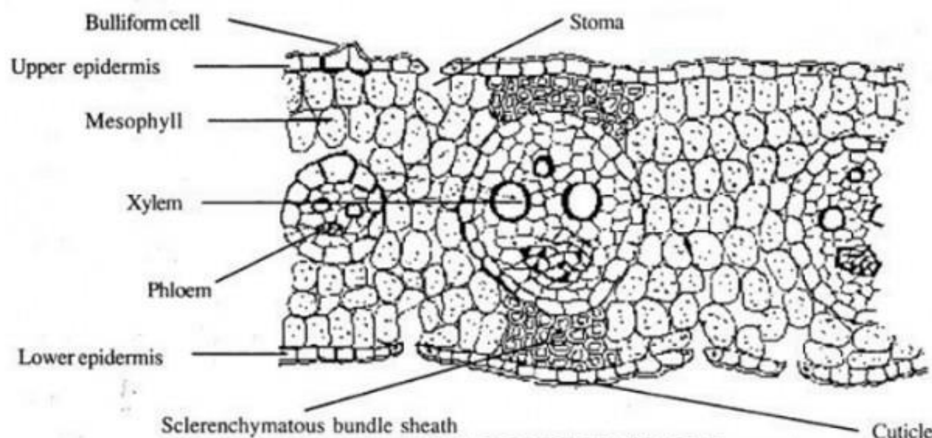


Fig. 12.12 T. S. leaf of Zea mays

12.5 ANATOMICAL INVESTIGATION OF ANOMALOUS GROWTH IN SOME IMPORTANT PLANTS

Anomalous structure: Some anomalous structures have been seen due to extraordinary activity of the cambium.

1. Anomalous structure in dicotyledons: *Boerhaavia* Stem

Observation: Cut a transverse section of the material stain in safranin and fast green combination and mount in glycerin. Outline appears almost circular in transverse section (Fig. 12.13).

Epidermis

1. It is an outermost single layer cell.
2. Outer surface has a thick cuticle layer.

Cortex

1. It is multilayered. The region is differentiated into outer collenchyma and inner parenchyma.
2. Collenchyma follows epidermis. It is 3-5 cells deep. The walls of neighboring cell are thickened.
3. Parenchyma follow the zone of collenchyma. It forms the rest of the cortex. It contains numerous chloroplasts. Intercellular spaces are present.

Endodermis and pericycle. These layers are indistinct.

Vascular tissue system

1. There are many vascular bundles which are arranged in rings. A zone of secondary tissues is also very distinct.
2. The outermost ring has many bundles. Due to secondary growth, phloem occurs in the form of crushed and obliterated patches. Abundant prosenchyma (conjunctive tissue) is present. Secondary phloem forms a complete ring.
3. Cambium that follows separates phloem and xylem. The primary xylem groups are situated close to the pith. Protoxylem is endarch and the vascular bundles are conjoint, collateral, endarch and open.
4. The innermost ring consist of two vascular bundles. Each bundle is conjoint, collateral, endarch and open. The bundle lies close to the pith, and are therefore kwon as medullary bundles. These bundles produce small amount of secondary phloem and secondary xylem in radial rows.
5. The middle ring consists of six or seven bundles. These bundles are smaller than those of the inner ring. Each bundle isconjoint, collateral, endarch and open.
6. **Pith:** In center a small parenchymatous pith is present.
7. **Identification**

8. Cortex well-differentiated; vascular bundles arranged in a ring; conjoint, collateral and open; presence of secondarygrowth .... **Dicotyledonous stem**

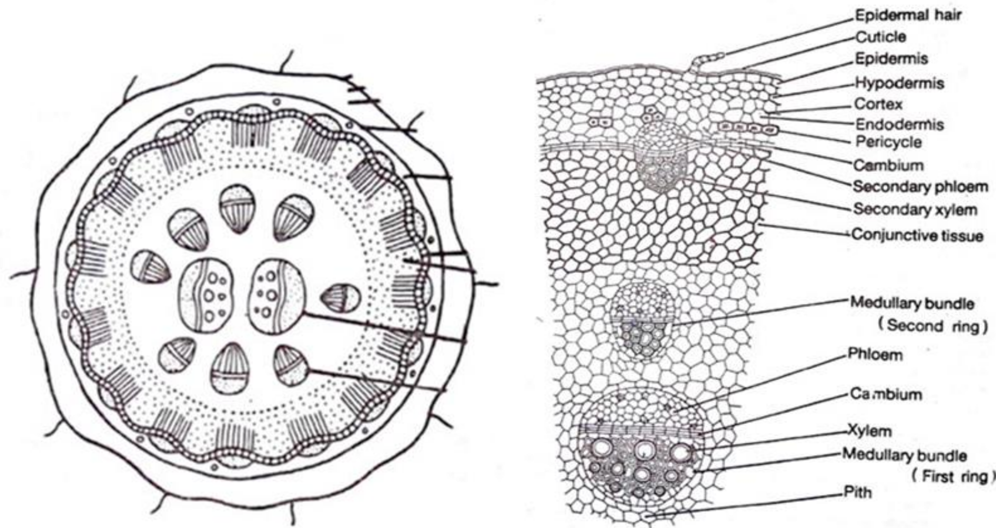


Fig. 12.13 T.S. of Boerhaaviastem showing anomalous secondary thickening

A. Diagrammatic B. Cellular

Points of Interest

1. **Medullary Bundles:** The vascular bundles are arranged in three rings. Out of these, two inner rings occur in the pith and are, therefore, known as medullary bundles. The medullary bundles possess fascicular (intrafascicular) cambium and produce a little amount of secondary tissues.
2. **Abnormal secondary growth:** In *Boerhaavia*, vascular bundles of the outermost ring have fascicular cambium. Later, intrafascicular cambium also develops, thus forming a complete ring. The cambial ring produces secondary xylem and secondary phloem. However, this cambial ring soon stops functioning. A new ring appears later in the region of pericycle. This ring of cambium, also, as in earlier cases functions only for sometimes. Such many accessory cambia are produced successively, much farther away into cortex every time. This results in the formation of successive alternate zone of secondary xylem and secondary phloem. These rings or zones are sometimes eccentrically developed. Cambia produced a very large amount of prosenchyma into which xylem remains scattered and at times becomes indistinguishable from it.

2. Anomalous structure in *Mirabilis* Stem

Observation: Cut a transverse section of the material stain in safranin and fast green combination and mount in glycerin. The transverse section is almost quadrangular in outline (Fig. 12.14).

Epidermis

1. This is the outermost single layer of rectangular cells covered with thick cuticle.
2. A few stomata and multicellular hairs are present in the epidermis.

Cortex

1. It is multilayered and differentiated into collenchyma, parenchyma.
2. Collenchymatous hypodermis is 4-5 layers deep, the cells are thickened at the corners.
3. Parenchymatous region follows hypodermis and forms a major part of cortex.The cells contain numerous chloroplasts.It is many layers deep and this region extends up to endodermis.

Endodermis

1. It separates cortex from the underlying vascular tissue.
2. The cells are parenchymatous.

Vascular tissue system

1. It forms a wide zone below the pericycle.
2. Pericycle is followed by conjunctive tissue in which secondary vascular bundles are embedded.
3. Immediately following the pericycle are small groups of secondary phloem.
4. Secondary phloem is separated from secondary xylem by 2-3 layered ring of cambium
5. Secondary xylem of secondary vascular bundle lies below the cambium. The amount of secondary xylem is much larger than the secondary phloem.
6. This secondary xylem is embedded in a zone of conjunctive tissue. The conjunctive tissue is a thick-walled parenchyma and almost indistinguishable from secondary xylem.
7. Numerous vascular bundles are scattered in the centrally located parenchymatous pith. These are primary vascular bundles, now called medullary bundles.

Pith

1. A little amount of secondary growth takes place in the medullary bundles.
2. Medullary bundles in the pith are conjoint, collateral, endarch and open.
3. The central part of the section is occupied by a large parenchymatous pith.

Identification.

Cortex is well-differentiated; vascular bundles are conjoint, collateral and endarch....**Stem**

Starch sheath is indistinguishable; vascular bundles arranged in a ring; secondary growth present...**Dicotyledonous Stem**

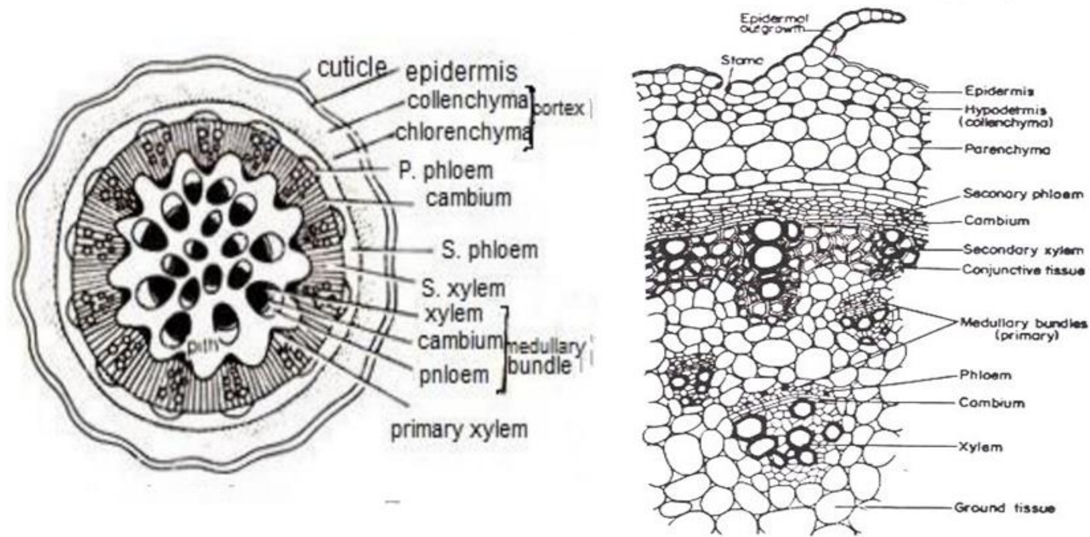


Fig. 12.14 T.S. of Mirabilis stem **A.**Diagrammatic **B.**Cellular

Points of interest: Young *Mirabilis* stem has many bundles which undergo secondary growth.Primary vascular bundles in a young stem are conjoint, collateral, endarch and open. The cambium of the bundles is active and each vascular bundle undergoes a little amount of secondary growth. The activity of this cambium stops after sometime. These vascular bundles come to lie in the pith and is called as medullary bundles.

Secondary growth starts with the formation of secondary cambium originating in the parenchyma closer to the pericycle. This cambium cuts off secondary xylem on its inner side. It remains embedded in thick-walled conjunctive tissues. A very small number of secondary elements are formed by the cambium on its outer side.

3. Anomalous structure in *Nyctanthes* stem

Observation: Cut a transverse section of the material, stain in safranin and fast green combination and mount in glycerine. The outline is almost circular with four angles protruded as bulges (Fig. 12.15).

Epidermis: This is the single outermost layer of cells with thick cuticle and multicellular hairs.

Cortex

1. It is few layered thick and is differentiated into outer region of collenchyma and inner parenchyma.
2. Collenchyma is several layers deep in the ridges and a few cells deep in the furrows.
3. Parenchyma forms rest of the cortex. The cells are thin and many intercellular spaces are present.
4. Cortex has four vascular bundles, one in each of the four bulges at four angles. Each one is conjoint, collateral, exarch and open. The protoxylem of the bundles is directed

towards the epidermis, hence the bundles are called inverted. The xylem of the bundles is situated closer to the epidermis and phloem away from it. Both these elements are separated by a cambium. The cambium is active and produces a little number of secondary tissues, these are known as cortical vascular bundles.

Endodermis: A distinct endodermis with casparian strips is absent.

Pericycle: It is present just below the cortical region and separates cortical region from the vascular tissues. It forms almost a complete ring of parenchymatous cells.

Vascular tissue system.

1. The structure shows secondary growth.
2. The tissues occur in the following sequence-primary phloem, secondary phloem cambium, secondary xylem and primary xylem.
3. The primary phloem occurs in small patches just below the pericycle.
4. Secondary phloem is in the form of a complete ring. It consists of phloem parenchyma, sieve tubes and companion cells.
5. The cambium is single-layered and is present between the secondary phloem and secondary xylem.
6. Secondary xylem occupies most off the region towards the centre of the axis.It consists of tracheid's, vessels and xylem parenchyma. A few vessels are very distinct. Annual rings are not very distinct.
7. Primary xylem lies close to the pith. The protoxylem is endarch. Primary vascular bundles are conjoint, collateral, endarch and open.

Pith: A large parenchymatous pith is present in the center.

Identification.

1. Vascular bundles are conjoint, collateral, endarch...**Stem**
2. Cortex well-differentiated; pericycle indistinguishable; vascular bundles arranged in a ring; secondary growth present; pith well- developed...................**Dicotyledonous stem**

Point of Interest: The stem has four cortical vascular bundles in the cortex, one each in four corners of the angular stem. These bundles are also called as leaf trace bundles becauseit arises at much lower a node. During the course of their upward passage, the bundles become inversely oriented. Thus, the phloem of the bundles is directed away from the epidermis while the endarch protoxylem is closer to the epidermis.

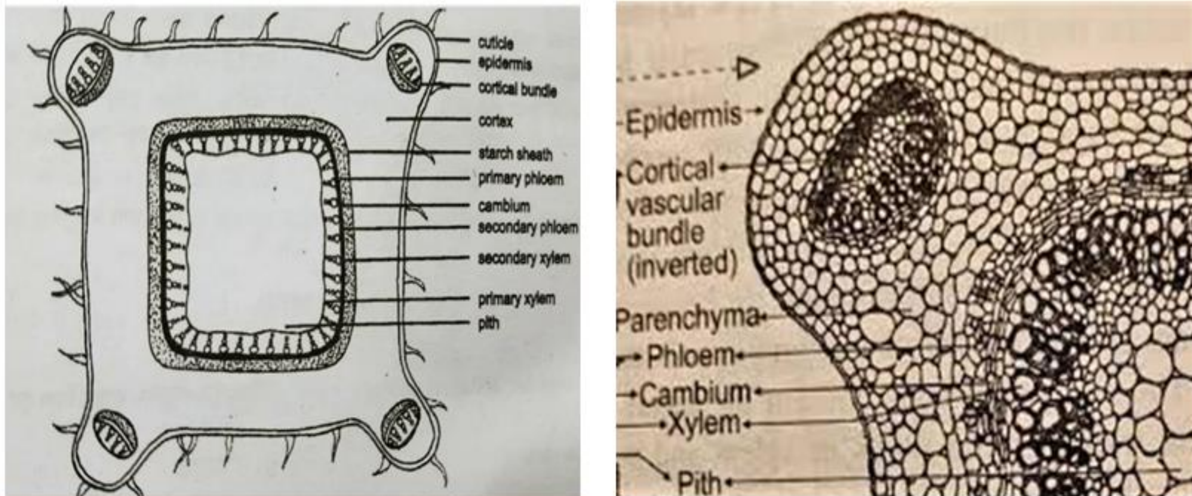


Fig. 12.15 T.S. of Nyctanthes stem A. Diagrammatic B. Cellular

4. Anomalous Secondary growth in *Dracaena* stem (monocot stem)

Observation: Cut a transverse section of the material, stain in safranin and fast green combination and mount in glycine. The outline of the transverse section is almost circular (Fig. 12.16).

Periderm

1. It is composed of cork (phellem) and secondary cortex (phelloderm).
2. A few lenticels also occur in the phellem.
3. The cells of the phellem are rectangular, thickly suberized and dead.
4. The cork cambium is one or two layers thick. The cells are thin walled and tangentially elongated.

Cortex: It is undifferentiatedparenchymatouscells filled with starch. Many intercellular spaces are present.

Meristematic tissue: It follows parenchyma and forms several layered deep zones. Cells are similar to cambium and almost rectangular, generally radially arranged.

Vascular system

1. Numerous vascular bundles lie scattered in the ground tissue.
2. The primary vascular bundles are present near the center of the axis. These are large in size and typically collateral closed.
3. Secondary vascular bundles are present near the periphery. These are small in size and remain embedded in the thin-walled tissue (sometimes thick walled due to lignification). Each vascular bundle is concentric (amphivasal) where phloem is surrounded by xylem.

4. Secondary phloem consists of short sieve tube elements.
5. Secondary xylem is composed of tracheids and xylem parenchyma.

Ground tissue: This tissue extends from the inner side in the meristematic zone and fill up the major part of the axis. Cells are parenchymatous, intercellular spaces are present.

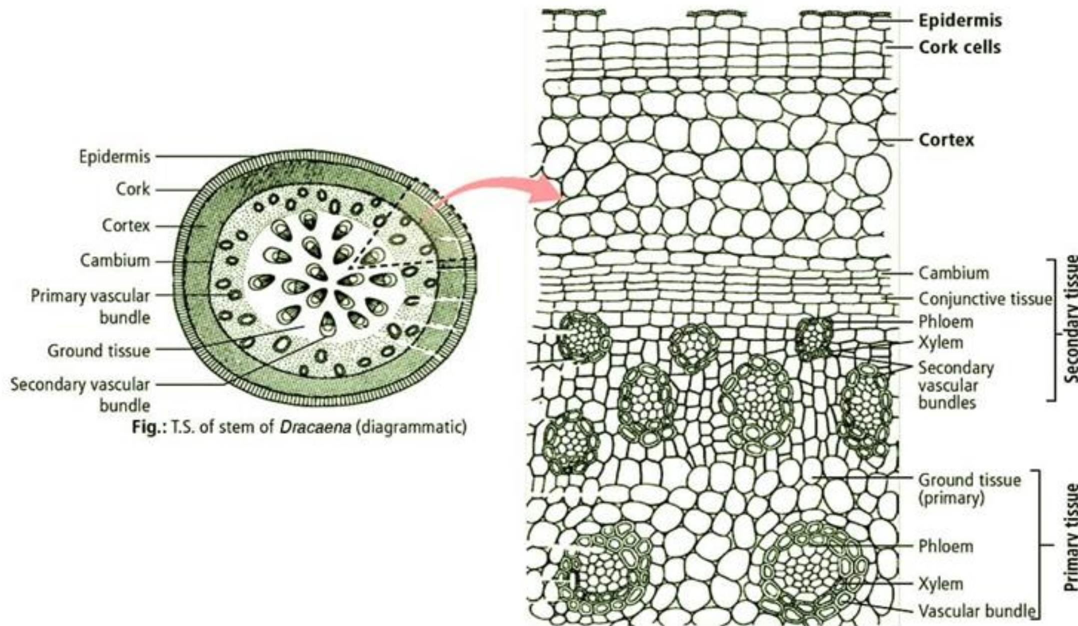


Fig.12.16 T.S. of Dracaena stem A. diagrammatic B. Cellular

Identification:

Vascular bundles are conjoint and collateral; ground tissue is present .... **Stem**

Endodermis and pericycle are absent, vascular bundles are scattered; cambium absent; pith is not well defined .... **Monocotyledonous stem**

Point of interest: At a very late stage during the development, a wide zone of secondary meristem (Cambium) develops outside the vascular bundles in the parenchymatous region. This meristematic tissue cuts off vascular bundles on its inner side. These are concentric (amphivasal) in contrast to the primary bundles which are collateral.

The cambium (meristematic zone) originates near the leaf primordia. The life of this zone or layer is limited. It stops functioning after sometime and the adjacent cells take over. Another important feature in contrast to cambium of dicotyledonous is that cambium in Dracaena cuts off both xylem and phloem on its inner side while on its outer face very little amount of parenchyma is produced.

12.6 MICRO-CHEMICAL TESTS FOR STORED FOOD MATERIALS

Following are some test of the major food material:

1. Cellulose

Objective: To detect the presence of cellulose.

Requirements: Microscope, slides, cover glasses, iodine solution, sulphuric acid 75%, water, paper/ cotton fibers, etc.

Procedure

1. Tear the paper or cotton in a way so that fibres are exposed.
2. Place the fibres in a drop of water on a slide.
3. Add a few drops of iodine and allow the fibers to take stain.
4. The fibres turn brown.
5. Add a drop of 75% sulphuric acids and then wash with water.
6. The colour of the fibres changes.

Results: The fibres turn blue.

Conclusion: The changes into blue indicate the presence of cellulose in the wall thickenings.This is because cellulose dissolves in cold concentrated sulphuric acid and is precipitated as amyloid on dilution.

2. Cutin

Objective: To detect the presence of cutin.

Requirement: Microscope, slides, cover glasses, razor/blade, watch glasses, water, potassium hydroxide, *Ficus*/ *Nerium* leaf etc.

Procedure

1. Cut the section of the leaf and place in water.
2. Treat the sectionwith KOH solution.
3. Observe the colour of outer of the outermost deposit on epidermis.

Results and conclusion: The yellow colour of the deposition on epidermis indicates that it is composed of cutin (a fat like substance).

3. Suberin

Objective: To detect the presence of suberin.

Requirements: Bottle cork/natural cork, Sudan IV (alcoholic), alcohol 50%, slides, cover glasses, water, glycerine, etc.

Procedure

1. Cut the thin slice of the material.
2. Leave the fresh section in Sudan IV to take stain for about 20 minutes.
3. Wash the excess of stain with 50% alcohol.
4. Transfer the section to water and mount in glycerine.
5. Observe the colour under the microscope.

Result and conclusion: The suberized portion become red stained indicating the presence of suberin in the wall.

4. Lignin

Objective: To detect the presence of lignin.

Requirements: Match shavings/ match sticks/ wood shavings, phloroglucin (1% alcoholic), hydrochloric acid (25%), 1% neutral aqueous potassium permanganate, ammonium hydroxide (sodium bicarbonate), slides, cover glasses, water, etc.

Procedure

Follow any of the two methods given below.

1. Method A: Prepare thin slices of the material and place them in 1% alcoholic phloroglucin. Cover the section with coverglass. Allow 25% hydrochloric acid to diffuse along the edges of coverglass.

2. Method B: Treat the section with 1% aq. neutral potassium permanganate for about 15-20 minutes. Wash with 2% hydrochloric acid followed by repeated washing with water. Add a few drops of either ammonium hydroxide or sodium bicarbonate.

Results and conclusion

1. In the first case red violet colour is taken by lignified walls.

2. In the second method, deep red colour develops in the lignified element of deciduous plants.

5. Mucilage

Objective: To detect the presence of mucilage.

Requirements: Linseed testa, copper sulphate (10%), potassium hydroxide (10%), water, slides, cover glasses, glycerine, etc.

Procedure

1. Cut thin section of linseed testa.
2. Soak the section in 10% copper sulphate solution for 20 minutes.
3. Wash the section in water and transfer to 10% potassium hydroxide.
4. Mount the section in glycerine and observe the colour.

Results and conclusion: The cells with mucilage are stained bright blue indicating that the material possesses mucilage.

6. Latex

Objective: To detect the presence of latex.

Requirements: Latex from Calotropis/ members of Euphorbiaceae/Apocyanaceae, sucrose, alcohol (concentrated), sulphuric acid, test tubes, test tube holder, water, etc.

Procedure

1. Prepare an alcoholic extract of latex.
2. Add an equal amount concentrated,sulphuric acid and sucrose to the latex extract.

Results and conclusion: The colour turns pinkish- purple indicating the presence of latex.

7. Hemicellulose

Purpose: To test for the presence of hemicellulose.

Requirements: Soya bean seeds, iodine, water, slides, cover glasses, glycerine, etc.

Procedure

1. Cut a thin section of the seed.
2. Observe the section under microscope.
3. Treat the section with iodine for a few minutes.
4. Observe the colour of the section.

Results and conclusion: The colour turns blue indicating the presence of hemicelluloses.

8. Glucose (Reducing Sugar)

Objective: To test for the reducing sugar; glucose (grape-sugar), etc.

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

Procedure

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

(A) Fehling's test

1. Take about 5ml of Fehling solution in a test tube.
2. Add few drops of glucose solution and boil.

(B) Benedict's test

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

2. Add a few drops glucose solution and boil.

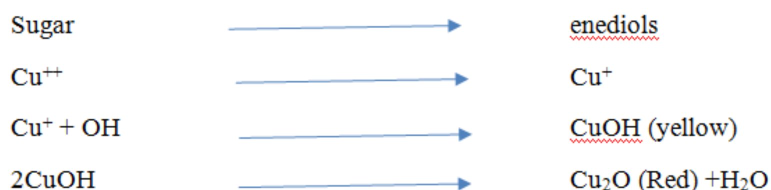
Results

A. The Fehling's solution gives brownish red precipitate.

B. Benedict's test give red yellow or green precipitate.

Conclusion: The tests reveal the presence of glucose.

Sugar when treated with alkali undergoes enolization to produce enediols. This being highly reactive reducing agents, are capable of reducing oxidizing Cu^{+++} ions. Both Fehling's and Benedict's solution contain soluble Cu^{++} ions in soluble form as complexes with citrate or tartrate. On coming in contact with enediols Cu^{++}(cupric) ions are reduced to Cu^{+} (cuprous) ions which later combine to precipitate yellow cuprous hydroxide. Yellow precipitate of cuprous hydroxide on heating gets converted to reddish cuprous oxide.



Overall reaction:

9. Sucrose (Non- reducing sugars)

Objective: To test for starch/sucrose (non- reducing sugars).

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

Procedure

1. Add to sugar equal volume of concentrated hydrochloric acid.
2. Boil the mixture for about five minutes.
3. Neutralize the resulting solution with sodium carbonate or bicarbonate.
4. Then subject the solution to the test of reducing sugar by adding Fehling's or/ and Benedict's solutions

Results

1. Fehling's solution gives brownish red precipitate.
2. Benedict's solution gives yellow red or green precipitate.

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

$$C_6H_{12}O_6 + C_6H_{12}O_6 \longrightarrow C_{12}H_{22}O_{11} + H_2O$$

(Glucose) (Fructose) (Sucrose)

10. Proteins

Purpose: To test the presence of protein.

Requirements: Gram flour/legumes/ soya

There are two methods for testing the presence of proteins.

(a) Xanthoproteic test

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

(b) Biuret test

1. Prepare a suspension of material in water.
2. Add 1 ml of sodium hydroxide (20%) and a drop of copper sulphate to the suspension.
3. Observe the developing colour. Add Millons reagent (a or b). Mix thoroughly and boil.
4. Note the change in colour in both cases – Millons reagent 'a' and / or 'b'.

Results

(a) Xanthoproteic test: The yellow colour reagent change in to orange.

(b) Biurete test: The colour developed is violet. On addition of Millons reagent 'b' it turns reddish on heating.

Conclusion: The colour changes indicate the presence of protein.

Biurete develops violet colour when treated with dilute $CuSO_4$ solution. This reaction is also shown by compounds containing- $CONH_2$ groups joined directly or by C or N atoms. Proteins also this reaction because they posses CO-NH-C-CO-NH- peptide bond architecture.

In Xanthoproteic reaction nitration of phenyl rings occurs to yield yellow substitution products which turn orange upon the addition of alkali (salt formation).

11. Fat or oils

Objective: To test for the presence of fat or oils.

Requirements: Seed of almonds/soya bean/ groundnut, Sudan III (alcoholic), water, osmic acid 1%, test tube, test tube holders, glycerine, microscope, slides, coverglasses, etc.

Procedure

Any of the two following methods could be followed.

1. Method I: Cut thin section of the material. Place the section in Sudan III for about 10 minutes. Wash the section with 50% alcohol. Mount in glycerine after repeated washes with water. Observe the colour under microscope.

2. Method II: Add a few drops of osmic acid to the material in the test tube. Observe the developing colour.

Results

1. The oil drops take red stain.
2. Black colour is developed.

12. Starch

Objective: To test the presence of starch.

Requirements: Starch, test tube, test tube holder, spirit lamp, iodine solution, HCl, Benedicts solution, NaOH (or sodium carbonate) etc.

Procedure

1. Follow any one of the two methods given below.

1. Method I: Take a pinch of starch and add water.Add a few drops of iodine.

2. Method II: Take a pinch of starch and add water.Boil it by adding HCl. Make this solution alkaline by adding NaOH or sodium carbonate. Test with Benedicts solution.

Results

1. Black colour appears.

2. Red precipitate is formed.

Conclusions: The test indicates the presence of starch

12.7 SUMMARY

- The plant morphology or phytomorphology is the study of the physical form and external structure of plants. Morphology is the backbone of plant identification and classification since ancient time. Morphology plays an important role in identifying plants it also provides us with range of variations found in different species of plants. Morphology

helps us to understand different aspects of plant life-like ecological adaptation and genetically appearance of traits.

- Anatomy is particularly useful in plant taxonomy identifying disassociated plants parts whether those parts be leaves, roots, stems, fruits or seeds of living or fossil plants.
- Microchemical analysis is the study of how tiny particles interact with other chemicals. A sample will react with different other substances in a same manner whether it is macroscopic or tiny.
- To assure product integrity, quality, and safe consumption, foods and beverages are chemically analysed. Chemical analysis is necessary to provide accurate food labelling and to protect customers from adulteration, food misbranding, and dangerous beverages.

UNIT-13: STUDY OF FORAGE PLANTS AND PLANT FIBRES

13.1 Objectives

13.2 Introduction

13.3 Study of Forage Plants

13.3.1 Grasses

13.3.2 Cereal and Forages crops

13.3.3 Legumes as Forages

13.3.4 Non Legumes Trees as Forages

13.4 Plant Fibres

13.4.1 Textile Fibre

13.4.2 Hard Fibre

13.4.3 Brush Fibre

13.4.4 Rough Weaving Fibre

13.4.5 Filling Fibres

13.5 Summary

13.6 Glossary

13.7 Self Assessment Question

13.8 References

13.9 Suggested Readings

13.10 Terminal Questions

13.1 OBJECTIVES

After reading this unit, student will be able to understand about the characteristics and utilization of fibre and fodder plant in specific reference to-

- General account on fibre and forage plants.
- Different types of forage plants and their uses
- Botanical characteristics and uses of forage plants.
- Different types of fibres, their sourcesand properties of natural fibres.
- Botanical characteristics and uses of fibre plants.

13.2 INTRODUCTION

The major parts of human basic needs are fulfilled by plant and plant products. Clothing and shelter are the other prime necessities of life. The fibre yielding plants hold the second position after the food plants in their economic importance. The agriculture and animal husbandry are interwoven as intricate part of the society in cultural, religious and economical ways, and daily life is not possible without using plant products. The mixed farming *i.e., to fulfill basic needs for food, fibre and forage*, and livestock rearing i.e., *for milk, dairy products, meat production, rural transport, manure, fuel etc.*forms an integral part of rural life.Some of the important and commonly used forage and fibre plants are discussed in this unit.

13.3 FORAGE PLANTS

The term Forage includes herbaceous plants and plant parts eaten by grazing livestock and wildlife animals, however, it also used to woody plants in practice. It is extended to forage crops, crop residue or immature plant residue and plants cuts for fodder or conserved as hay or silage. It also includes grasses, herbs, shrubs and tree plant species either grow under cultivation or in wild state. Forages may be annuals or perennials and are produced in pastures *i.e.*, controlled grassland used as feed for grazing animals or in protected fields *i.e.,* forage cut and carry to animals.The most important forage plants are grasses family *i.e.,* members of Graminae, includes about 620 genera and more than 10000 species.Forage crops are important because they can grow rapidly and often can be produced continuously, and the crop residue i.e. straw, stover, chaff, husks etc. constitute over half the harvestable biomass of widely grown food crops and cereals, serve as principal sources of feed for a number of different kind of domestic animal.Furthermore, grasses are useful in preventing erosion, and legumes can increase the nitrogen availability in the soil for other crops.

Salient features of forage

Forage is the edible parts of plants can provided feed or harvested for feeding to grazing animals. It includes browse, herbage and forage crops. Browse is the leaves and twig growth of shrubs, trees and other non-herbaceous vegetation available for animal consumption.Whereas,

herbageincludes the herbaceous plants aboveground biomass also including edible roots and tubers. Forage crops refers to the cultivated plants, other than separated grains produced to be grazed or harvested or agricultural byproducts, used for animal feed.

A range of forage crops are available and the right crop will depend on (feed quality and quantity requirements) soil type, climate, water availability, drainage, weeds and diseases. The important characteristics or criteria of good forage are as follows-

- Wider adaptability with capacity to grow under stress condition
- Good forage should grow well and survive with a minimum of care, and should also resist in the dry season of the year with continuing to grow or maintaining foliage and nutritive value.
- Good forage should be palatable to the animals.
- Short growth period with high seed rate.
- High persistency and regeneration.
- Crop cultivation should be used for subsequent or multi cuts.
- Improve soil health through addition of higher amount of organic residue in the soil.
- Forage crops can also play an important role in maintaining ground cover, preventing erosion, accumulation of nitrogen in soil and improving land condition.

Indian subcontinent is mega center of biodiversity. India has abroad spectrum of eco-climate ranging from humid temperate to alpine and tropical to semi-arid, which possess a wide range of genetic diversity in forage resource *i.e.,* in form of grasses, legumes, trees, crop plants etc.The major sources of forage are forests and pasture grassland, crop residue and cultivated fodder. In this unit, we discuss the common plants specifically used as forager plants.

13.3.1 Grasses

Grasses, as forage serve as important resource i.e., natural feed of the herbivorous or grazing animals, and this form provides most of the feed of ruminantsthroughout the year. They make up the bulk of the plants found in many mixtures of natural vegetation used as forage. About 75% forage consumed in tropics is grasses and includes about 620 genera and 10000 species. Nearly 350 or more grasses are cultivated as forage, while a relatively small number of grasses are considered as principal forage species.Tropical grasses are propagated by seeds, rhizomes (modified underground) or stolons (prostrate aerial stems).

The grasses are considered suitable as forage plants for grazing and soil and water conservation, due to-

1- Grasses have wider range adaptability to the climate, topographic and edaphic factors.

2-Grasses provide sustainability by readily spread through rapid growth and ground coverage, which provide soil and water conservation.

3- Pasture grasses possess the quality of higher productivity, palatability, high nutritive value.

Some of the important grasses and herb plant grow in forests or uncultivated land, and commonly used by villagers of hill region of Uttarakhand are listed in Table-1.

Table 13.1 List of important grasses and herbs plants used as forage.

S.No.	Scientific Name	Famiy	Native Name
A.	**Fodder Grasses**		
1.	*Bothriochloa pertusa*	Poaceae	Jargi
2.	*Chrysopogon gryllus*	Poaceae	Lamguchii
3.	*Chrysopogon fulvus*	Poaceae	Godia
4.	*Heteropogon cotortus*	Poaceae	Kumaria
5.	*Imperata cylindrical*	Poaceae	Siru
6.	*Pennisetum orientale*	Poaceae	Bhimalsa
7.	*Saccharum spontaneum*	Poaceae	Kans
8.	*Saccharum bengalense*	Poaceae	Manju
9.	*Vetiveria zizanioides*	Poaceae	Khas
B.	**Fodder Herbs**		
1.	*Asparagus curillus*	Liliaceae	Jhirna
2.	*Achyranthus aspera*	Amaranthaceae	Apamar
3.	*Cassia occidentalis*	Caselpiniaceae	Banar
4.	*Caucalis anthriscus*	Umbelliferae	Kokali
5.	*Crotalaria prostrate*	Papilionaceae	Khadkhadi
6.	*Desmodium gyrans*	Papilionaceae	Chamalii
7.	*Desmodium polycarpum*	Papilionaceae	Boner
8.	*Excaecaria acerifolia*	Euphorbiaceae	Dudila
9.	*Indigofera cylindracea*	Papilionaceae	Sakina
10.	*Phryma leptostachya*	Vervenaceae	Phirma
11.	*Pueraria tuberosa*	Papilionaceae	Sirala
12.	*Wikstroemia canescens*	Thymelaceae	Chamliya

The natural grasses supplies from various forests, natural grazing lands, pastures, alpine grassland orchards etc. are potential sources of green and dry fodder. In Uttarakhand, high productive hybrid grasses i.e., Napier grasses, setaria, anjan, guinea, broom etc. are growing successfully and some of the important grasses are discussed here.

13.3.1.1 Guinea Grass

Botanical name	:	*Megathyrsus maximum* Jacq.; Syn. *Panicum maximum*
Family	:	Poaceae
Eng. Name	:	Guinea grass
Hindi name	:	Ginii Ghas

Botanical characteristics

Guinea grass is a large perennial, fast growing and highly palatable leafy grass. Guinea grass has a broad morphological and agronomic variability, and suitable for all types of soil with well drainage. The plant of Guinea grass grow as erect clums which attain height from 0.5-4.0 m and 5-10 mm in diameter, having short creeping rhizome. Leaves are blade-shaped, globous to pubescent upto 35 mm broad with panicle inflorescence.

Uses

1. Guinea grass is suitable for pasture, silage and hay.
2. It's a highly palatable to livestock with a good nutritional value (crude protein range from 4-14%).

A **B** **C**

Fig. 13.1 Grasses as forage (A) Guinea Grass; (B) Napier Grass; (C) Tiger Grass

13.3.1.2 Napier Grass

Botanical name	:	*Pennisetum purpurem* Schumach
Family	:	Poaceae
Eng. Name	:	Napier grass
Hindi name	:	Elephant grass ; Hathi Ghas

Botanical characteristics

Napier grass is commonly known as Elephant grass and is popular fodder crop. Napier grass is perennial, fast growing and deep rooted grass. Napier grass forms dense thick clumps, upto 1 m in diameter. It can attain height upto 4 m, forming large clumps which branched above.Napier grass can spread by underground stems to form thick ground cover. It has a vigourous root system which developed from the nodes of creeping stolons. The leaves are flat, linear, upto 100-130 cm in length and 1-5 cm wider with hairy base. The inflorescence is 10-15 cm long stiff terminal bristly spike. Napier grass is tolerant to drought and flood. Napier grass can grow in almost all soil but prefer deep, fertile, well drained soils.

Uses

1-Napier grass is a high productivity forage with palatability, and used after cutting and fed in stalks. It is not suitable for direct grazing.

2-It is also used to make silage or hay.

3-Napier grass can be harvested multiple times in a year.

4-Napier grass can grow along with maize or sorghum as mixed fodder crop.

5-Napier grass is planted in marginal land and slopes, which reduces soil erosion and increase soil fertility.

13.3.1.3 Tiger Grass

Botanical name	:	*Thysanolaena latifolia* (Roxb. ex Hornem.) Honda;
		Syn. *T. maxima* (Roxb.)Kuntze
Family	:	Poaceae
Eng. Name	:	Tiger grass; Broom grass
Hindi name	:	Ghas

Botanical characteristics

Tiger grass is an evergreen, large, perennial, fast growing and large clumpsforming grass, and popularly known as Broom grass. Broom grass grows in clumps and deep roots. Broom grass grows a of height upto 2.5 m. The leaves of Broom grass are linear, narrow, large, heart shaped,

taper to a fine point and alternatively arranged which vary 20-30 cm in length and 5-7 cm wide in size. The inflorescence is panicle, in which flowers are arranged in minute spikelet, in large branched clusters.The panicle are has an average length of 50cm and preferred for soft-broom making. Broom grass distributed upto an altitude of 2000m and studies revels that it can thrive at low to medium elevation but can grow faster in higher elevation. Broom grasscan be grow in many types of soil but prefer moist and well-drained soil.

Uses

1- Broom grass provides green forage to animalscan be harvested multiple times in a year.

2- Broom grass has potential to generate income from the harvesting panicles after flowering.

3- Broom grass is planted in marginal land and slopes which promote soil conservation.

13.3.2 Cereal and Forage Crops

The coarse cereals play a major role in animal feed supply and four major cereals viz. maize, barley, sorghum and pearl millet accounts 44% of total cereals. Some of the cultivated cerealscropsand forage used for commercial cereal production as well as supply as alternate fodder to livestock, are described below.

13.3.2.1 Maize

Botanical name	:	*Zea mays* L.
Family	:	Poaceae
Eng. Name	:	Maize, Corn
Hindi name	:	Makki, Makka

Botanical characteristics

Maize is annual, herbaceous, fast growing plant with leafy stalk, attaining a height upto 3 m. The stem is unbranched and differentiated into nodes and internodes. Leaves are long and grow from node and leaf size upto 4 feet in length and 4 inch in width. Maize, particularly stalks, leaves and ears, is used as green forage and is an energy rich feed for ruminant livestock. Maize forage mature within three months and harvested only once. The maize plant is monoecious, and the male inflorescence (tassel) found at the apex of stem while the female inflorescence commonly called husk is tightly enveloped by several layers of ear leaves. Maize plant cultivation dependent on soil moisture because it is cold intolerant and most sensitive to drought and prefer well drained, fertile, heavy loamy soil and humus rich soil.

Uses

1-Maize is used for green forage and cattle feed.

2-Maize is also used as grain crop and dried kernels are used as feed.

3- Maize used for silage, is harvested while the plant is green and the fruit is immature.

4-The whole Maize plant after ear harvesting, dried and store as shocks or stooks (an arrangement of sheaves of cut grain-stalk keep the grain heads-off the ground for drying purpose before threshing), used for weeks to several months.

13.3.2.2. Bajra

Botanical name	:	*Pennisetum glaucum* (Burm.) Stapf. And Hubbard
Family	:	Poaceae
Eng. Name	:	Pearl Millet
Hindi Name	:	Bajra

Botanical characteristics

Bajra is cultivated for grains as well as fodder in India and traditionally is an indispensable component of dry farming system. Pearl Millet is an annual herb and mostly grown in kharif season from June to October. The Pearl Millet has remarkable ability to withstand and grow in harsh environment, and is mainly confined to low fertile and water deficit soils. Pearl Millet is an erect annual grass, reaching upto 3 m high, profuse root system, culms are slender. The leaves of the plant are alternate, simple, blade linear, pubescent and minutely serrated, upto 1.5 m long and 8 cm wide.

Uses

1-Pearl Millet is considered as a staple food. The straw of pearl millet plant i.e., remains after grain harvesting, is a low nutrient value, fibrous byproduct and used for forage purpose.

2- Bajra is known to tolerate acid sandy soils and is able to grow on saline soils.

13.3.2.3. Sorghum

Botanical name	:	*Sorghum vulgare* (L.)Moench
Family	:	Poaceae
Eng. Name	:	Sorghum
Hindi Name	:	Jowar

Botanical characteristics

Sorghum is an annual plant and very popular as green forage in most parts of north India and planted during kharif. While in summer, multicut sorghum is very popular under irrigated conditions. The stem of Sorghum plant is tillers. Roots are fibrous, branched and not penetrate deeply, inflorescence panicle, spikelets occurs in pairs i.e., sessile and pedicellate.

Uses:

1- Sorghum is worldwide crop and used for food, fodder, biofuel, alcohol beverages etc. Jowar grain’s used as one of the foodstuff for poor and rural people.

2- The palatable stem and leaves of sorghum are used as cattle fodder.

13.3.2.4. Lablab

Botanical name	:	*Lalalb pupureus* L.; Syn.-*Dolichos lalalb*L
Family	:	Fabaceae
Eng. Name	:	Hyacinth bean, Kidney Bean
Hindi Name	:	Lobhia, Sem

Botanical characteristics

Hyacinth bean is an edible legume or pulse used as pulse, as well as a forage crop. The Hyacinth bean is either annual or perennial, drought resistant legume, growing vegetatively as long and vigorous vines, usually twining to reach 2-10 m but bushy, semi erect or prostrate forms exists.Hyacinth bean grow best in tropics and subtropics, and growwell in variety of drained soil i.e. sand to clay.

Uses

1. The Hyacinth bean usually used in pastures, often with grasses.
2. The leaf is very palatable but the stem is not palatable.
3. Hyacinth bean leaves are less often used as hay or silage.

13.3.2.5 Finger Millet

Botanical name	:	*Eleusine coracana*(L.) Gaertn.
Family	:	Poaceae
Eng. Name	:	Finger Millet
Hindi Name	:	Ragi, Madua

Botanical characteristics

Finger Millet is an annual herbaceous cereal grass plant and has both cultivated as well as wild form (*Eleusine coracana* subsp. *africana*). The stems and leaves (2-3 feet long and 2 cm broad) of Finger Millet are usually green, with a panicle inflorescence having 4-20 finger like spikes. Finger Millet can be cultivated in rain fed area under adverse climatic and soil conditions. Finger Millet prefers to silt loams soil and grow well in all well drained soil.

Uses

1- Finger Millet is cultivated as grain and fodder grass for livestock.

2- Finger Millet straw after grain harvesting can be directly grazed or feeded by the animals.

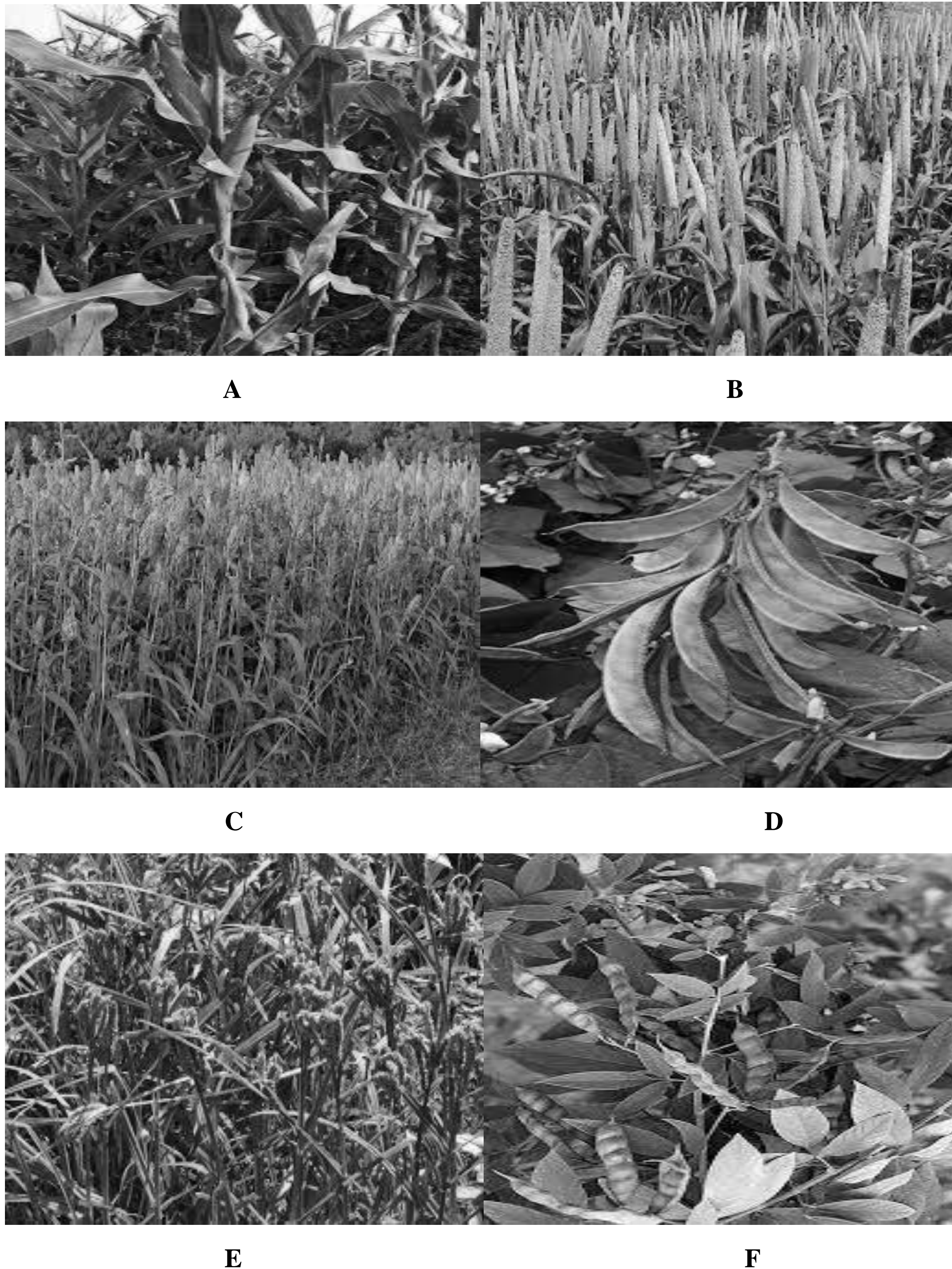


Fig.13.2 Cereal and Forage Crops: (A) Maize; (B) Bajra; (C) Sorghum; (D) Lablab; (E) Finger Millet and (F) Pigeon pea

13.3.2.6. Pigeon pea

Botanical name : *Cajanus cajan* (L) Mill

Family : Fabaceae

Eng. Name : Pigeon pea

Hindi name : Toor Dal

Botanical characteristics

Pigeon pea is a perennial, erect and highly branched shruband grown for its edible pods and seeds. The pigeon pea attains a height upto 4 m and stem is slenderwith a woody base and deep tap root. The leaves are compound, trifoliate (central leaflet longer than lateral leaves) with small stipules and hairy lamina. The inflorescence is an axillary raceme often forming a terminal panicle with distinct papallionaceous flower. The fruit of pigeon pea is a pod varies in length. The seeds are round or oval shaped with white or molten brown seed coat and yellow coloured cotyledons. The pigeon pea is tolerant to drought conditions and grows best in hot humid climate. It can grow in a wide range of soils but prefer well drained soil.

Uses: The pigeon pea plant grows vegetatively during most of the year and serves as good forage crop for the dry season when forage is scarce. The pigeon pea plant's leaves, pod and residues are used feed for livestock, and the nutritional value is high which will result in good animal weight gains.

13.3.3 Legumes as forages

Legume forages are dwarf type with dense foliage and can be grow in pure stand or mixed. Legumeshas advantages over other forages because legumes are rich in nutritious crude protein with an average of 12-16% of dry weight, and also enrich soil fertility by fixing nitrogen in presence of appropriate bacterium i.e., *Rhizobium*. Legumes have relatively high nitrogen content in the vegetative matter. Legume forage with grasses are used either as hay, silage or as pasture, on the other hand pure stand legume forages generally used as hay. With reference to Uttarakhand, some legume fodders such as white clover, red clover, perennial lucerne, centro, desmodium etc. are successfully grow in the forest.

Some of the important forage legumes are described below-

(a) Herbaceous legumes

13.3.3.1 White Clover

Botanical name : *Trifolium repens* L.

Family : Fabaceae (Leguminoseae)

Hindi name : Garila

Eng. Name : White Clover, Dutch Clover

Botanical characteristics

White clover is herbaceous, perennial broad leaved legume plant with prostrate growth and spread by stolons, roots found at nodes. The leaves are palmately trifoliate, borne on long petiole and with oblong to wedge shaped serrated margins leaflets. The inflorescence is a spherical head borne on long penduncles with 20-100 white to pinkish flowers. It grows in humid region and irrigated area, and prefer to clay soil. White clover is less tolerate to saline soil, but sensitive to shade, drought and frost.

Uses

1- White Clover is used as excellent feed for livestock.

2- White Clover produces high quality pasture, hay and silage, when planted with a grass.

3- White clover varieties favored and tolerate to moving and close grazing because of their stoloniferous habit.

13.3.3.2. Alfalfa

Botanical name : *Medicago sativa* L.

Family : Fabaceae (Leguminoseae) also placed in Papilonaceae

Hindi name : Lusan Ghas, Lucerne

Eng. Name : Varigated Lucerne, Alfalfa

Botanical characteristics

Alfalfa is a drought resistant, herbaceous, perennial flowering plant i.e., live upto 20 years with erect, woody base, 30-120 cm long stem and a deep tap root system.. Leaves of alfalfa are trifoliate; stipulate trifoliate, 5-15 cm long; petiole pubescent, 5-30 mm long; inflorescences dense racemes with 10-35 flower. It grows well in well-drained, sandy loam soil.

Uses

1. The alfalfa plant resembles to clover plant, and widely used for grazing, hay, silage, green manure and cover crop.
2. Lucerne is highly palatable and among one of the first forage crop to be domesticated.
3. Alfalfa is used as multi-purpose forage, being able used for both grazing and conservation (hay, silage). It can be shown as a pure stand or in mixed with other forages or grasses.

Fig.13.3 Herbaceous legumes as forages (A) White Clover; (B) Alfalfa and (C) Barseem

13.3.3.3. Barseem

Botanical name	:	*Trifolium alexandrium* L.
Family	:	Fabaceae
Hindi name	:	Barseem
Eng. Name	:	Egyptian Clover

Botanical characteristics

Barseem is a prominent legume and fastest spreading fodder species in recent time. Barseem is an annual, sparsely hairy, erect forage legume, 30-80 cm in height. Barseeem is a fast growing, high quality forage that is cut and fed as green chopped forage. Barseem makes most digestible and palatable green fodder to the cattle, especially the milch animals. It provide green forage with high tonnage over a long period i.e., from November to may in 5-6 cuts. Barseem should be cut 50-60 days after planting and then 30-40 days. It has 2-0-24% crude protein and 70% dry matter digestibility.

Uses

1. Barseem is high quality green forage for milk producing cattle and also known as milk multiplier.
2. Barseem is shown in early autumn and valued as a winter crop, thus provide feed during colder months.
3. As a green manure, barseem intercropped with oats and maize.
4. It is nitrogen fixing legume and increase soil fertility.

(b) Tree legumes

13.3.3.4 Silk tree

Botanical name : *Alibizia lebbeck* (L.) Benth.

Family : Fabaceae

Hindi name : Siris

Eng. Name : Lebbeck tree, Women tounge, Silk tree Mimosa

Botanical characteristics

Lebbeck tree are found in forests and they are also cultivated in farming system along the field boundaries and roadside probably for the purpose of shade and to improve soil fertility from litter breakdown. *Alibizia* sp. tree can attain a height of 15-30 m with a trunk 0.5-1 m in diameter. Silk tree Mimosa leaves are8-15 cm long bipinnately compound, pinna in 2-4 pairs with oblong to elliptic-oblong leaflet. The flowers are white in colour and fruit is a pod.

Uses

1. Lebbeck plant provide forage as stock feed directly.
2. Lebbeck plant used as drought reserve and as protein replacement for feeding weaners (to replace mother milk by other nourishment for a child mammal).
3. The leaves and waste leaves after forage uses of Lebbeck plant is valuable as mulch and green manure.

13.3.4 Non Legumes Trees as forages

Non Legumes are tall growing crop with good foliage resulting in higher biomass production. They are usually high yielders, rich in carbohydrate and the crude protein content is 5-8% on dry weight basis.Non Legumes are annuals or perennials (usually for 3-4 yrs or more), and cereals, millets and other grasses belongs to family Graminea are considered as Non Legumes fodder.

The contribution of leaves as forage, produced from forest trees, forest bushes and herbaceous plants is remarkable. In special reference of Uttarkhand, fodder treesare found as pure forest or mixed forest, and certain popular fodder trees include mainly to Banj, Moru, Kharsu, Banj-oak, Sal, Bhimal, Timla, Kharik, Mulberry, Bitain, Burans etc. Some of the important perennial bushesand tree species used as fodder in hilly region of Uttarakhand is listed in table 2 & 3.

Table 13.2 List of important bushes used as fodder

S.No.	Scientific Name	Family	Native Name
1.	*Aechmanthera tomentosa*	Aecanthaceae	Jamila
2.	*Bauhinia vahlii*	Caesalpiniaceae	Malu
3.	*Berberis aristata*	Berberidaceae	Kilmora
4.	*Crataegus crenulata*	Rosaceae	Ghigaru
5.	*Ficus infectoria*	Moraceae	Pilkhan
6.	*Ficus foveolata*	Moraceae	Beduali
7.	*Hypercium cernuum*	Hyperiacaceae	*Peuali*

8.	*Prinsepia utilis*	Rosaceae	Bhaical
9.	*Rubs ellipticus*	Rosaceae	Hisalu
10.	*Ribes glaciale*	Glossulariaceae	Darvia
11.	*Rosa macrophylla*	Rosaceae	Kunja
12.	*Strobilanthes alatus*	Acanthaceae	Janu
13.	*Viburnum cariaceum*	Caprifoliaceae	Titmueia

Table 13.3 List of important fodder yielding tree species

S.No.	Scientific Name	Famiy	Native Name
1.	*Albizzia lebbek*	Mimosaceae	Siser
2.	*Albizzia lebbek*	Minosaceae	Siris
3.	*Acacia mollissima*	Mimosaceae	Acacia
4.	*Acer oblongum*	Aceraceae	Putli
5.	*Aesculus indica*	Sapindaceae	Pangar
6.	*Alnus nepalensis*	Betulaceae	Uttish
7.	*Bauhinia variegate*	Caesalpiniaceae	Kachnar, Kaweral
8.	*Buxus wallichiana*	Euphorbiaceae	Papari
9.	*Celtis australis*	Ulmaceae	Khadig
10.	*Diploknema butyracea*	Sapotaceae	Chuira
11.	*Ficus nemoralis*	Moraceae	Dudhila
12.	*Ficus palmate*	Moraceae	Beadu
13.	*Ficus roxburghii*	Moraceae	Timila
14.	*Faxinus micratha*	Oleaceae	Angu
15.	*Grewia optiva*	Tiliaceae	Bhimal
16.	*Melia azedarach*	Miliaceae	Bakain
17.	*Moras alba*	Moraceae	Sahtuat
18.	*Moras serrate*	Moraceae	Kemu
19.	*Prunus cerasoides*	Rosaceae	Padam
20.	*Pyrus pashia*	Rosaceae	Mehael
21.	*Prunus cornuta*	Rosaceae	Jamun
22.	*Quercus dilitata*	Fagaceae	Moru
23.	*Quercus leucotrichophora*	Fagaceae	Banj
24.	*Quercus semicarpifolia*	Fagaceae	Kharshu
25.	*Salix tetrasperma*	Salicaceae	Bains
26.	*Salix wallichiana*	Salicaceae	Gadbains
27.	*Stranvaesia nussia*	Rosaceae	Gadmahel
28.	*Ulmus wallichiana*	Ulmaceae	Chamarmora

Men have three basic necessities of life *i.e,* food, clothing and shelter, and the adequate supply mainly derived from plant and plants product. Animals are just as dependent on plants as men himself and a range of forage crops have feed valueare available to producers. Fodder trees and shrubs have been used as multipurpose resources for food, fodder, timber, wood and live fences across the farms and ecological zones.A large number of trees and non-leguminous are listed but only a small number apparently have a real feed value. The choice of alternate food resources should not be restrictive but must fit within the existing farming system, and be adopted the economic realities of the farmers.

13.4 PLANT FIBRES

The fibre may occur almost in each part of the plant *i.e.,* stems, root, fruit, seed. The fibre yielding plants ranked second after food yielding plants in their importance to human beings. The majority of economically fibre yielding plants belongs to families are- Malvceae, Poaceae, Tiliaceae, Bombaceae, leguminosae, Palmae, Liliaceae, Moraceae etc. Fibres are specialized sclerenchymatous cells, usually long with thick walls with small cavities and pointed ends, chiefly contains cellulose (64-94%) alongwith trace amount of lignin, hemicellulose.

The plant fibres are classified mainly on the basis of morphological nature, structure, origin and uses. On the basis of use and commercial utilization, fibres are grouped into six categories as follows-

Fibre type	Utilized for
Textile fibres	Manufacturing of fabrics, netting & cordage etc. On the basis of origin and structure textile fibres includes- (i) Surface fibre- small hairlike outgrowth on seeds. (ii) Soft orbastfibre- originated from extrasteler region. (iii) Hard fibre or mixed fibre- found chiefly in the leaves of monocots.
Brush fibres	Making of brushes & brooms.
Rough weaving fibres	making of baskets, chairs, mats, thatched roofs of houses etc.
Filling fibres	used for padding cushions, pillows, upholstery mattresses etc.
Natural fibres	Used as coarse clothing or cloths without weaving.
Paper making fibres	Manufacturing of paper.

On the basis of origin of fibres are grouped into bast (soft) fibres, hard (structural) fibres and surface fibres.

1-Surface (short) Fibre: The surface fibre originated from seed or fruit surface. Fibre separation is done by the process of ginning and mechanical extraction. e.g., Cotton.

2- Bast (Soft) fibres: These fibres are exogenous in origin and are generally more durable, resistant to retting, bleaching and other processing treatments. They are associated with vascular tissues, such as phloem, pericycle and cortex. Example: jute, hemp, flax, ramie etc.

3-Structural (hard) fibres: Structural primarily associated with monocotyledonous plants are shorter, lignified cells surrounding vascular tissue. They are endogenous in nature, coarse, weaker, hard and brittle and thus less durable than bast fibres. Example: Manila hemp, Sisal and kittul fibres. The separation is done by mechanical methods using simple rollers, washing, beating and thrashing process is usually applied to make them into shreds.

13.4.1 Textile Fibre

13.4.1.1 Cotton

Botanical name	:	*Gossypium* Linn.
Family	:	Malvaceae
Eng.	:	Cotton
Hindi	:	Kapas
Habit	:	Perennial shrub
Part used	:	seed hairs

Botanical characteristics

Cotton is the leading fibre crop and is grown commercially as a cash crop worldwide. *G. arboreum* and *G.herbaceum* are diploids and considered as old world cotton. *G. hissutum* and *G. barbadense* are tetraploids cotton species and known as new world species, which cover more than 80% of the world's cotton cultivation area.Cotton is an annual or perennial shrub(grow as annual shrub in temperateclimate; like a perennial tree in tropical climate) usually 1-2 m tall, leaves are simple, stipulate and palmately lobed. The flower is solitary axillary, regular, borne singly and bisexual. The fruit are 3-5 locular capsule, triangular pods that mature within 55-80 days, known as cotton boll. During maturation, the seeds and their attached hairs develop within the ball. The seed hairs or fibre, reaching a maximum length of about 6 cm in long fibre verities, is known as lint. Seed hairs are used as fibre and after processing made into various products.Cotton grow on sandy damp soil of humid region.In India, black alluvial soil of Deccan plateau is considered the best for cotton.

Cotton fibres are single celled and thick walled tubular delicate epidermal prolongation of seed coat, formed by outer epidermis of outer integuments. The mature cotton fibre is a translucent, collapsed hollow tube. The fibre of cotton composed of about 90% of cellulose.

Uses

1. The fibre collected from seeds *Gossypium* sps., and have to undergo several processes, thereafter processed fibre used for the manufacturing of various products.

2. Cotton is vastly/chiefly used either alone or in combination with other fibres in textile industry.
3. The byproduct or waste of good grade cotton is used for making cotton sheets, towels, blankets etc.
4. Cotton is used for padding for upholstery, wadding, bed quilts, stiffing the pillow and cushions etc.
5. Cotton is also used in rubber tier fabrics.
6. The stalks also contain stem fibre and used for pressed paper and cardboard making.

13.4.1.2 Jute

Botanical name	:	*Corchorus* Linn.
Family	:	Tiliaceae
Eng.	:	Jute
Hindi	:	Patsan
Habit	:	Annual Herb
Part used	:	Bast fibre

Botanical characteristics

Jute fibre is also known as Golden fibre and extracted from the stem *i.e.,* runs along the length of the stem in the form of lacework sheath. Two species of *Corchorus* are grown commercially viz., *C. capsularis* known as white jute produces better quality fibre than the *C. olitorius* (tossa or dark jute).Jute is an annual herbaceous, 1.5-4 m tall plant, with cylindrical, branched or unbranched stem. Jute is a long, soft, shiny fibre, and grows well on sandy, deltaic loam alluvial soil and requires high temperature, heavy rainfall and humid climate. The jute fibre obtained from secondary phloem by retting the stem *i.e.,* soaked stem into waterto get loosened the fibre by decomposition, and then beaten and separated the fibre.

Jute fibres are soft or bast fibre is not very strong, consists of smaller stands made up of many elongated cells with tapering or pointed ends. Each individual fibre cell is about 2-5 mm long with a wide lumen. Jute fibre stand are 1.5-3 m long and possess a silky lustre. Jute fibre is composed of primarily of cellulose (65.2%), hemicellulose (22.2%) and lignin (10.8%), with trace amount of or fats or wax (0.3%).

Uses

1. Jute fibre, as world's chief material, is used for manufacturing textile for bags, sacks and canvases.
2. Jute fibres are used for manufacturing curtains, carpets, gunny bags, twine and ropes.
3. Jute fibre is widely used in the manufacturing of coarse cloth, rugs, upholstery etc.
4. Jute buts i.e. short fibres isolated from lower ends of stalks, used to paper and paperboard.

13.4.1.3 Linen

Botanical name	:	*Linum usitatissimum* Linn.
Family	:	Linaceae
Eng.	:	Flax
Hindi	:	Alsi
Habit	:	Annual Herb
Part used	:	bast (phloem) fibre from the stem

Botanical characteristics

Linen is one of the oldest and popularly used fibres from stalk of the flax. Flax is an annual plant, attaining 0.5-1.2 m inheight; having slender stem and bearing small leaves. The fibre obtained from flax straw by retting *i.e.,* either soaked to the stem into water or chemically, and then scotching and separated the fibre. The flax fibres are hair like fibres and they form fibrous strands by aggregation of many long pointed cells, having thick cellulose walls, which held together by a gummy substance known as pectin lying beneath the bark. Linen fibre is composed of cellulose (60-65%), hemicellulose (15-18%) and pectin (2-3%).

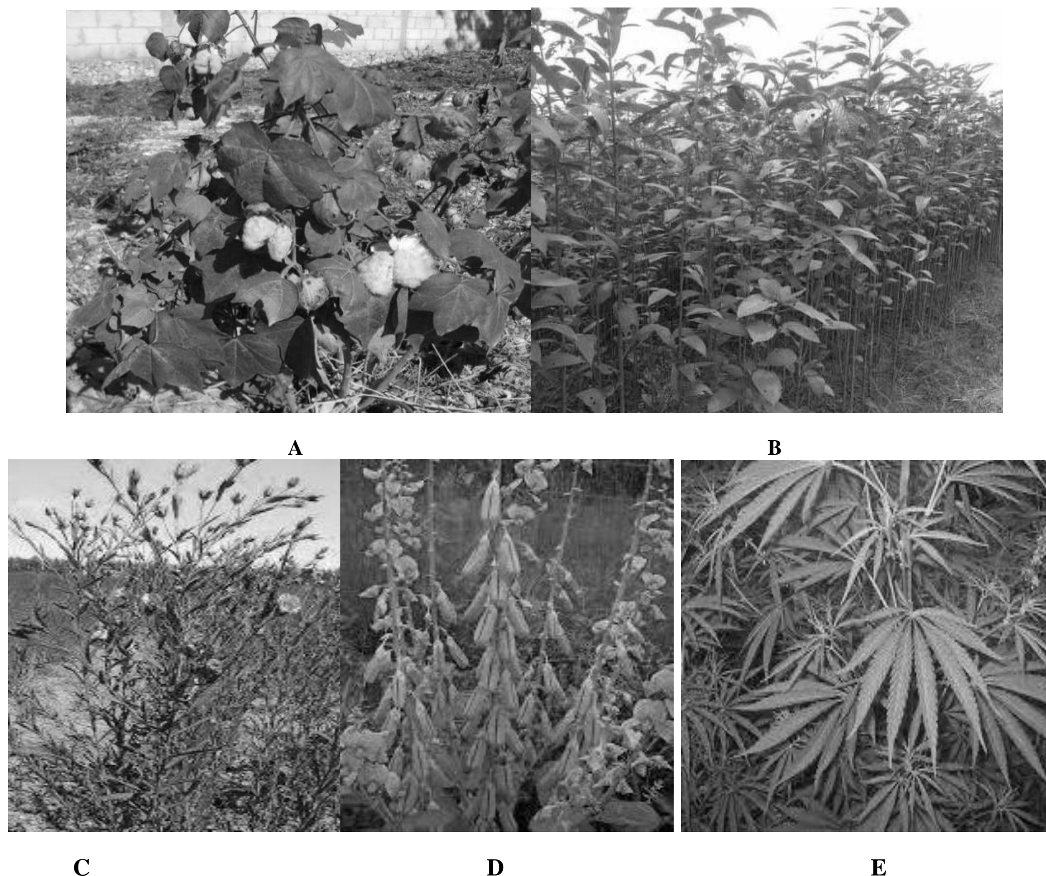
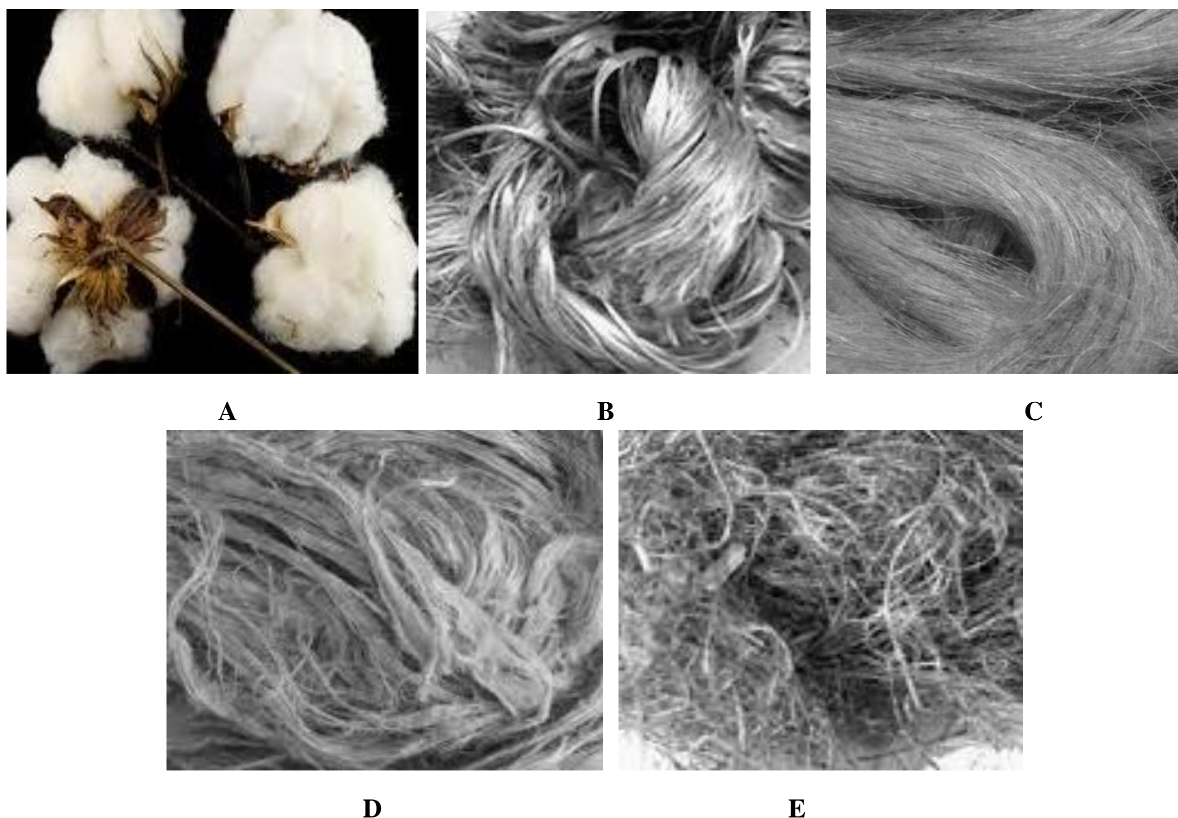


Fig.13.4 (i) Plants used as Textile Fibres: (A) Cotton; (B) Jute; (C) Linen; (D) Sunn-Hemp and (E) Hemp

Uses

1. Flax fibres are stronger and durable than cotton, and are used for preparation of cloth that has soft lustre, flexibility and high water absorbency, known as linen. Flax fibre is resistant to high temperature as such it is cooler than cotton and improves the feel of dresses/cloths in hot and humid weather.
2. Linen fibre is preferably used to oil paintings.
3. Flax products also used as sail and tent canvas, fishing nets, book binding and leather working threads.
4. Flax fibres are used for tablecloth, bath towels, bed sheets, upholstery, sewing threads, wallpapers etc.



13.4 (ii) Plant Fibre **(A)** Cotton; **(B)** Jute; **(C)** Linen; **(D)** Sunn-Hemp and **(E)** Hemp

13.4.1.4 Sunn-Hemp

Botanical name	:	*Crotalaria juncea* Linn.
Family	:	Papilionaceae
Eng.	:	Sunn-Hemp
Hindi	:	San, Sunn
Habit	:	Annual Herb

Part used	:	Bast (phloem) fibre from the stem

Botanical characteristics

Crotalaria juncea is an annual erect shrubby herbaceouslegume plant growing to a height of 1-4 m, stem are cylindrical, ribbed and upto 2 cm in diameter; leaves are simple, ovalor elliptical in shape and spirally arranged along the stem. Sunnhemp grows well in the high sandy soil. It is a drought resistant species and productive with relatively high humidity, and prefer to light textured (sandy loam or loam) soil.

Sunnhemp fibre is a bast fibre obtained from the stem of plant is slightly lignified, light in colour, coarse and strong, lustrous and 1.2-1.5 m long ribbon like strands. Sunnhemp fibres obtained by retting the stem and later on separate the fibre. The sunnhemp fibre are chiefly composed of cellulose and thickened with lignin and with blunted end.

Uses

1. Sun hemp, a native of cordage trade and chiefly utilized in the manufacturing of ropes, cords, marine cordage etc.
2. Sun hemp fibre also used in the manufacturing of coarse cloth, sacking bags, canvas, fishing nets etc.
3. Sun hemp fibre contains high cellulose and low ash content, and used for cigarette paper and wrapping paper.

13.4.1.5. Hemp

Botanical name	:	*Cannabis sativa* Linn.
Eng.	:	Hemp
Hindi	:	Bhang, Ganja, Charas
Family	:	Cannabinaceae
Habit	:	Annual Herb
Part used	:	Bast (phloem) fibre from the stem-white fibre formed in pericycle

Botanical characteristics

Hemp fibre is one of the strongest natural fibres and obtained from stem of*Cannabis sativa*. Hemp is an annual herbaceous plant grow over 4-5 months and attain upto 5 m height, and stem is oval to cylindrical, branched, 5-6 cm in diameter with palmate leaves.

Hemp grows best in mild humid climate and humus rich loam soil. Hemp fibre is white coloured bastfibre, and develops in the pericycle of the stem, lignified and varied in length form 3-15 feet.

Uses

1. Hemp fibre is used for manufacturing of carpets, sail cloth, sack, bags, ropes etc.

2. Good quality or fine grade hemp fibre is used for weaving cloth looks like coarse linen and denim cloths and trousers.
3. Hemp fibre resistant to deterioration in water and used for manufacturing fishing nets and marine cordages.

13.4.2 Hard Fibre

13.4.2.1 Coir

Botanical name	:	*Cocos nucifera* Linn.
Eng.	:	Coconut
Hindi	:	Nariyal
Family	:	Palmaceae/Arecaceae
Habit	:	Perennial
Part used	:	Mesocarp

Botanical characteristics

The coconut palm is an important natural fibre yielding plant and obtained fromits fruit. It is a perennial tall plant growing upto 30 m in height, and bearing a cluster of 4-6 m in long pinnately compound leaves at the apex. The coconut palm prefers shoreline areawith abundant sunlight and regular rainfall with humid climate, and thrives on sandy soil. It has tolerance to salinity.

Coir fibre obtained from coconut fruit. Coconut fruit is a fibrous drupe fruit with a tough, greenish exocarp and a brown coloured fibrous mesocarp. Coir fibre is a fibrous material found between the hard, internal shell and the outer coat of a coconut. The individual fibre cells are narrow and hollow, with thick walls cellulose. The immature coir fibre are pale in colour, and later on maturation, fibre become hardened and yellow in color due to deposition of lignin on their walls. Mature processed coir fibre are 10-30 cm in length, contain more lignin than cellulose and are stronger with less flexibility, which makes the fibre enough elastic to twist without breaking. Coir fibres are broadly categories into two type's viz., Brown colour coir and White colour coir fibres. The dark brown colour coir fibre are obtained from ripened coconut husks, while white or light brown colour coir fibres recovered from immature or early harvested coconut husks. The coir fibre yield from coconut by retting process i.e., the husk are socked into saline water for several months to loosen the fibre, and then beaten, washed and dried.

Uses

1. Coir fibre is used for making bristles of cordage, matting, floor rugs, doormats, rugs, sacks, rope making, stuffing etc.
2. Coir fibre used for manufacturing of modern days mattresses and seating cushion in form of 'Rubberised coir' by mixing a proportionate blend of coir and rubber latex.

3. Coir fibre or dust can hold large quantity of water and used for the replacement of traditional peat in soil mixtures, known as ‘coco peat’ featuring high water retention, suitable aeration and antifungal benefits. Coir dust, byproduct of coir industry, used as manure and soil surface mulch.
4. Coir fibre utilized in the production of activated carbon, paper pulp, roofing tiles, writing board etc.
5. Coir dust in combination of cement forms an excellent thermal insulator agent.
6. Coir bags are used for collecting tea leaves and for transportation in tea estates.
7. Coir mats or fibres used for commercial packing purpose and transporting gas cylinders.

13.4.3 Brush Fibres

13.4.3 Coir

Botanical characteristics: as described in 13.4.2.1.

Uses

1. Coir green leaves are used for basket making and mid-ribs of leaves are used for broom.
2. The leaves are used for roofs of houses by villagers.

Fig. 13.5 Coir

13.4.4 Rough Weaving Fibre

13.4.4.1 Bamboos

Botanical name	:	*Dendrocalamus hamiltonii* Nees&Arn.
Eng.	:	Hamilton’s Bamboo or Tama Bamboo
Hindi	:	Kaghzi bans, Maggar
Family	:	Gramineae
Habit	:	Annual Herb

Part used : Clum fibre or Clum

Botanical characteristics

Tama bamboo, also known as Himalayan Miracle Bamboo, is an evergreen, tall, clump forming bamboo with woody clums, can grow 30-50 cm, grow in moist tropics at elevation upto 1000m, chiefly distributed in Dehradun. The culms are 9-20 cm in diameter, walls 12-20 mm thick and with internodes distributed 30-50 cm apart. The wild stands are endangered by overexploitation for paper pulp and harvesting from wild as a source of food and sources of materials

Uses

1. Bamboo is used for window curtain.
2. Bamboo is used for manufacturing of mats, and baskets.
3. The culms are widely used as raw material for pulp to make papers.
4. Bambooculms are used for temporary construction (house, bridge etc.).

A **B**

Fig. 13.6 Rough Weaving Fibre: **(A)** Bamboos and **(B)** Solid Bamboo

13.4.4.2 Solid Bamboo

Botanical name : *Dendrocalamus strictus* Nees MP

Eng. : Solid Bamboo, Male Bamboo

Hindi : Bans Kaban

Family : Gramineae

Habit : Perennial

Part used : Clum fibre or Clum

Botanical characteristics

Male Bamboo, one of the two most important bamboos, is a perennial, clump forming, fast growing, evergreen producing woody culms grow upto 6-20 m in height and 25-75 mm in

diameter, with 30-45 cm long internodes. Individual culms are matured within 3 years but can live upto 15 years. It grows in all types of soil, but prefer well drained with sandy loams, coarse and stony soil. This species of bamboo is widely adapted to high as well as to low temperature.

Uses

1-Bamboo is used for manufacturing of walking sticks, furniture and baskets.

2- Bamboo culms are used to make papers.

3- Bamboo culms are used for temporary construction poles, bridge, agricultural implements etc.

13.4.5 Filling Fibres

13.4.5.1Redsilk Cotton

Botanical name	:	*Bombex ceiba*Linn.
Eng.	:	Redsilk Cotton
Hindi	:	Semul
Family	:	Bombacaceae
Habit	:	Perennial Tree
Part used	:	hairs from side of the seedpods

Botanical characteristics

Bombax cotton, is seed floss of trees of *Bombax* genus, also known as tree cotton. Bombax is a tall tree grows upto a height of 25 m or more. The bombax plant commence flowering after 8-10 years after planting, and the flowers are scented and produced when the tree is leafless, to attracts the pollination agents. The bombax flowers having 5 red coloured petals, appears in spring before new foliages. It produces a capsule, which contains white fibres on ripening. The bombax plant is a fast growing plant which prefers deep, well-drained soil and tolerates a wide range of environmental conditions. Semal tree are well known to drought and fire resistant.

Red silk cotton is silky floss, soft and pale yellow to brown in colour which obtained from inner wall of the seed. The individual fibre is 0.5- 3.25 in length and 20-40 µm in diameter. Bombax cotton fibre grows from side of the seedpod, instead of from the seed itself as in cotton. Bombax cotton fibre is weaker and less elastic because it contains woody substance lignin, which makes it unsatisfactory to use as a textile fibre.

Uses

1. The seed floss is used as a studding material for pillow, cushions, mattress etc.
2. The redsilk fibre also used for packing fragile materials.
3. The redsilk fibre is used as an insulating material in refrigerators, sound-proof walls.

13.4.5.2 Kapok

Botanical name	:	*Ceiba pentandra*(Linn.) Alston
Eng.	:	Kapok,Java cotton
Hindi	:	Safed Simal
Family	:	Malvaceae
Habit	:	Perennial Tree
Part used	:	inner wall of fruits

Botanical characteristics

Kapok is a huge individual, perennial tree, attaining a height up to 70 m and having trunks often be up to 3 m or more in diameter. The trunk and branches often crowded with large simple thorn, with a wide crown of foliage. The foliage is composed of palmate leaves, in which 5 to 9 leaflet are arranged each upto 20 cm long. The tree produces a number of capsular fruit or pods (15 cm in length) having seeds surrounded by a fluffy and white or pale-yellowish fibre.

Kapok fibre is also known as WhiteSilk cotton, which is lustrous, up thrust, resistant to water and made up ofcellulose (60-65%)and lignin (10-14%), has a wide lumen and thin walls having water proofing wax. The fibre forms on the inner epidermis of the epicarp. Individual fibres are cylindrical with bulbous base and 0.8-3 cm in length. The hollow core of fibre and a sealed tail makes fibre extremely light i.e., eight times lighter than cotton by volume, short length and smooth surface of fibre, lead to it desirable for functional textiles.

Uses

1. Kapok fibre (fragile and smooth surface) is difficult to spin and, hence, used as an alternative to filling and stuffing purposes.
2. Kapok fibre is used to sacking life jackets due to its wax coating and water proofing quality.
3. Silk cotton fibre is light, very buoyant and used to filling mattresses and blankets, stuffing toys, pillows, cushions etc.
4. Red silk fibre, sometimes, also as an insulating material in refrigerators and has sound-proofing properties.

13.5 SUMMARY

Animals are dependent on plants as men himself and a range of forage crops have feed value are available to animals. Forage refers to the plant resources and plant material includes parts of plant that consumed by grazing livestock andwildlife animals. The edible part of plants of forest and cultivated crops or edible part after separated the grains from cereals and millets, crop residue or immature cereal crops, that can provide feed for grazing animals or that can harvested for feed, play a vital role to fulfill the feed requirement of animals. Since forages are grown and used with livestock, includes many types of grasses and legumes, and harvested in various ways. The forage may be preserved and used in form of hay, silage, strains etc.

Beside the natural forage i.e., includes grasses, the forage resources include cultivated leguminous and non-leguminous crop residue, fodder trees and shrubs.Forage from forest is an alternative feed source for ruminant animals. Fodder trees leaves from forests, agriculture lands and pastures, are rich in protein, carbohydrate, minerals and vitamins, and play important role as alternative forage resources. Fodder trees and shrubs have been used as multipurpose resources for food, fodder, timber, wood and live fences across the farms and ecological zones.The right crop will depend on the quality and quantity of feed required to meet the enterprise production objective as well as other factors such as soil type, climate, water availability, drainage, water and diseases.

Among the plant species commonly used by man the fibre yielding plants hold the second position after the food plants in their economic importance. The plant fibres are extracted from different parts such as stem, leaf, petiole, roots, fruits and seeds. The fibres are grouped into bast fibres, hard fibres and surface fibres on the basis of their origin. The plant fibres are variable in characteristics with respect to strength, durability, length texture, plant part in which present, chemical composition, pigmentation, resistance to water etc. The bast fibre has been of maximum use for extraction of fibres in comparison to other plant parts. The fibre yielding plant are grown under cultivation andsemi- cultivation or exploited from wild state.Besides the utilization of fibre in clothing or textile industry, the plant fibres have specific qualities such as thermal insulation, resistance to water and other desirable traits.

The durability of fibres depends largely on the chemical substances and nature of the deposits along with location in the plant tissue whereas the strength of fibre is mainly due to arrangement or clustering of fibre, purity of cellulose and thickness of the cell wall. The fibres are used for textile and paper manufacturing, filling, making ropes, fishing nets and cordages, thatch, hats and other weaving materials and brush making. The bast fibres are commercially used for items like gunny bags, ropes, cordages, fishing nets, and mainly extracted from *Corchous* spp., *Hibiscus* spp, *Linum* spp. The items made from bast fibres are more durable than those from the structural fibres.

The rice straw, young bamboos, bark of paper mulberry, grasses and sedges are regular sources of soft and flexible fibres, and are generally used for paper making. The fibre is also used for filling of articles like pillows, matresses and toys e.g., *Gossipium* sp., *Bombax ceiba* etc. whereas the fibre extracted from *Phoenix sylvestris* and *Ceiba pentandra* are used as thermal insulator.The hard or structural fibres includes parts like the leaves, green spathe and dried stalks, are mainly used for making articles like basket, mats, hats and burshes. So, the raw material collected from forestry based plants products getting fibre for small scale or cottage industry, andplay an important role to generate rural employment and income especially in the rural and tribal areas of the country.

13.6 GLOSSARY

Aftermath: Forage grown following a harvest.

Agroforestry: Land-use system in which trees are used for forest products combined with agricultural crops.

Crop residue: Plant materials, with variable forage potential, remaining on the cultivated land after seed grain.

Fodder: Coarse grasses such as corn and sorghum harvested alive green plant with seed and leaves, then cured and feed to domesticated livestock in their entirely as forage

Forage: The edible parts of plants that can provide feed for grazing animals or can be harvested for feeding.

Forage crop: A crop of the cultivated plantsproduced besides the separated grains, used as feed for animals.

Forb: Any herbaceous, dicotyledonous broad leaved plant used as animal feed.

Legume: Members of the plant family Fabaceae

Linters: Cotton fibre more closely connected to the seed and considerable shorter than seed hairs, result from second growth beginning, are known as linters.

Ginning: Ginning is the process which separate leaf matter, traces and seed from cotton fibre which is than made into a bale.

Grass: Members of the plant family Poaceae.

Grassland: An area refers to an imposed grazing-land ecosystem. The vegetation of grassland includes grasses, legumes and other forbs.

Grazable forestland: Forestland that understory vegetation can be grazed at least periodically.

Hay: Hay is the preserved harvested forage by drying, generally to a moisture content of less than 200 g/kg.

Micronaire: A measure of fibre strength.

Meadow: A natural or semi-natural grassland often associated with the conservation of hay or silage.

Pastureland: Land devoted to the production of introduced or indigenous forage for harvest by grazing, cutting or both.

Prairie: The originally treeless grassland or with a few scattered trees, and usually on fertile trees.

Residue: Forage remains on the land after harvest.

Retting: Retting is a process by which the fibres present in the bark get loosened and separated from the woody stalk due to the removal of pectin, gums and other mucilaginous substances.

Silage: Silage is the preserved harvested forage containing high moisture content (500g/kg) by organic acid production during partial anaerobic fermentation.

13.7 SELF ASSESSMENT QUESTION

13.7.1 Fill in the blanks:

1. Short fibres are known as ………… (lint/flint/fluff)

2. Long fibres are known as ………… (lint/flint/fluff)

3. *Gossypium arboreum* is an example of …………… world cotton. (old/new)

4. Removal of lint and fuzz is known as ……………….. (lapping/ginning)

5. Cotton fibres are made of ……………………. (cellulose/pectin)

13.7.2 Select the correct answer:

1. Jute Agriculture Research Institute (JARI) is situated at

a) Dehradun (UK) b) Jhansi (UP)

c) Bhopal (MP) d) Barrackpore (W. Bengal)

2. Indian Grassland & Fodder Research Institute (IGFRI) is situated at

a) Dehradun (UK) b) Jhansi (UP)

c) Bhopal (MP) d) Barrackpore (W. Bengal)

3. Central Institute of Cotton Research is situated at

a) Dehradun (UK) b) Jhansi (UP)

c) Bhopal (MP) d) Nagpur (Maharashtra)

4. The fibres are

a) parenchyma tissue b) collenchyma tissue

c) sclerenchyma tissue d) chlorenchyma tissue

5. Botanical fibres are used for making cloth and paper are found mainly in

a) cortex b) seeds

c) fruit d) Vascular tissue

6. Hemp (*Cannabis sativus*) is used to make

a) linen b) paper bags

c) news print d) rope

7. Sunhemp is obtained from

a) phloem b)xylem

c) seeds d)leaf

8. Forage that has been cut and dried until it contain low level of moisture

a) pasture b) hay

c) silo d) silage

9. Which of the following is not a plant fibre?

a) flax b) coir

c) silk d) hemp

10. The preserved harvested forage containing high moisture content by organic acid production during partial anaerobic fermentation is known as

a) pasture b) hay

c) silo d) silage

Answer keys:

13.7.1: 1-Fluff, 2- lint, 3-old, 4- ginning, 5- cellulose

13.7.2: 1-(d), 2-(b), 3-(d), 4-(c), 5-(d), 6-(d), 7-(a), 8-(b), 9-(c), 10-(d)

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13.10 TERMINAL QUESTIONS

Q.1. Write a detailed account on forage crops.

Q.2. Give a detailed account on forage plants.

Q.3. Describe plant fibres and their types. Discuss any five plant fibres with their commercial utilization.

Q.4. Write an essay on plant fibres used in textile industry.

UNIT-14: STUDY OF OIL YIELDING, MEDICINAL AND AROMATIC PLANTS

14.1 Objectives
14.2 Introduction
14.3 Study of oil yielding plants
- 14.3.1 Soyabean oil
- 14.3.2 Linseed oil
- 14.3.3 Sunflower oil
- 14.3.4 Peanut oil
- 14.3.5 Mustard oil
- 14.3.6 Corn oil
- 14.3.7 Coconut oil
- 14.3.8 Palmoil

14.4 Medicinal plants
- 14.4.1 Drug obtained from roots
- 14.4.2 Drug obtained from stem
- 14.4.3 Drug obtained from bark
- 14.4.4 Drug obtained from stem and wood
- 14.4.5 Drug obtained from leaves
- 14.4.6 Drug obtained from flower
- 14.4.7 Drug obtained from fruit
- 14.4.8 Drug obtained from seeds

14.5 Aromatics plants
14.6 Summary
14.7 Glossary
14.8 Self assessment question
14.9 References
14.10 Suggested readings
14.11 Terminal questions

14.1-OBJECTIVES

After reading this unit, student will be able to understand about the characteristics and utilization of edible oil, medicinal and aromatic plant in specific reference to-

- General account on oil and oil types
- Botanical characteristics of important edible oil yielding plants and uses
- Botanical characteristics of medicinal and aromatic plants, and uses in healthcare
- Advantages of cultivation of important medicinal and aromatic plants

14.2-INTRODUCTION

India is unique among geographical regions of world with high biological diversity to valuable plant resources. Oil and fats are indispensable substances to mankind in nutritional and industrial respect. Plant contains two types of oil. The first type of oil which usually stored in seeds and used for energy needs. The second type is plant essential oil which are produced as self-defense compounds. Many of the plant produces seeds containing fats which are used as a food reserve for developing seedling and they are quite often present in sufficient quantities to make their extraction in form of oil. Vegetative oils are produced from nuts, seeds, grains and beans, and referred as fixed oils because they are not as volatile as essential oils. India is also considered a treasure of medical and aromatic plant species. Medicinal plants have played a significant role in Ayurvedic and Unani system in India, and associated with human, livestock and plant healthcare. The derivatives of medicinal and aromatic plants have no or least side effects and used for pharmaceutical, cosmetics and aromatherapy products.

14.3 STUDY OF OIL YIELDING PLANTS

Oils are complex chemical compounds which have triester of glycerol with the long chain of organic acid. The oil is remains present within various plant parts like other substances. The oil is very important part in daily life of human beings. The oil extracted from plants used either as edible (fatty) oil or essential oil. Essential oils are distinguished from fatty oils by the fact that they evaporate or volatize in contact with air and process a pleasant taste and strong aromatic order without any change in composition. Fatty oils are non-volatile and consist of glycerin in combination of fatty acid. The large amount fatty oils in association with proteins are stored in seeds, and to a less extent found in stem, tuber, fruit and other plant organs.

On the basis of ability of oil to absorb oxygen, vegetative oils are classified into four categories-

Drying oil: These oils readily form a dry thin elastic or solid film after a period of exposure to air and are rich in unsaturated fatty acids, especially linotic and linolenic acid. e.g., Linseed oil, Tung oil etc.

Semi-Drying oil: Semi-drying oils absorb atmospheric oxygen slowly and produce a soft film even after a long exposure. These are fairly rich in linoleic and saturated acids but do not contain linolenic acid. *e.g.*, Cotton seed oil, Sunflower oil, Corn oil etc.

Non-drying oil: Non-drying oils do not absorb atmospheric oxygen and fairly rich in saturated acids and oleic acids. These oils does not harden and unable to forming elastic films even after long exposure to atmospheric oxygen. e.g., Groundnut oil, Olive oil etc.

Vegetable fats: These fats are rich in saturated fatty acids and semi-solid at ordinary temperature. e.g., Coconut oil, Palm oil etc.

Some of the commercially cultivated and important medicinal and aromatic plants are discussed below-

14.3.1. Soyabean Oil

Botanical name	:	*Glycine max* (L.) Merr.
Family	:	Papilionaceae
Eng. Name	:	Soyabean
Hindi name	:	Bhat

Botanical characteristics

Soyabean contribute 22% of total fat production in the world and largest oilseed crop. The soyabean is an erect, herbaceous, annual, leguminous and fast growing plant. The soyabean plant is usually an erect bush with woody stem and grows upto 1m in height. Roots bear nodules and symbiotically associated with *Bradyrhizobium japonicum*. The leaves are trifoliate, alternately arranged and leaflets are oval to lanceolate. The fruit are pods and with 2-4 seeds per pod, containing yellow, round seeds with a helum colour. Soyabean oil is low in saturated fats without any trans-fat and contains high amount of poly & mono-saturated fats.

Uses

Soyabean oil is the most widely used edible oil. Soyabean oil has good emulsifying ability and also used in mayonnaise, salad dressing and baked goods. Soyabean oil are abundant in ‘good’ unsaturated omega-3 and omega-6 fatty acids, it slow down LDL (low density lipoprotein-deposited & clog blood vessels and interface with blood circulation leading to heart attacks or strokes) accumulation and reduce the risk of hypertension and heart diseases. Soyabean oil is rich in vit. E and K, also have antioxidant and anti-inflammatory properties, boosts bone health and vision.

14.3.2. Linseed Oil

Botanical name	:	*Linum usitatissimum* Linn.
Family	:	Linaceae

Eng. Name : Linseed

Hindi name : Alsi

Botanical characteristics

Linseed or flax oil is obtained from ripened seeds of the flax plant. Flax is an annual plant, attaining 0.5-1.2 m in height; having slender stem and bearing small leaves. The oil obtained from flax seeds by pressing or sometimes extracted by solvent method. The Linseed oil is drying oil and colourless to pale yellowish in colour. Linseed oil is distinctive for its usually large amount of ∝-linolenic acid. Linseed oil has nearly 70-90% unsaturated fatty acids (50-55% linolenic acid& 15-20% oleic acid) and 9-11% saturated fats.

Uses

Linseed oil is an edible oil in demand as a nutritional supplement, as a source of α-Linolenic acid (ALA). α-Linolenic acid is a form of omega-3 fatty acid. Omega-3 fatty acid are essential to health and have been associated with benefits like reduced inflammation, improved heart health and protection for the brain against aging. Linseed oil exploit for its hydrophobic and drying properties, and makes suitable for industrial uses. It is used in manufacturing of paint and varnishes. The oil makes the material water resistant and adds glow to wood, used to protect metal products from corrosion and furniture from water. Linseed oil is used to making of waterproof cloths.

14.3.3 Sunflower Oil

Botanical name : *Helianthus annuus* L.

Family : Compositae (Asteraceae)

Eng. Name : Sunflower

Hindi name : Surajmukhi

Botanical characteristics

Sunflower oil, content ranges between 46-52%, is an important source of edible oil with non-cholesterol and anti-cholesterol properties. Sunflower is a large annual herb, has an erect rough hairy stem, grow upto 3m in height. The leaves are broad, coarsely toothed and alternate. The inflorescence of sunflower is capitulum or pseudanthium or composite. The center of the flower of the head is made up of disk flowers, surrounded by ray flowers. The disk flowers mature into fruit. Sunflower oil consists of low saturated oil and high amount of Vit. E. Sunflower oil are two types- Linoleic i.e., common cooking oil with low level of trans fat (11% saturated fatty acids, 20% monosaturated fatty acids and 69% polyunsaturated fatty acids) and Oleic (12% saturated fatty acids, 84% monosaturated fatty acids and 4% polyunsaturated fatty acids).

Uses

The Sunflower oil is used for cooking purposes at low to extremely high temperature. Unrefined Sunflower oil is a traditional salad dressing. Sunflower oil is also use as biodiesel. Sunflower oil is directly applied to the skin for joint pain, dry skin and arthritis as massage oil. Sunflower oil lower total cholesterol and LDL cholesterol in blood and used by heart patients.

14.3.4 Peanut Oil

Botanical name : *Arachis hypogaea* Linn.

Family : Fabaceae

Eng. Name : Groundnut, Peanut

Hindi name : Mungphali

Botanical characteristics

Groundnut is an annual herb, has cylindrical or angular, rough hairy stem, grow0.3-0.6m in height. The leaves are pinnately compound, quadrifolate with slender, grooved petiole and flowers are borne in the axils of leaves. After fertilization, the flower bearing branches are pushed underground, at the depth of nearly 2.5cm in the soil, and the fruit formation takes place geotropically (below the soil). The fruit is an elongated, oblong pod containing 2-4 ovate seeds with red coloured paper like seed coat. Groundnut grows in warm region of tropical and sub-tropical regions, and prefers well drained sandy loam soil. Groundnut oil contains 17% saturated fatty acids, 46% monosaturated fatty acids and 32% polyunsaturated fatty acids.

Uses

Groundnut oil is used for cooking purposes and an excellent source of Vit. B, Vit. E, dietary minerals like manganese, magnesium, phosphorus and dietary fiber. The oil is also use as emollinent and laxative. Groundnut oil is high in monosaturated fat and low in saturate fat, and help to prevent heart disease and lower cholesterol. It is used for the preparation of 'vegetable ghee' by hydrogenation. Groundnut oil is directly applied to the skin for arthritis, joint pain, dry skin and scalp crusting. The oil is medicinally used as laxative, emollient and in ointments.

14.3.5

Mustard Oil

Botanical name : *Brassica compestris* L. (Yellow sarson); *B. juncea* L. (Lahi)

Family : Cruciferae (Brassicaceae)

Eng. Name : Mustard, Yellow sarson

Hindi name : Sarson

Botanical characteristics

Mustardoil is a popular vegetable oil and obtained from seeds of mustard. Mustard plant is an annual herb, slender, erect, branched and attains a height upto 1.5 m. The leaves are simple

lyrate, lobed and arranged alternately and inflorescence is corymbrose racemes. The fruit is a siliqua with small, spherical and yellow or brown seeds on maturity. The characteristic pungency and flavor of mustard oil is due to allylisothiocyanate. Mustard oil has about 60% monosaturated fatty acids (42% erucic acid & 12% oleic acid), 21% polyunsaturated fats and 12 % saturated fats.

Uses

Mustard oil is generally used for edible purposes. Mustard oil act as appetizer and is a very strong stimulants. It can stimulate digestion by the production of bile and gastric juice from liver and spleen. Mustard oil used for massaging and stimulate circulation very effectively. Mustard oil due to presence of fatty acids i.e., linoleic acid & oleic acid, makes an effective hair vitaliser and also enhance blood circulation of scalp.

14.3.6 Corn Oil

Botanical name : *Zea mays* L.

Family : Gramineae (Poaceae)

Eng. Name : Corn

Hindi name : Makai

Botanical characteristics

Maize is annual, herbaceous, attaining upto 3 m in height. The stem is unbranched and differentiated into nodes and internodes. Leaves are long and grow from node and leaf size upto 4 feet in length and 4 inch in width. The maize plant is monoecious, and the male inflorescence (tassel) found at the apex of stem while the female inflorescence commonly called husk is tightly enveloped by several layers of ear leaves. The fruit is the corn kernel and called caryopsis. Corn oil is extracted from seeds obtained from ears. Maize plant is cold intolerant and most sensitive to drought and prefer well drained, fertile, heavy loamy soil and humus rich soil.

Uses

The refined Corn oil is mainly use in cooking and bakeries. Corn oil can help to control inflammation and excess cholesterol due to low amount of saturated fats. Corn oil is a key component of some margarines or flavouring agent. Corn oil is also used in soap, skin care and low grade paint industries.

14.3.7 Coconut Oil

Botanical name : *Cocos nucifera* L.

Family : Palmaceae (Arecaceae)

Eng. Name : Coconut

Hindi name : Nariyal

Botanical characteristics

The coconut palm is important oil yielding plant and obtained from the kernel of its fruit. It is a perennial tall plant growing upto 30 m in height, and bearing a cluster of 4-6 m in long pinnately compound leaves at the apex. Coconut fruit is a fibrous drupe fruit with a tough, greenish exocarp, a brown coloured fibrous mesocarp and the kernel- a fairly thick coating of white milky flesh. The coconut palm prefers shoreline area with abundant sunlight and regular rainfall with humid climate, and thrives on sandy soil. It has tolerance to salinity. Coconut oil has about 82 % saturated fatty acids, 6% monosaturated fatty acids and 1-2% polyunsaturated fats.

Uses

Coconut oil is used in refined form as edible oil. Higher content of saturated fats in coconut oil provide incredible benefits if taken in limited quantity and it also lead to healing capabilities. Coconut oil is use as a feedstock for biodiesel. Coconut oil is used in cosmetic and soap preparations. Coconut oil is the directly applicable and easily available remedy for any kind of skin infection due to its anti-fungal and anti-bacterial property, and used in cracked heal, shedding skin, diaper rashes, skin and hair massage.

14.3.8. Palm Oil

Botanical name	:	*Elaeis guineensis* Jacq.
Family	:	Palmaceae (Arecaceae)
Eng. Name	:	Oilpalm
Hindi name	:	Palm

Botanical characteristics

The oil palm is important oil yielding plant and obtained from the kernel of its fruit. The oil palm is perennial, tall, monoceious plants grow upto 20 m in height, and leaves are pinnate and 3-5m long. The fruit is a large plum, oval drupe, reddish, grows in large branches with a single seed. The fruit is made up of an oily pericarp or fleshy outer layer and a seed. The mesocarp of fruit *i.e.,* fiberous pulp of the drupe, is the source of palm oil. Palm oil has about 49% saturated fatty acids, 37% monosaturated fatty acids and 9% polyunsaturated fatty acids.

Uses

Palm oil is used as cooking oil. It is also used in the manufacturing of margarine, non-dairy creams and ice-creams. Palm oil used in the manufacture of lubricants, candles, soaps and detergents. Palm oil used in pharmaceutical, cosmetics and personal care.

14.4 MEDICINAL AND AROMATIC PLANTS

Medicinal and Aromatic plants are known for their aroma and flavour and considered as important component of indigenous system of medicines i.e. Ayurveda, Siddha, Unani etc. India

is blessed varied climatic, topographic and environmental conditions and hiving high biodiversity and availability of rare species. The Medicinal and Aromatic plants provide raw materials for use in the pharmaceutical, cosmetic and drug industries. Even, the basic component in many of the modern medicines is derived from medicinal and aromatic plants, and due to easy availability, low cost, lasting curative and least side effect have become accepted medicines.

The Ministry of Environment and Forest, Govt. of India has reported over 9500 species of medicinal and aromatic plants significant for the pharmaceutical industry, and 2000-2300 plant species out of the documented are used in traditional medicine. Of these, nearly 150 species are used for commercial purpose.

The over exploitation of medical and aromatic plants has led to the cultivation, it have better economic opportunities as against the traditional field crops as raw material to the pharmaceutical industries. The commercial cultivation of medicinal and aromatic plants has many advantages, including-

(i) Help to conserve endangered species in their natural habitats

(ii) Provide better returns and price to the farmer

(iii) Provide better environment through utilizing unproductive and waste lands

(iv) Providing continuity of supply

The classification of drug might be based on the chemical nature or therapeutic nature of plant products, the natural affinities of various species, or the morphology of plant organs from which drug is obtained. The active chemicals are present in the storage organs of the plant, particularly in root and seeds, and a lesser content in leaves, bark, wood and other part of plants. On the basis of morphology of plant organs from which drug is obtained, the drug plants are classified into following categories-

- Drugs obtained from roots
- Drugs obtained from underground stems
- Drugs obtained from bark
- Drugs obtained from stem and wood
- Drugs obtained from leaves
- Drugs obtained from flowers
- Drugs obtained from fruits
- Drugs obtained from seeds
- Drugs obtained from all parts of plant

The medicinal value of medicinal plants is due to the presence of chemical substances in the plant tissue that produce a definite physiological action on human body. The most important

constituents in these substances are the alkaloids *i.e., compounds of C,H and O*, alongwith glucosides, essential oils, fatty acids, resins, mucilages, tannins and gums.

Some of the commercially cultivated and important medicinal and aromatic plants are discussed below-

14.4.1 Drugs obtained from roots

14.4.1.1 Aconite

Botanical name	:	*Aconitum heterophyllum* Wall.
Family	:	Ranunculaceae
Eng. Name	:	Aconite
Hindi name	:	Atis, Ativisha

Botanical characteristics

Aconite is a perennial plant and roots are used as medicine. It grows to a height of 0.6-1.5 m. Aconite has a dome shaped blue or purple coloured flowers. A number of alkaloids have been isolated from aconite and are highly toxic cardiotoxins and neurotoxins. The major alkaloid derived from Aconite is aconitine.

Uses

Aconite has been used as medicines and poison from centuries. The extract of Aconitum spp. have been given orally in traditional medicine to reduce fever associated with colds, pneumonia and asthma; to promote sweating; for pain, inflammation and high blood pressure; as a diuretic. Root extract of Aconite used as arrow poison. In homeopathy, Aconite is used to treat fear, anxiety and restlessness.

14.4.1.2 Belladonna

Botanical name	:	*Atropa belladonna* Linn.;
		Atropa acuminate Royle ex Lindley (Indian belladonna)
Family	:	Solanaceae
Eng. Name	:	Belladonna
Hindi name	:	Sag-angur

Botanical characteristics

Atropa spp. is tall, erect, branched, perennial herbs grow upto 1.6m in height with ovate leaves. The flowers are bell shaped with dull purple and green tinges. The fruits are berries and shiny black coloured on ripening. The whole plant, especially the root is extremely poisonous. The toxins are concentrated in the ripe fruit. Belladonna prefers well drained soil with sandy and loamy soils and good for cultivation.

Uses

Belladonna contains the alkaloids atropine, hyoscyamine and bellodonnine, which are used as a sedative, antispasmodic, in convulsive disorders and as antidots for poisoning. The drops prepared from belladonna plant used in ophthalmology to dilate pupils. Belladonna is used to relieve pain and internally to check excessive sweat and cough.

14.4.1.3 Ceylon Leadwort

Botanical name	:	*Plumbago zeylanica* Linn.
Family	:	Plumbaginaceae
Eng. Name	:	Ceylon leadwort, Wild leadwort, Doctorbush
Hindi name	:	Chitraka

Botanical characteristics

Plumbago is a herbaceous shrub with glabrous, climbing, prostrate or erect stem growing upto 2m in height. The leaves are simple, petiolate, flower sessile and spirally arranged. The inflorescence is racemes. Plumbago prefers well drained sandy soil.

Uses

Earlier Plumbago is used as folk medicine and the crushed plant as an abortifacient as well as a treatment for leprosy. Plumbago has been reported to be a powerful irritant to the smooth muscles and uterus. Plumbago plant especially the chewing root produces copious salivation and used for toothache treatment.

14.4.1.4 Ashwagandha

Botanical name	:	*Withania somnifera* Dunal.
Family	:	Solanaceae
Eng. Name	:	Indian Ginseng, Poison Gooseberry
Hindi name	:	Ashwagandha

Botanical characteristics

Ashwagandha is a short, tender perennial shrub. The berries and roots of Ashwagandha are used to make medicine. It is growing upto 0.7m in height. The cultivated plant of Ashwagandha is distinct from wild plant not only in morphology but also in the therapeutic properties.

Uses

Ashwagandha commonly used as a medicinal herb in Ayurvedic medicine. It is used for arthritis, anxiety, asthma, bronchitis, tuberculosis and liver disease. Ashwagandha is used to reduce fat and sugar in blood.

14.4.1.5 Serpentine

Botanical name : *Rauvolfia serpentina* (L.) Benth. Ex Kurz

Family : Apocynaceae

Eng. Name : Serpentine

Hindi name : Sarpagandha, Chandrabagha, Chota chand

Botanical characteristics

Serpentine is native to tropical and subtropical regions, an evergreen plant and the roots are used for medicine. Serpentine is an erect glabrous, perennial shrub and usually grows to 60-90cm in height with tuberous, cylindrical roots. The leaves are found in whorls of three, which are lanceolate and elliptical, pale green in colour. The inflorescence of Serpentine is corymbose cymes and the fruit are drupe type, oval fleshy and tiny which turn shiny purple on ripening.

Uses

Serpentine is used as a major constituent for preparation of many pharmaceutical drugs. Serpentine tuber is the main source of alkaloids i.e., ajmaline, ajmalicine, indobine, serpentine, serpentinine etc. and the major alkaloids is reserpine. Serpentine is effective for treatment for hypertension. It is widely used for high blood pressure and also as sedative and transquillizing agent. It is quite effective herb for dysentery, colic and cholera. Serpentine is mentioned in ancient literature including work of Charakas (1000-800B.C.) where it is described by its name Sarpagandha and used as antidotes to the stings and bites of insects and poisonous reptiles.

14.4.1.6 Indian Madder

Botanical name : *Rubia cardifolia* Linn.

Family : Rubiaceae

Eng. Name : Indian Madder

Hindi name : Majith, Manjistha

Botanical characteristics

Manjistha is evergreen, climbing or scrambling plant, with red rhizomatous roots. *Rubia* spp. plant has been cultivated for a red pigment derived from roots. The stem is slender, four angled and grow upto 1.5m. The leaves are rough, evergreen, arranged in whole of 4-7 leaves around stem at nodes with 5-10 cm long and 2-3 cm wide and oval to heart shaped. The inflorescence is dense racemes and fruits are red to black berries. Manjistha prefers humus rich loamy soils.

Uses

The roots of Manjistha is astringent, alternative, deobstruent and tonic. It is given as a decoction in jaundice, paralysis, urinary troubles, inflammation of chest etc. The extract of Manjistha from a constituent of the drug Septelin, used for rhinosinal infection.

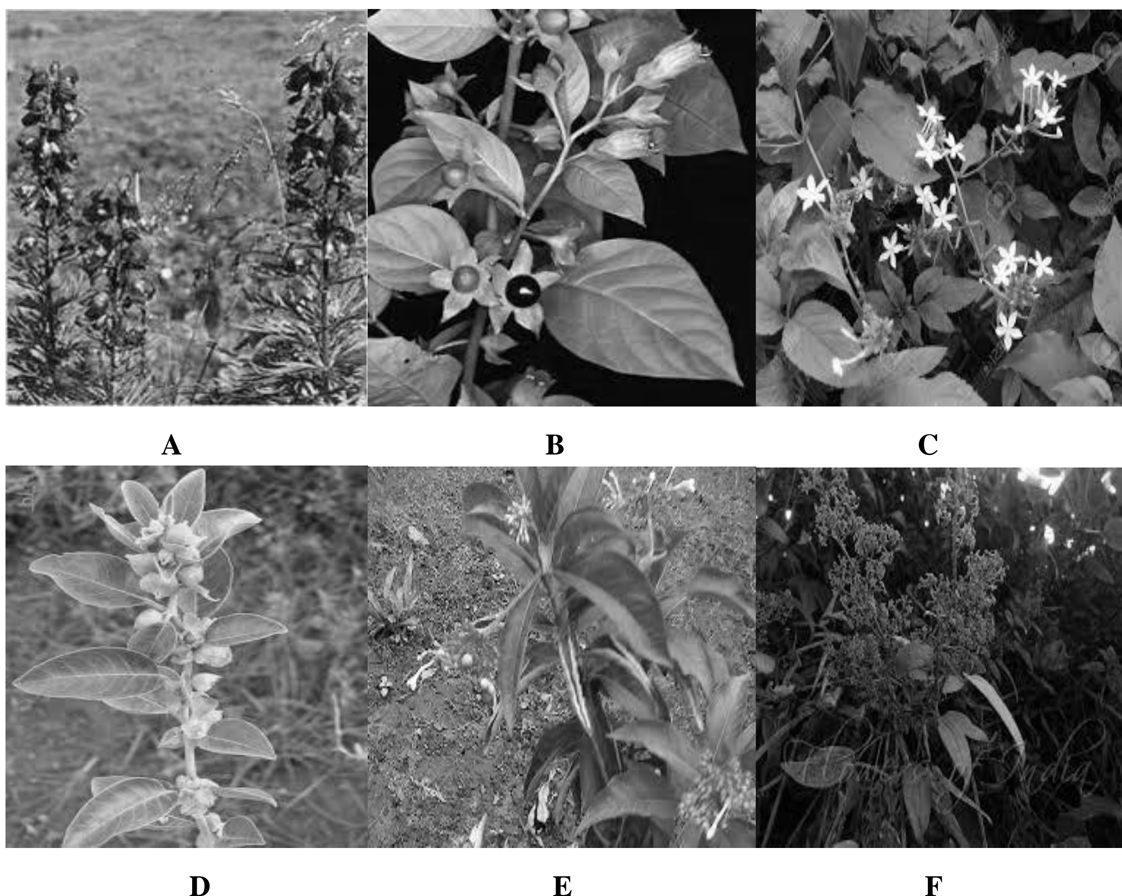


Fig.14.1 (A) Aconite; **(B)** Belladonna; **(C)** Ceylon Leadwort; **(D)** Ashwagandha; **(E).**Serpentine and **(F).** Indian Madder.

14.4.2 Drugs obtained from underground stem

14.2.2.1 Ginger

Botanical name	:	*Zingiber officinale* Rosc.
Family	:	Zingiberaceae
Eng. Name	:	Ginger
Hindi name	:	Adrak

Botanical characteristics

Ginger is a perennial herb. The plant has underground stem or rhizome, herbaceous tuberous and aromatic. Ginger is a slender, 30-50 cm tall pseudostem with palmately branched rhizome bearing leafy shoots, used as raw or processed and valued as spice. Ginger grows on a wide range of soil and prefers laterite loam soil with well distributed rain fall during growing season.

Uses

Ginger is used in culinary and ginger oil preparations. Dried ginger powder or juice with honey is used as remedy against cough and asthma. Ginger is widely used in pharmaceutical, ayurvedic and unani drug preparations as well as traditional remedies. The rhizomes are used as stimulants, carminative and flavouring agent. Ginger is used as an adjunct to many tonic and stimulatory remedies. Ginger is used for preparing drugs for disorder of the digestive system, rheumatism, pulmonary, neurological diseases etc.

14.2.2.2 Turmeric

Botanical name	:	*Curcuma domestica* syn. *Curcuma longa* L.
Family	:	Zingiberaceae
Eng. Name	:	Turmeric
Hindi name	:	Haldi

Botanical characteristics

Turmeric plant is a herbaceous perennial, 0.5-1m high, a short pseudo stem formed by leave sheath and 7-12 tufted alternate leaves, arranged in two rows. The inflorescence is a central spike of 10-15 cm length with 25-30 flowers. Turmeric is the boiled, cleaned, dried and polished rhizomes of the plants. Turmeric is a tropical cultivated crop grown in warm and moist regions and prefers well drained fertile loam and alluvial soils.

Uses

Turmeric is most effective nutritional nutrient and specifically valued for its flavour, colour and medicinal uses. Turmeric is very rich source of many essential Vitamins such as pyridoxine (B_6), niacin, choline, riboflavin, Vit. C (water soluble). Turmeric contains many compounds called Curcuminoids. Curcumin is the major compound known for medical properties and has strong anti-inflammatory and anti-oxidant properties. It is stimulatory antiperiodic tonic and carminative. It is given in diarrhea, high blood pressure, strokes, jaundice, liver disorder and urinary troubles. The fresh juice of ginger is used as amthelmintic (cleaning foul ulcers) and antiparasitic for skin affection.

14.2.2.3 Onion

Botanical name	:	*Allium cepa* L.
Family	:	Liliaceae
Eng. Name	:	Allium, Onion
Hindi name	:	Piyaz

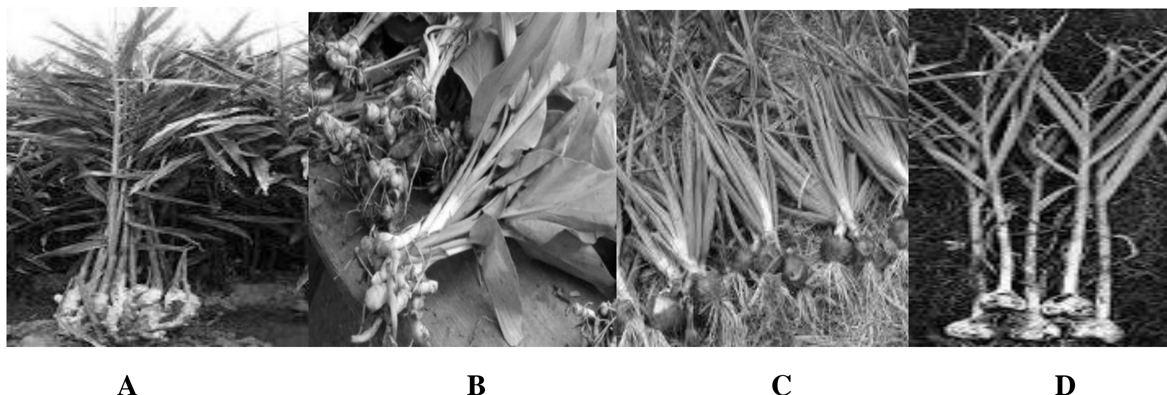
Botanical characteristics

Onion is an herbaceous, herb, biennial plant but usually treated as an annual because harvested in its first growing season, and cultivated for its edible bulb. The plant is attain a height upto 0.5m

with stem having a flattened disc at the base and tubular leaves form a pseudostem where their sheaths overlap. The leaves are erect or oblique and 4-8 per plant. The bulb is oval to spherical in shape and made up of several layers, each corresponding to afresh modified scale (leaf) that envelop a central bud at the tip of the stem. It prefers well drained, fertile, sandy and clay loamy soil for commercial cultivation.

Uses

Onion produces various sulfur containing organic compounds and provide defense through their antibacterial and antifungal properties, and also be benefit in preventing and treating heart diseases, atherosclerosis, diabetes, cancer and asthma. It is more commonly used for cold treatment. Onion contains chemicals that are used to reduce swelling or inflammation, effective in asthma, and to reduce cholesterol and sugar in the blood.



A **B** **C** **D**

Fig.14.2 Plant with rhizome and bulbs of (A) Ginger; (B) Turmeric; (C) Onion and (D) Garlic

14.2.2.4 Garlic

Botanical name	:	*Allium sativum* L.
Family	:	Liliaceae
Eng. Name	:	Garlic
Hindi name	:	Lahsun

Botanical characteristics

Garlic is a herbaceous, annual, bulbous plant grow upto 0.5m in height with short, woody central stem or a softer pseudostem made up of overlapping leaf sheath and possess long and blade like leaves. The compound bulb of Garlic consists of several bulblets or cloves. Garlic can grow in different types of soil, however well drained, organic rich, sandy, silt and clay loamy soil are recommended for cultivation.

Uses

The Garlic is contains antibiotic substances that check or inhabit the growth of certain fungi and bacteria. Garlic contains many nutrients including Vit. B_6, C, as well as dietary minerals,

calcium, potassium, manganese, iron and zinc. The health benefits are caused by sulfur containing organic compounds allicin and allisatin. Garlic is used for earaches, abnormal cholesterol level, stomach ulcer and dialated bronchitis. It is given for the treatment of fever, cough, headache, stomach ache, joint pain, diarrhea, tooth sensitivity, high blood pressure, and hypertension and throat infection.

14.4.3 Drugs obtained from bark

14.4.3.1 Quinine

Botanical name : *Cinchona officinalis* L.

Family : Rubiaceae

Eng. Name : Calisaya

Hindi name : Sinakona, Kunaina

Botanical characteristics

The genus *Cinchona* belongs to the family Rubiaceae native to the Andes of South America and in India grown in Nilgiris and Anamalai hills of Tamil nadu. *Cinchona* plants include 23 species of trees and shrubs with evergreen foliage, growing 5-15m in height. The leaves are opposite, petiolate, triangular to ligulate and the inflorescence is terminal, with many pink or purple coloured, sessile to pedicellate flowers and capsular fruit containing numerous seeds. Cinchona prefers porous, well drained, humus rich fertile soil with high water holding capacity.

Uses

The bark of *Cinchona* plant has been the source for the febrifuge chemical quinine, effective against malaria.

14.4.3.2 Arjun

Botanical name : *Terminalia arjuna* (Roxb.) Wigth & Am.

Family : Combretaceae

Eng. Name : Arjun tree

Hindi name : Arjuna

Botanical characteristics

Arjuna is large, deciduous tree, with vast spreading crown and branches drop downwords, and grows upto 35m in height. Its leaves are usually broad, sub-opposite, conical with 10-15 cm long and 4-7cm broad in size. The flowers are sessile and occur in simple or panicled spikes. The tree prefers humus rich alluvial loamy or black soil with good drainage and water holding capacity.

Uses

The bark of Arjuna is astringent, sweet, acrid, cooling, aphrodisiac, urinary astringent, and expectorant, but, chiefly use as cardio tonic as it improves blood supply to heart. The bark of Arjuna tree has inflammatory properties used for the treatment of ulcer, asthma, cirrhosis of liver and hypertension. The powdered dry bark taken with honey is used to strength the fractured bones. The extract of the Arjuna bark considered to be a potent reducing agent to diabetes.

14.4.3.3 Barberry

Botanical name : *Berberis aristata* DC

Family : Berberidaceae

Eng. Name : Indian Barberry

Hindi name : Daruhaldi

Botanical characteristics

Indian Barberry is a much branched shrub 2-4m in height, with pale yellow branches, and thick rigid evergreen shining, ovate to elliptical leaves with spiney margins. The flowers are produced singly or in racemes, pale yellow, and fruit is a small berry, oblong-ovoid shaped and dark purple in colour.

Uses

The roots of Indian Barberry are used for treating ulcers, ophthalmia, jaundice and fever. The fruit is edible, rich in Vit. C used as cooling and laxative. The extract of bark and wood is used as antibacterial, laxative and tonic.

14.4.4 Drugs obtained from stem and wood

14.4.4.1Ephedra

Botanical name : *Ephedra gerardiana* Wall.

Family : Gnetaceae

Eng. Name : Ephedra

Hindi name : Khanda

Botanical characteristics

Ephedra is erect, procumbent or climbing gymnosperm, shrub or vines and a total of 65 species has been reported. The stem is green and profusely branched. The leaves are reduced to scales about 1cm long, are opposite or in whorls of three, connate at base to form a sheath, nodes of green branchlets that resembles to the horsetail. The species of Ephedra grow on shores or in sandy soils with direct sun exposure.

Uses

Ephedra plants have traditionally been used by indigenous people for a variety of medicinal purposes. Ephedra plants contain many alkaloids. The ephedrine and pseudoephedrine are active constituents of Ephedra. The stems are the main source of the famous and wonder drug-Ephedrine. It is used to relieve nasal and sinus congestion and also for the treatment of night wetting. The extract of stem is used for treatment of asthma, hey fever and cold.

A **B**

Fig. 14.3 (A) Ephedra, (B) Giloe

14.4.4.2 Giloe

Botanical name	:	*Tinospora cordifolia* Wild Miers ex. hook
Family	:	Meninspermaceae
Eng. Name	:	Indian Tinospora, Heartleaved Monoseed
Hindi name	:	Giloe, Giloy

Botanical characteristics

Giloe is a large, perennial, glabrous, deciduous climbing shrub. The stem is succulent with long filiform fresh aerial roots from the branches with membranous, cordate with a broad sinus. The bark is gray-brown or creamy white. The flowers are small, yellow or greenish yellow with drupe ovoid, glossy and succulent fruit.

Uses

Giloe plants are rich in protein and minerals like calcium, phosphorous etc., and traditionally been used for a variety of medicinal purposes. Giloe plants contain many alkaloids including tinosporol, tinosporon, giloin, berberine etc. and are the active constituent. The plant is used in diabeties, dyspepsia, fever and urinary disease. The young leaves bruised in milk, and used as a liniment in erysipelas. The Giloe are used for treatment of gout. The watery extract of roots is used for treatment of leprosy. The dried powder of fruits used for the treatment of jaundice and rheumatics disease.

14.4.5 Drugs obtained from leaves

14.4.5.1 Aleovera

Botanical name	:	*Aloe vera* (L.) Burm.f.
Family	:	Liliaceae
Eng. Name	:	Aleo
Hindi name	:	Ghigver, Ghrita-kumaari, Gheekuvar

Botanical characteristics

Aleovera is a shrubby, arborescent, perennial, succulent and xerophytic plant. The plant grows upto 1m in height with a very short thick, cylindrical and woody stem. It has a triangular, thick, fleshy leaves with serrated small white teeth on margins. The flowers are borne in cylindrical, terminal racemes on scape and produced on a spike

Uses

Aleo vera plant is best known and use for health, medicinal and skin care properties. Aleo vera leaves has translucent gel, which is bitter in taste and contains 75 potentially active constituents including vitamins (Vit. A, B_1,B_2, B_6, B_{12}, C & E), enzymes, minerals, sugars and amino acids (eight essential amino acids) and believed to help in sustaining youth, due to positive effect on skin, and hence known as *Gheekumaari* or *Gheekuvar*. Aleovera extract has healing, anti-inflammatory, antiviral, antitumor and laxative properties and used in skincare, pharmaceutical and cosmetic industries. The pulp of leaves are used for the treatment of fever, cold, cough, skin disease, constipation, skin disease, enlargement of liver and rheumatic affection. Aleo is regarded as valuable in the treatment of piles and rectal fissures.

14.4.5.2 Holy Basil

Botanical name	:	*Ocimum sanctum* Linn.
Family	:	Labiateae
Eng. Name	:	Holy basil
Hindi name	:	Tulsi

Botanical characteristics

Tulsi is widely grown sacred plant in India. Tulsi is a branched, erect, fragrant, herb or subshrub, annual or perennial plant, attains a height upto 1m with hairy stem. The leaves are oval to elliptical in size with the margins being entire or toothed and flowers are arranged in verticillester inflorescence as close whorls on elongated racemes.The fruit of Tulsi is caeruleus. Tulsi can grow better in rich loam to poor laterite soils with proper drainage. The long day and high temperature conditions enhance the growth and higher oil production. The leaves of Basil are offered for the worship of lord Vishnu in Hindu mythology/rituals.

Uses

Ocimum species are diversely used in indigenous system of medicine for its antifungal, antibacterial and insecticidal properties. Eugenol with linalool and estragole are the major constituent responsible for therapeutic potential of Tulsi. The species of Holy basil contains economically important essential oil used in perfumery and cosmetics industries. Tulsi has been used in Ayurveda for treatment of various diseases. Tulsi is an ingradient of Tefroli, a propriety medicine for viral hepatitis. Tulsi plant is used in case of insect bite, fever, skin, bronchial asthma, bronchitis, diarrhea, dysentery, cardiac disorder, eye diseases etc. Basil leaves juice with honey is good for obese person.

A **B**

Fig. 14.4 (A) Aleovera; (B) Holy Basil

14.4.6 Drugs obtained from flower

14.4.6.1 Saffron

Botanical name	:	*Crocus sativus* L.
Family	:	Iridaceae
Eng. Name	:	Saffron
Hindi name	:	Kesar

Botanical characteristics

Saffron is the dried tri-lobed stigmas, obtained from flower of *Crocus*. Saffron is a small bulbose, 15-20 cm in height, perennial, with an underground corm and the flowers are large, blue or violet coloured and arising directly from corms. The flowers have tri-lobed orange coloured stigma used as spice and is the part of commerce. Crocin, a carotenoid chemical compound, is the chief colouring while sarranal is the main flavouring agent. Saffron can grow well drained sandy to clay loam, and tolerate frost and snowfall.

Uses

Saffron is used for medicinal and flavouring purposes. Saffron is an important ingredient of Ayurvedic and Unani medicine. Saffron is popularly known as stimulant, used in urinary,

digestive and uterine trouble. Saffron contains water soluble carotene called crocin, which is responsible to trigger apoptosis in different types of human cancer. Saffron is a stimulant tonic and effective for the treatment of cold and fever.

14.4.7 Drugs obtained from fruit

14.4.7.1 Bel

Botanical name : *Aegle marmelas* (Linn.) Corr.

Family : Rutaceae

Eng. Name : Indian Bael

Hindi name : Bel

Botanical characteristics

Indian Bael is a deciduous tree, attains 6-8m height with trifoliate leaves. The leaves are alternate, trifoliate (terminal leaflet with long petiole and two lateral leaflets almost sessile, and oval to lanceolate in size), serrate at margins and tapering at apex. The inflorescence is auxillary and terminal, and the flowers are borne on clusters in lateral panicles.The fruit is globous, oblong pyriform in shape, with grey or yellow rind and hard shell. Bael is considered as a sacred plant and its leaves have been offered for the worship of lord Shiva in Hindu rituals.

Uses

Bael fruits are used in the treatment of chronic diarrhea, constipation and peptic ulcers, as a laxative. Bael fruits are nutritive fruit, rich in riboflavin and used to prepare candy, squash, murabba and juice.

14.4.7.2 Opium

Botanical name : *Papaver somniferum* Linn.

Family : Papaveraceae

Eng. Name : Opium Poppy

Hindi name : Afim, Posta

Botanical characteristics

Opium poppy is an annual herb native to Turkey and attains a height to 1m. The stem and glaucous (waxy coated) silver green leaves bears a sparse distribution of coarse hairs. The leaves are simple alternate, exstipulants, ovate-oblong or linear-oblong, sessile and lobed. The fruit is a hairless, rounded, capsule topped with 12-18 radiating strigmatic rays. Opium poppy is cultivated for the production of poppy seeds (eaten by human beings) and for the production of opium mainly used in pharmaceutical industry. The poppy grown on almost all types of soils but the plant prefer well drained humus rich sandy loam type.

Uses

The unripe fruit of Opium poppy is used for the production of milky latex sap contains isoquinoline alkaloids, mainly the baine, morphine and oripavine classified as a narcotic which refers to the pain-relieving and sleep-inducing properties, and processed by pharmaceutical industry into drug such as codeine and oxycodone. Morphine is acetylated to produce diacetylmorphine, better known as heroin. Morphine is used as a narcotic, sedative, anodyne, antispasmodic, hypnotic, and used in many cough medicine, for allaying diarrhea and vomiting and to reduce blood pressure and bleeding. Codine is a mild analgesic. The Poppy seeds are free form narcotics, and used in bakery food items *i.e.,* bangels, mufflis and cakes and for seasoning. Paperverine is an antispasmodic and also used as vasodilator to relief pain in coronary and cerebral thrombosis. Paperverine provides relief in asthma.

14.4.7.3. Emblic

Botanical name : *Emblica offcinalis* Gaertn.

Family : Euphorbiaceae

Eng. Name : Emblic, Indian gooseberry

Hindi name : Amla

Botanical characteristics

The tree is small to medium size tree grow to 9-12m in height with a crooked trunk and spreading branches. The branchlets are glabrous or finely pubescent, usually deciduous, with the leaves simple, pinnate, subsessile, and closely set along the branchlets. The flowers are greenish-yellow, borne in axillary fascicles, and produce globous shaped, six lobed, quite hard, translucent shiny skin fruit. The raw fruit contain high acidic nature and astringent taste, is considered unacceptable for fresh consumption.

Uses

The Indian Gooseberry is quite tart, nutritional and fibrous to eat and used as such, powder, juice, oil, spices and in combination with other plant products. Amla is abundant in Vit. A and C, also contains minerals i.e., calcium, potassium, phosphorous, iron, carotene, magnesium etc. and folic acid. Amla has antioxidant, anti-inflammatory, diuretic effect and used to enhance immunity and to boost skin and hair health. Gooseberry contains chromium, which has therapeutic values for diabetic patients. Amla is used as a main component in Ayurvedic formulations, triphala churna and chavanprash. Amla is also used to boost vitality and vigor and has ability to re-energize the liver. Amla seeds have aphrodisiac and antipyretic properties, and are useful in treatment of biliousness, leucorrhoea, vata and vomiting.

14.4.7.4 Long Pepper

Botanical name : *Piper longum* Linn.

Family : Piperaceae

Eng. Name : Long Pepper

Hindi name : Pipli

Botanical characteristics

Long Pepper is a slender, much branched, creeping undershrub. The fruit of pepper consists of many minuscule fruit embedded in the surface of a flower spike. The spike of Long Pepper contains piperine and piplaritine alkaloids. Long Pepper flourishes well in organic matter rich, well drained loamy soil.

Uses

Long Pepper is used to improve appetite and digestion, as well as treat stomachache, heartburn, indigestion, intestinal gas, diarrhea and cholera. Long Pepper is also used with other herbs in Ayurvedic medicine and has haematinic, diuretic and digestive properties. In Ayurveda the root is used as a carminative, tonic to the liver, stomach-ache. Long Pepper is help in eliminating toxins from the liver. It is also used for the treatment of headache, toothache, beriberi, fever, muscle pain, nasal discharge and intestinal worms.

14.4.7.5 Black Pepper

Botanical name : *Piper nigrum* L.

Family : Piperaceae

Eng. Name : Black Pepper

Hindi name : Kali Mirch

Botanical characteristics

Black Pepper plant is a perennial, woody, climber, evergreen plant, attain 5-9 m in length and the vines branches form the nodes. The black pepper leaves are simple, round or obtuse, alternate, smooth, lanceolate and varied in leave shape. The inflorescence is spike type, 3-15 cm long with 60-150 minute white to yellow flowers, and the fruit is a one seeded berry, sessile, globose to oval in size and matured within 6 months. Black Pepper is a crop of humid tropics with adequate rainfall and humidity; prefer to planting towards the eastern slopes with well drained humus rich laterite or alluvial soil. The alkaloid piperine is major constituent responsible for bitter taste of black pepper with other major pungent alkaloids viz. chovicine and peiperidine.

Uses

Black Pepper has extensive medicinal value and used for home remedies and Ayurvedic medicine. Black Pepper used in medicine as a carminative and febrifuge, for digestion and common cold cure. Black Pepper considered as a remedy for various anatomical disorders. It is used with honey as traditional approach, helping to expectorate and dry up mucus membrane and prescribed as an effective cure for cold and flu remedy. Black pepper is a rich source of minerals

i.e., potassium, calcium, magnesium, phosphorous, sodium, vitamins *i.e.,* thiamin, riboflavin, niacin, B_6 and dietary fiber. Black pepper is used for treatment of sinus, asthma and nasal congestion. The antioxidant of Black pepper can prevent or repair the damage caused by free radicals and help to reduce the risk of cancer, cardiovascular diseases and liver problems.

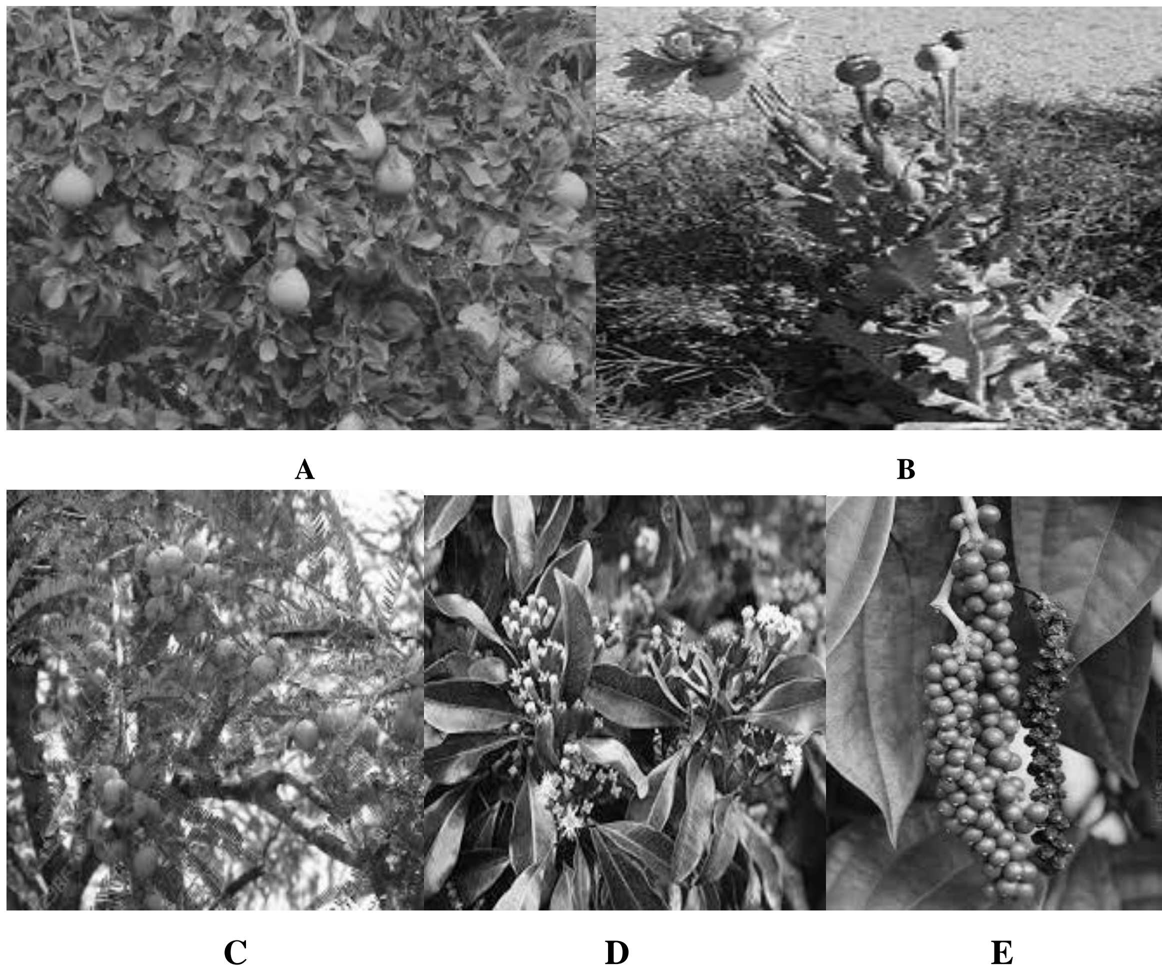


Fig. 14.5 Medicinal Plants with fruits (A) Bel (B) Opium (C) Emblic (D) Long Pepper (E) Black Pepper

14.4.8 Drugs obtained from seeds

14.4.8.1. Castor

Botanical name	:	*Ricinus communis* L.
Family	:	Euphorbiaceae
Eng. Name	:	Castor
Hindi name	:	Arand

Botanical characteristics

Castor is a fast growing, perennial, suckerling shrub or a small size tree with erect, herbaceous, branched and hollow stem, young branches covered with hair like outgrowth. The leaves are

petiolate, exstipulate, alternate and deeply palmately lobed. The inflorescences of castor are raceme or panicle and terminal, and fruit is covered by spinous outgrowth and called regma.

Uses

Castor seeds are the source of castor oil and have a number of uses. Castor oil is a viscous, pale yellow, non volatile and non drying oil, very high boiling point with a bitter taste. The seeds contain contains 40-60% oil that is rich in triglycerides mainly ricinolein, a toxic alkaloid ricinine and very toxic albumen called ricin. The castor oil is used as a laxative. The castor oil is also used skin and hair care.

14.4.8.2 Fenugreek

Botanical name	:	*Trigonella foenum-graccum* L.
Family	:	Fabaceae
Eng. Name	:	Fenugreek
Hindi name	:	Methe, Methi

Botanical characteristics

Fenugreekis an annual herb, grow 30-90 cm in height, and has pinnately trifoliate, light green leaves. The plant produces 8-12 cm long, slender pods, contains 10-20 small, hard, oblong and yellow colour seeds. Fenugreek is cultivated as a leafy vegetable. Fenugreek is fairly frost tolerant plant grow in the tropics and temperate regions and can be cultivated in all types of well drained and humus rich soils.

Uses: Fenugreek is considered as important medicinal plant and its leaves and seeds have been used in various illnesses and as a health tonic since very long time. Fenugreek is used to boost immunity and treatment of cold, cough and flu. Fenugreek is used for stomach inflation, arthritis, obesity and antherosclerosis (hardening of arteries). The water of Fenugreek seed soaked is excellent forregulating blood sugar level, boosting metabolism, digestive system and to promote lactating mothers. Fenugreek seed can nourish, moisturize and anti-aging effect, and used to the skin and hair care. Methi seeds are good source of magnesium.

A **B**

Fig. 14.6 (A) Castor and (B) Fenugreek

14.5 AROMATIC PLANTS

Aromatic plants possess pleasant or distinctive odorous volatile substances in plant parts, as a result of a chemical combination of acids and alcohol that produces an essential oil. Aromatic plants are cultivated for the production of essential oils which are synthesized and accumulated in oil cells, in secretion ducts or cavities or in glandular hairs of plants. An essential oil is a mixture of chemical compound, chiefly containing terpenoids and evaporates when exposed to air at ordinary temperature. The most widely used procedure for extracting essential oils from the plant is steam or water distillation. Solvent extraction processes may be used when plants contain small amount of essential oil. The essential oil extracted from aromatic plants is used in perfumery, food industry, beverages as well as pharmaceutical and aroma therapeutic products.

For the sake of practical convenience the crops are classified into four groups-

Aromatic grasses e.g., lemmon grass, vetiver etc.

Aromatic herbs and shrubs e.g., mint, fennel, ginger, ajowain, cumin etc.

Aromatic trees e.g., sandlewood, cinnamon, nutmegetc.

Aromatic flowers e.g., rose, jasmine, champacaetc.

Some of the commercially cultivated and important aromatic plants are described below.

14.5.1 Lemmon Grass Oil

Botanical name : *Cymbopogon flexuosus* (Steud.) Wats.

Family : Poaeceae

Eng. Name : Indian lemon grass

Hindi name : lemon grass, Nimbu Ghas

Botanical characteristics

Indian lemongrass is a perennial grass, grown for its fragrant leaves and stalks which are used as flavouring agent. Lemon grass grows as dense clumps grow upto 1.8m in height, and has several stiff stems and slender blade like leaves which droops towards the tips. The leaves are long blade like, blue-green in colour and have a strong lemon fragrance. It produces large compound flowers on spikes. The essential oil extracted from lemon grass containing 75% of aldehyde, chiefly citral.

Uses

Indian lemongrass is used commercially for lemon scent and flavouring in a wide array of food, beverages and pharmaceutical products. The antimicrobial and antioxidant activities of essential and its chemical component like vetivone, zizanal, epizizanal etc. are well known. The young or dried leaves are used to flavour dishes, in soups, tea, stews or other drinks. The essential oil

extracted from lemongrass has anti-inflammatory and anti-cancer properties. The oil is used for the preparation of Vitamin A. It is also used in perfumery, soap and cosmetic industries.

14.5.2 Jasmine Oil

Botanical name	:	*Jasminum auriculatum* Vahl. (Juhi)
		Jasminum officinale Linn. *var. grandifolrum* Linn.. (Mogra)
Family	:	Oleaceae
Eng. Name	:	Jasmine
Hindi name	:	Bela, Mogra

Botanical characteristics: Juhi is a stunning, small, climbing, bushy shrub plant with simple, ovate, dark green coloured small leaves, and white flower plant. The leaves are alternate, pinnate or trifoliate, opposite, oval shaped and short spiny pointed. The flowers are in many flowered cyme and fragrant. The Juhi plant can grow in full sunny and moderate shade and dry to moist soil, but it prefer a fertile humus rich, well drained moist soil. Juhi plant is cultivated for its essential oil and traditionally used for medicine. Jasmine flower is a rich source of volatile oil, linalol and jasminol with other alkaloid, acids, terpenoides and flavonoids.

Uses: Juhi leaves are used in the treatment of mouth ulcer. In Ayurveda, Juhi is mentioned for treatment of wounds, headache and disease of the oral cavity. The flower is held sacred to all forms of Goddess Devi and used as sacred offering during Hindu religious ceremonies. The essential oil of Juhi is used in perfumery and skin care products to provide scent.

14.5.3. Field Mint

Botanical name	:	*Mentha arvensis* Linn.
Family	:	Labiatae
Eng. Name	:	Field Mint
Hindi name	:	Pudina

Botanical characteristics

Field Mint is a herbaceous, perennial herb attaining a height upto 0.5m . The leaves are in pair at stem axis, simple, opposite, oval shaped, hairy with coarsely serrated margin. The flowers are pale purple and present in whorls on the stem at the base of the leaves. Field Mint contains many alkaloids i.e., menthol, methanone, isomenthone, piperitone etc.

Uses

Field Mint is traditionally used as carminative, refrigerant and stimulants. Field Mint is used as appetizer and use in stomach and gastric trouble. Menthol, commercially alkaloid extracted, is used in dental care as antibacterial agent, while the oil extracted fromfield mint is widely used in pharmaceutical, beverages and tobacco.

14.5.4 Khus Oil

Botanical name	:	*Vetiveria zizanioides* (L.) Nast
Family	:	Poaeceae
Eng. Name	:	Vetiver
Hindi name	:	Khas

Botanical characteristics

Vetiver, commonly known as Khas grass, is a densely tufted, glabrous and perennial grass. The plant grows in large, erect culms with much branched root stock and plant grows 0.5-1.5m in height. The leaf blades are relatively stiff, glabrous, long and narrow. The inflorescence is spike like branches, racemose and rachis disarticulating at base of sessile spikelet. The Khas grass prefers loamy alkaline soil and required well distributed rain fall throughout the year.

Uses

Vetiver roots contain fragrant essential oil, and mainly used in perfumes, cosmetics, aromatherapy, food and flavouring industries. The Khas oil is diaphoretic, stimulant and refrigerant, and use in colic, flatulence and abstinate vomiting.

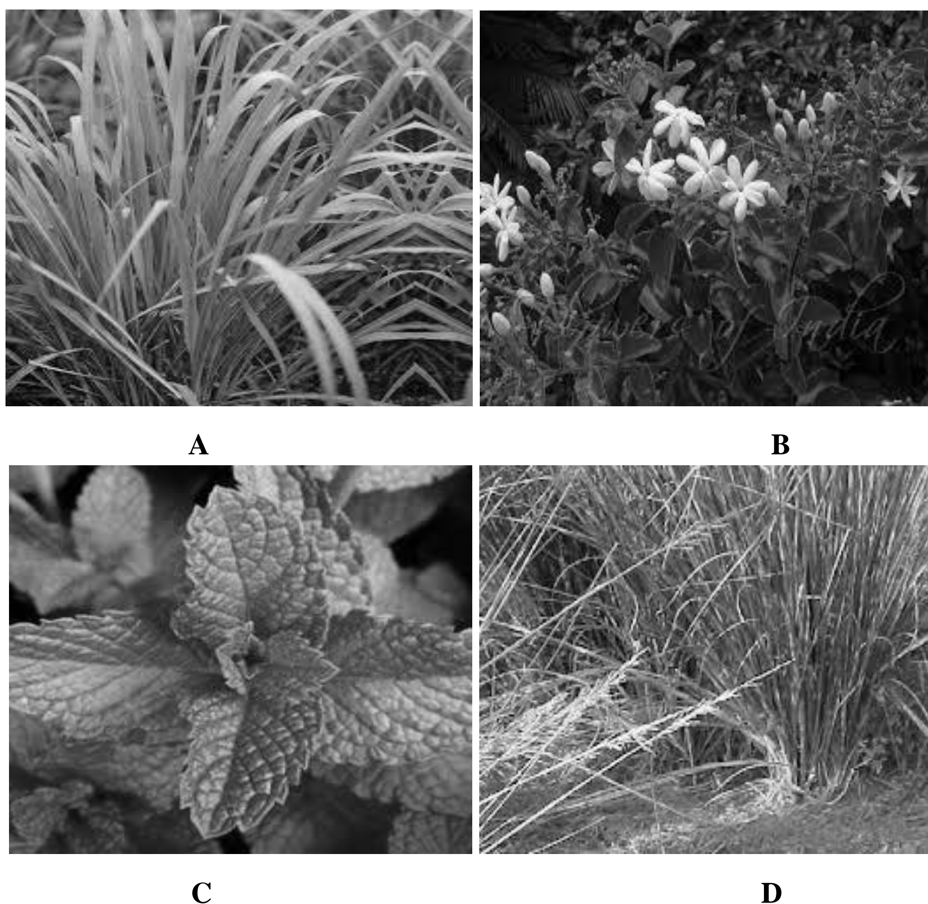


Fig. 14.7 Aromatic Plants (A) Lemmon Grass; (B) Jasmine; (C) Field Mint and (D) Khus

14.6 SUMMARY

Fats and Oils are important energy sources in food and recognized as essential nutrient in both human and animal diets. In plants, the fatty oils are stored as reserve food mainly in seeds, along with a less extent to other plant parts. Fats and Oils are concentrated sources of energy and provide essential fatty acids, which are the building blocks for the hormones needed to regulate bodily system and are a carrier for the oil soluble vitamins A, D, E and K. They also enhance the texture and imparting flavour to the food. Majority of oils are marketed and consumed in refined form which is physically and chemically treated. While a small part of edible oil i.e., pressed oil, is used in raw form only after purification.

Chemically, the main component of edible fats and oils are triglycerides of complex organic fatty acids i.e., consists of three fatty acids attached to one glycerol molecule, which is either saturated or unsaturated. They are known as fixed oils as they are non-volatile in nature. The minor components include monoglycerides and diglycerides, free amino acids, phosphatides, sterols, fat-soluble vitamins, tocopherols, pigments, waxes and fatty alcohols. Other than the free fatty acids, crude vegetable oils contain approximately two percent of these minor components. On the basis of absorption of atmospheric oxygen, plants fats and oils are categorized into four categories- drying oils, semi-drying oils, non-drying oils and vegetable fats. The refined grades of drying and semi-drying oils are commercially used as edible oil. Whereas inferior grade drying and semi-drying oils along with non-drying oils are used in preparation of soap, varnish, paint, lubricants, candle etc.

Edible oils and fats serve as a source of nutrients and important to consistency and flavor to foods. They act as tenderizing agents, facilitate aeration, carry flavors and colours, and provide a heating medium for food preparation. Vegetative oil has been used in cosmetic and skin care products, due to their healing and nurturing properties.

India is unique among geographical regions of world with high biological diversity and has been considered a treasure of valuable medical and aromatic plant species. Medicinal plants have played a significant role in many ancient traditional or indigenous system of medication such as Ayurvedic and Unani system in India, and it has been in existence for several centuries which is associated with human, livestock and plant health. The derivatives of medicinal plants used for pharmaceutical, cosmetics and aromatherapy products with no or least side effects. The extracted chemical compounds of plant used in drugs are merely metabolic byproducts and hard to ascribe any biological significance to the plants except protective function, but they are so valuable to the mankind in the treatment of diseases.

Medicinal and Aromatic plants are more recently used, in a slightly broader sense, distinguishing the fragrant constituent-containing group of medicinal plants. The demand of raw material is regularly increased for industrial processing coupled with the loss of natural habitats of most medicinal plants, hence large scale cultivation of promising species has been required to fulfill demand, conserve natural habitats and utilize waste lands.

14.7 GLOSSARY

Analgesic: A substance that relieves pain. (Examples: aspirin, Balsam Poplar.)

Antifungal: An agent that hill or inhibit fungal growth.

Antioxidant: A substance that prevents or inhibits oxidation (a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms). It can slow down the damage caused by free radicles, unstable molecules that the body produced as a reaction to environmental and other pressure.

Antiphlegistic: Any plant or plant derivative has capacity to reduce fever or inflammation.

Antipyretic: A drug that reduces fever.

Aromatic: A drug which is fragrant, spicy and mildly stimulant.

Asthma: Achronic disorder that can cause coughing and wheezing and shortness to breath.

Bronchitis: Inflammation of the mucus membranes on the bronchi, usually caused by an infection, allergies or chemical irritations.

Crude oil: The oil obtained from extraction either mechanical or solvent based extraction.

Diuretic: A substance that increases the flow of urine, either by increasing permeability of the kidneys' nephrons, increasing blood supply into the nephrons, or increasing the blood into each kidney by renal artery vasodilation.

Essential oil: An volatile organic oil derived from a plant, processing the order and other characteristics properties of plants. They are primarily composed of terpenes with other organic compounds and used in perfumes, flavouring and pharmaceuticals.

Gum Resin: Gum resin is natural exudation from plants and trees consisting gums and resins with trace amount of essential oils.

Iodine value: Iodine number denotes the amount of unsaturation in fatty acids. A higher iodine number indicates a higher degree of unsaturation.

Odour: The property of a substance which stimulate and is prescribed by the olfactory sense.

Oil: Esters of fatty acids and glycerol which normally are liquid at room temperature.

Oleogum resin: The exudation from tree trunks or barks of trees and characterised by the fact that consist of mainly oil and resin.

Refining: The process of removing impurities from crude oil by way of physical and chemical treatment.

Soap: Soap are water soluble sodium or potassium salts of fatty acids. Soap are made from fats and oils or their fatty acids, by treating them chemically with a strong alkali.

Smoke point: The temperature at which oil from a thin continuous stream of smoke on heating.

Terpenes: Any of a group of hydrocarbons that are made up of building blocks of isoprene (C_5H_8) or similar five-carbon units, with a monoterpene made up of two units (example: limonene and pinene), a sesquiterpene made up of three units (example: humulene, a Hops aromatic), and a diterpene made up of four units.

Tincture: Tincture is a cold alcoholic extract of natural fragrant material of vegetable origin, and the solvent being left in the extract as a diluent.

Tonic: A substance taken to strengthen and prevent disease, especially chronic disease.

Ulcer: An open sour on the skin.

Whooping cough: An acute contagious infection in respiratory track that cause a cough with sneezing and low fever.

14.8 SELF ASSESSMENT QUESTION

14.8.1 Select the correct answer

1. The nutmeg tree is the source of both nutmeg and

a) cinnamon b) black pepper

c) mace d) clove

2. Which of the following drug is used by opthamologists to enlarge the pupils of the eyes

a) caffine b) digitalis

c) belladonna d) papvarin

3. The opium poppy is the source of

a) morphine b) codeine

c) heroin d) all of these

4. The fatty oils which do not absorb atmospheric oxygen are known as

a) drying oil b) semi-drying oil

c) non-drying oil d) vegetable fat

5. Central Drug Research Institute is situated in

a) New Delhi b) Lucknow

c) Dehradun d) Hyderabad

6. Which of the following is an oil seed crop

a) *Arachis hypogea* b) *Solanum tuberosum*

c) *Cajanus cajan* d) *Triticum aestivum*

7. Opium is obtained from

a) dried leaves b) roots

c) latex of unripe fruit d) leaves

8. Asfortida is obtained fromexudation of

a) stem b)fruit

c) root d) leaves

9. Clover oil is obtained from which part of *Syzygium aromaticum*

a) leaves b) flower bud

c) rhizome d) wood

10. Which of the following is not an essential oil

a) Clove oil b) Eucalyptus oil

c) Sandle wood oil d) Groundnut oil

Answer key: 14.8.1: 1-(c), 2-(c), 3-(d), 4-(c), 5-(b), 6-(a), 7-(c), 8-(c), 9-(b), 10-(d)

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14.11 TERMINAL QUESTIONS

14.11.1: Very short answer type questions

1. Ephedrine is obtained from?
2. Write botanical name of Bellodona.
3. Write botanical name of Aconite.
4. From which plant reserprine is obtained?

14.11.2: Short answer type questions

1. What is the economics importance of Gileo.
2. Write botanical name of three medicinal plants with family and uses.
3. Differentiate between medicinal and aromatic plants.
4. Write botanical name and family of three aromatic plants.
5. Write botanical name and family of three medicinal plants from which drug is obtained from bark.

6. Write botanical name and family of three medicinal plants from which drug is obtained from seeds.
7. Write botanical name and family of three medicinal plants from which drug is obtained from fruits.
8. Write botanical name and family of three medicinal plants from which drug is obtained from roots.
9. Write botanical name and family of three medicinal plants from which drug is obtained from underground stem or tubers.
10. Differentiate between drying and non-drying oils.
11. Differentiate between saturated and non-saturated fatty acids.

14.11.3: Long answer type questions

1. Define vegetable oil and its types. Describe any four edible oil yielding plants with botanical description and their uses.
2. Write a detailed account on Medicinal and aromatic plants.
3. Describe any five medicinal plants of your locality with botanical description and economic importance.

UNIT-15: TEST AND MICRO TEST FOR DETECTING ADULTERATION IN IMPORTANT FOOD PRODUCT

15.1 Objectives
15.2 Introduction
15.3 Impact of adulterant on health
15.4 Experiments for adulteration
15.4.1 Test to detect adulteration in milk and milk product.
15.4.2 Test to detect adulteration in sugar, jaggery, honey and its products
15.4.3 Test to detect adulteration in flour, gram flour and its products.
15.4.4 Test to detect adulteration in spices.
15.4.5 Test to detect adulteration in tea and coffee.
15.4.6 Test to detect adulteration in oils and ghee.
15.4.7 Test to detect adulteration in fruit and fruit juice.
15.4.8 Test to detect adulteration in different vegetables.
15.5 Methods of preparation of different reagents in test.
15.6 Chemicals(formula) list.
15.7 Equipment used in experiments.
15.8 Precaution during experiments.
15.9 Summary
15.10 Glossary
15.11 Self assessment questions.
15.12 References
15.13 Suggested Readings

15.1 OBJECTIVES

From this unit you will be able to-

- Know about basic concept of adulteration.
- Know about the common food adulterant present in different food.
- Know about the Impact of adulterant on health.
- Perform different test and micro test for identification of adulterant.
- Induce awareness in society.

15.2 INTRODUCTION

"WE ARE WHAT WE EAT"

Pure, fresh and healthy food is the basic necessities for sustenance of life.The original ingredient of food is important because of its nutritional value, toxicological potential and interactive effect. The quality of food has been changed in whole world specially developing countries like India due to climatic change, man- made menace of adulteration. In the recent times, media has highlighted many cases of adulteration of food and food products with various types of adulterants. In generally every food industry in India is facing problem of adulterated food. The practice of adulterating food and food products is one of the important problems that stand against the progress of food industry in India and it may have also detrimental effect on export of food and food products.

Adulteration can be defined as the addition and deletion of any prohibited substance(vital/non-vital) to or form food and food products so that the natural composition and quality of food substance become deteriorate. An adulterant is a substance which is used in adulteration process. Food adulteration may occur at any stage of food processing from production to selling. Food adulteration is of many types like intentional adulteration, non-intentional/accidental adulteration, natural adulteration, metallic adulteration, packaging/ handling adulteration. The image of food and food products has been considerably deteriorated due to its adulteration with harmful chemicals. In the recent past, the menace of adulteration has taken serious proportion as highlighted by many media report as well as FSSAI report.

Over the years, efforts have been made to develop simpler methods to detect adulteration of food and food products. This unit provides the overview of analytical methods covering various aspect of adulterant being added to the food and food product. It includes various tests and micro-tests (qualitative) that will help students to identification of adulteration in food. Qualitative detections are advantageous because these are simple, rapid and very easy toperform. These methods may be suited to a small laboratory and a limited number of samples to analyse. Procedures generally require chemicals and equipment routinely available in an analytical laboratory and do not require expensive instrumentation.

15.3- IMPACT OF ADULTRANT ON HEALTH

Adulterant addition is only beneficial to the producers/ manufacturers always harmful to the consumers.Some of adulteration are very harmful in nature and produce negative impact on public health. Adulteration causes chronic as well as acute disease.

Mislabeled food product consumption may cause allergies. Consumption of adulterated food causes various disorder in human like cancer, insomnia, neurological problems, liver disorder, diarrhea, stomach disorder, lathyrism, vomiting, dysentery, joint pain, heart diseases, food poisoning, abortion, weaken the immune system, brain damage etc.Adulteration in food also influence the nutritional value of food thus may produce nutritional deficiencyin our body.

15.4 EXPERIMENT TO DETECT ADULTERATION

15.4.1 Test to detect adulteration in milk and milk products

Requirements: Sample of milk and milk productsand

Chemicals: Absolute alcohol/ Ethanol, Aminobezaldehyde solution(DMAB), Tincture iodine, Furfural solution, Chromotropic solution, Soyabean powder, 0.5% Bromothymol solution, HCl, H_2SO_4, Ferric chloride, Rosalic acid solution, Lemon juice, Resorcinol solution, Methylene blue dye, Chloroform, 2% Diphenyl amine solution, Mercuric chloride solution, Nessler's reagent, 10% NaOH, 0.1N Silver nitrate, 10% Potassium chromate etc.

Equipment: Test tubes, Test tube holder, Flask, Bunsen burner/ Sprit lamp, Test tube stand, Pipette, Filter paper, Litmus paper, Beaker, Measuring cylinder, Turmeric paper strip, Glass rod, etc.

Test to detect adulteration in milk and milk products

S.N.	Added adulterant	Experiment	Observation	Result
1	Urea in milk	Take milk sample in test tube. Add soyabean powder in it. Mix properly and left for 5 min. After that dip litmus paper for 30 seconds.	Colour of litimus paper will change from red to blue.	Positive
		Take equal amount of milk sample and tri chloroacetic acid in a test tube. Mix and filter. Take 3ml filtrate in another test tube and add 3 ml DMAB.	Colour will change white to bright yellow.	Positive
2	Starch in milk and various	Take 5 ml milk/melted sampleof milk product in the test tube. Boil	Colour will change white to blue.	Positive

	milk product like paneer, butter, ghee etc	for 4 min, after cooling add 1-2 drops of tincture iodine and shake.		
3	VANASPATI/ Refined oil in milk and milk product like ghee, butter etc.	Take 3 ml milk/melted ghee/ butter sample in test tube than add 10 drops of HCl and mixand add one tea spoon sugar and shake gently. Take 100 ml melted sample of ghee/ butter in test tube. In another test tube mix 0.1 ml furfural solutions with 10 ml conc. HCl. Now mix melted sample with furfural acid solution. Shake well and allow mixture to stand for 10 min.	Red colour will appear after few minutes. Crimson colour will appear.	Positive Positive
4	Formalin in milk	Take 10 ml milk sample in test tube. Add 5ml sulphuric acid (containing little amount of ferric chloride) from the wall of test tube without shaking. Take 1 ml sample milk in test tube. Add 1 ml chromotrophic acid and mix well.	Violet or blue ring will appear at the junction of two layers. Yellow colour will appear.	Positive Positive
5.	Coal tar dye in ghee, cheese, condense milk, khoa, milk powder etc.	Take melted sample of milk product in a test tube and add 5 ml of dil. H_2SO_4 or dil. HCl than shake well.	Pink colour will appear in case of H_2SO_4 and crimson colour will appear in case of HCl appear.	Positive
6	Blotting paper in Rabri	Take a teaspoon of rabri in test tube and add approximately6-10 ml of dil. HCl/ dil. H_2SO_4. Stir the mixture with glass rod. Remove the rod and examine.	A fine fiber is visible on the glass rod.	Positive
7.	Neutralizer in milk	Take 5 ml milk sample in test tube, add 5 ml ethanol and mix well than add 2-3 drops of 0.1% rosalic acid solution.	Rose red colour will appear.	Positive

8.	Washing soda in ice-cream	Add few drops of lemon juice to ice cream.	Frothing will appear	Positive
9.	Sugar in milk	Take 1 ml milk sample in test tube, add 1ml resorcinol solution and mix gently.	Red colour will appear	Positive
10.	Detergent in milk	Take 1 ml of milk sample in test tube. Add 1 ml methylene blue dye followed by 2 ml chloroform in the test tube. Vortex the contents for about 15 sec and centrifuge at 1100 rpm for 3 min.	More intense blue colourwill produce in the lower layer	Positive
11.	Nitrate (pond water) in milk	Take 2 ml milk sample in test tube. Rinse the test tube with the milk and drain the milk from the test tube than add 2-3 drops of that2% diphenylamine solution.	Deep blue colour will appear	Positive
		Take 10 ml milk sample in beaker. Add 10 ml mercuric chloride ($HgCl_2$) solution in it. Mix and filter through filter paper. Now take 1 ml sample in test tube and add 4 ml of diphenyl amine solution.	Blue colour will appear	Positive
12.	Ammonium salt in milk	Take 5 ml milk sample in test tube. Add 1 ml of Nessler's reagent and mix well.	Yellow or grey colour will appear.	Positive
		Take 5 ml milk sample in test tube. Add 1 ml of 10% NaOH solution in such manner that should not touch the rim of the test tube and mix well. Place a piece of turmeric paper on the rim of the test tube and keep the test tube undisturbed.	Pinkish red colourwill appear	Positive
13.	Common salt in milk	Take 5 ml of milk sample in the test tube. Add 1 ml 0.1N silver nitrate solution. Mix thoroughly and add 0.5 ml 10% potassium chromate solution.	Yellow colour will appear.	Positive

15.4.2 Test to detect adulteration in sugar, jaggery and honey:

Requirement: Honey, sugar and jaggery samples and

Chemicals: Ether, 1% Resorcinol solution, Conc. HCl, Water etc.

Equipment: Mortar and Pastel, Evaporating dish, Test tubes, Beaker,Porcelain dish, Cotton wick, Glass, Bunsen burner/Sprit lamp, Match stick, Pipette, Dropper, Measuring cylinder, analytical balance etc.

Test to detect adulteration in sugar, jaggery and honey

S.N.	Test	Experiment	Observation	Result
1.	Sugar in honey	Take 5 ml honey with 5ml ether in pestle and mortar. Mix and decant off the extract in evaporating dish than pour into porcelain dish. Evaporate the ether gently on water bath, add 2drops of 1% resorcinol and 1drop of conc. HCl in residue. Take a glass of clean water. Add a drop of honey sample.	Cherry red colour will appear. Honey will disperse in water	Positive Positive
2.	Diluted sugar or (sugar + water) in honey	A cotton wick dipped in pure honey and lighted with a match stick.	Will not burn if burn will produce a cracking sound.	Positive
3.	Washing soda in jaggery/ brown sugar	Take melted jaggery/ brown sugar in a test tube and add few drops of conc.HCl solution.	Effervescence will appear	Positive
4.	Chalk powder in jaggery /brown sugar	Take 5 ml of jaggery/ brown sugar in test tube and dissolve it in water. Take a melted jaggery in test tube and add few drops of conc. HCl.	A powder will settle down. Effervescence will visible	Positive Positive
5.	Chalk in sugar	Dissolve approximately 10 gm of sugar sample in a glass of water and allow setting	Chalk will settle down	Positive

6.	Metanil yellow in jaggery/ brown sugar	Take appx. 5 mg jaggery/ brown sugar sample in test tube and add small amount of water to make jiggery solution. Add 5 ml of conc. HCl in it Take approx. 5 mg jaggery/ brown sugar sample in test tube and add 5 ml of ethyl alcohol. Shake vigorously and prepare solution than pour approx. 10-15 drops of conc. HCl solution	Magenta red colour will appear. Pink colour will appear.	Positive Positive

15.4.3 Test to detect adulteration in flour, gram flour, rice and its products

Requirements: Samples of wheat flour, Refined wheat flour , Gram flour, Rice sample.

Chemicals: Dil./Conc. HCl, Distilled water, Turmeric paper strip, 20% Salt solution, Ethyl alcohol.

Equipment: Test tubes, Test tube stand, Test tube holder, Beaker, Bunsen burner or Sprit lamp, measuring cylinder.

Test to detect adulteration in flour, beson, rice and its products

S.N.	Test	Experiments	Observation	Result
1.	Chalk in flour	Take a teaspoon of flour in test tube and shake it with 10 ml distilled water. Warm 20 ml of HCl in another test tube at 40-50°c and add it in the test tubecontaining flour.	Gas bubbles will release	Positive
2.	Boric acid in wheat flour or refined wheat flour	Take a small amount of sample in test tube. Add some water and shake. Then add few drops of conc. HCl and dip a turmeric paper strip in that solution.	Turmeric paper strip will turn Red.	Positive
3.	Ergot (fungi) in cereal grains	Soak sample cereal grains in 20% salt solution for some time.	Some grains of dark colour will float	Positive
4.	Kesari dhal	Take 5 mgsample of gram flourin	Pink colour will appear	Positive

	in gram flour	test tube and 3 ml ethyl alcohol. Mix gently add 5 ml of water with few drops of conc. HCl.		
5.	Metanil yellow in sella rice (parboiled rice)	Rub few grains in palm. Take few rice grains in test tube. Add dil. HCl and mix	Yellow colour will reduce or disappear. Pink colour will appear	Positive Positive

15.4.4 Test to detect adulteration in spices

Requirement: Different spices samples and

Chemicals: Water, Ethyl alcohol, 10% Ferric chloride solution, Conc. HCl,Tincture Iodine, potassium iodide solution etc..

Equipment: Glass, Burner/ Sprit lamp, Test tubes, Test tube holder, Test tube stand, Pastel and mortar, Burner/ Sprit lamp, Filter paper,Watch glass, Measuring cylinder, Analytical balance etc.

Test to detect adulteration in spices

S.No.	Test	Experiment	Observation	Result
1.	Papaya seeds in black pepper	A little amount of sample seeds dip in a glass of water. A little amount of sample dip in ethyl alcohol	Some seeds float over water surface. Some seeds will sink.	Positive Positive
2.	Argemone seeds in mustard seeds	Take few sample seed and crush them.	Some seeds are not yellow in its inner surface.	Positive
3.	Resin galbanum, colophony in asafoetida	Take pinch of Asafoetida sample in test tube and add few drops of water in it. Shake well. Take a small quantity of asafetida sample in a spoon. Burn it Take powder asafetida in test tube. Add ethyl alcohol in test tube and shake gently to form	Milky solution will form Sample will not produce bright flame like camphor. Olive green colour will appear.	Positive Positive

		solution. Filter the solution. Add few drops of 10% $FeCl_3$		Positive
4.	Metanil yellow in turmeric powder	Take approx. 5mg of sample in test tube and add 5 ml dil. HCl. Take a small amount of sample in test tube. Add 3 ml alcohol and shake to mix content thoroughly. Add approximately 10 drops of HCl.	Violet colourwill appear instantly which become persist. Pink colour will appear	Positive Positive
5.	Extraction of essential oil from clove	Put some clove in a glass of water.	Clove will float on the surface of water	Positive
6.	Starch in powder spices	Take 5mg spice sample in test tube and add approx. 5ml tincture iodine or iodine solution.	Blue colour will appear	Positive
7.	Lead chromate inturmeric	Take 5 gm turmeric sample in test tube, add 5 ml water and few drop of conc. HCl.	Pink colour appear	Positive
8.	Red lead in chilli powder	Take a pinch of chilli powder sample in test tube, add dil. HNO_3. Filter the solution and add the 2-3 drops of potassium iodide solution in filtrate.	Yellow ppt will form	Positive
9.	Brick powder in chilli powder	5 mg of sample of chilli powder take in test tube, add50 ml water in it	Some powder will set at the bottom.	Positive
10.	Dried tendril of maize cob in saffron	Take few tendrils in watch glass and try to break.	Tendril will break easily	Positive

15.4.5 Test to detect adulteration in tea and coffee

Requirement: Tea sample, Coffee sample.

Chemicals: Water, $Ca(OH)_2$**,** Ethyl alcohol, Conc. HCl,Potassium hydrochloride, 1% Sodium carbonate solution, Basic lead acetate solution, Potassium permanganate solution, 1% Aqueous solution of iodine, Mercuric acid solution etc.

Equipment: Blotting paper, Glass plate, Beaker, Test tubes, Blottingpaper, Watch glass, Magnet, Sprit lamp/ Burner, Beaker and Measuring cylinder etc.

Test to detect adulteration in tea and coffee

S.N.	Test	Experiment	Observation	Result
1	Processed used tea leaves and colour in tea	Take some moisten tea leaves and place them on blotting paper.	Red or brown colour will be imparted to the blotting paper.	Positive
		Take a glass plate and spread a small amount of $Ca(OH)_2$ than sprinkle tea dust over it.	Orange red colour will appear	Positive
2	Artificial colour in tea	Take half-filled clean water. Dip some sample tea leavesin it and stir.	Water colour will change	Positive
	Azo dye in tea	Take a tea sample in test tube. Add few drops of conc. ethyl alcohol. Allow to evaporate alcohol and make sample dry then add water.	Red/ yellow/ orange colour will appear.	Positive
	Coal tar dye in tea	Take small amount of tea sample in test tube. Add 5 ml conc. HCl.	Pink or crimson colour will appear	Positive
3	Tamarind**,** date,chicory seeds powder in coffee powder	Take small amount of coffee powder sample on blotting paper and sprinkle potassium hydrochloride along with water.	Edges become brown	Positive
	Chicory powder in coffee powder	Put a small coffee sample on filter paper. Spray a 1% sodium carbonate over sample.	Red color will appear on the filter paper.	Positive
4.	Iron filling in tea/coffee	Take tea/coffee sample on watch glass and magnet come over the sample.	Some particle will attract by the magnet	Positive
5.	Starch in coffee	Take a small amount of coffee sample in test tube. Add 3 ml distil water and mix it. Light up	Blue color appears	Positive

		the sprit lamp and heat the solution to colourise. Add approximately 30 ml potassium permanganate and mercuric acid solution (1:1) to decolourise the mixture. Then add 1% iodine solution.		

15.4.6 Test to detect adulteration in different types of oils and vegetable ghee

Requirements: Oils, vegetable ghee and fat sample

Chemicals: Petroleum ether, Conc. HCl, Conc. HNO_3, Acetic anhydride solution, Conc. H_2SO_4, Acetic acid, Chloroform, Alcoholic potash, Ice salt mixture, Water etc.

Equipment: Test tubes, Test tube holder, Test tube stand, Refrigerator, Measuring cylinder, Bunsen burner/ Spirit lamp etc.

Test to detect adulteration in different types of oils and vegetable ghee

s.no.	Test	Experiment	Observation	Result
1.	Castor oil in edible oil	Take 5 ml sample oil in test tube than add 5 ml petroleum ether and allow cooling in ice salt mixture.	Quick turbidity appears	Positive
2.	Prohibited colour in edible oil	Take 5 ml sample in test tube. Add equal amount of conc. HCl. Shake gently and leave it stand for 5 minutes.	Color will separate in the upper layer of solution.	Positive
3.	Other oil in coconut oil	Take coconut oil sample in a small bottle and put it in refrigerator for some time.	Un-solidifying layer will separate	Positive
4.	Argemone oil in edible oil	Take a small amount of sample oil in test tube, add few drops of conc. HNO_3 and shake gently.	Red colour will appear in the acid layer	Positive
5.	Paraffin wax and hydrocarbon in vegetable ghee	Take small amount of melted sample of vegetable ghee, add approx.10 ml of acetic anhydride solution.	Droplet of oils will float over the surface of unused acetic acid	Positive
6.	Dyes in fat	Take 1 ml fat sample in test	Red /Pink colourwill	Positive

		tube, heat it with the mixture of 1 ml conc.H_2SO_4 and 4 ml acetic acid	appear	
7.	Mineral oil in vegetable oil	Take 2 ml oil sample in a test tube. Add same amount of alcoholic potash in it. Warm the sample on low flame burner for about 10 min than add water.	Turbidity will appear.	Positive

15.4.7 Test to detect adulteration in fruit and fruit juices

Requirement: Sabudana and Fruits samples.

Equipment: Stainless steel spoon, Bunsan burner/ Sprit lamp, Knife, Cotton ball.

Test to detect adulteration in fruit and fruit juices

S.No	Test	Experiment	Observation	Result
1.	Talcum powder in sabudana	Take small amount of sabudana on a steel spoon and burn it over the flame	After burning sample will leave ash.	Positive
2.	Wax over the fruit surface	Scratch the fruit surface with knife	Waxy layer will peel off	Positive
3.	Erythrosine in watermelon	Cut the sample water melon into two halves.Take a dry, clean cotton ball and rub it on the succulent part of watermelon	Cotton ball will turn red	Positive
4.	CaC_2 over fruit wall	Cut a ripe fruit with the clean knife.	Fruit willmore soft from periphery than center	Positive

15.4.8-Test to detect adulteration in vegetable and its products

Requirements: Vegetable samples

Chemicals: Water, Paraffin wax.

Equipment: Glass bowl of suitable size, Cotton wick.

Test to detect adulteration in vegetable and its products

S.No.	Test	Experiment	Observation	Result
1.	Rhodamine B in sweet potato	Take a sampleof sweet potato and rub a wet cotton ball over the surface of sweet potato	Cotton ball turn reddish violet	Positive
2.	Malachite greenon green vegetable	Take few provided green vegetable in a dish. Soak a cotton ball in liquid paraffin. Rub this cotton ball on the surface of provided green vegetable.	Cotton ball will turn green	Positive
		Take some clean water in a suitable size container. Sample vegetables dip into the dish water and stir gently	Water will turn green	Positive

Note: 1. Result positive means sample containing Adulterant.

2. Fresh solution should be made for those chemicals which exist solid in natural state.

3. Method of freshly form chemicals are given in 15.5 section of the unit.

15.5 METHODS OF PREPRATION OF DIFFERENT REAGENT USE IN TEST FOR FOOD ADULTERATION

Acetic anhydride solution: It is prepared by adding 100ml of water in 10ml of pure acidic anhydride.

Alchoholic potash: It is a solution of potassium hydroxide in ethanol.

Chromotrophic solution: Dissolve saturated solution of 1,8-dihydroxynaphthalene 3,6-disulphonic acid in 72% sulphuric acid.

2% Diphenyamine solution: Dissolve 2gm Diphenylamine in suphuric acid to make volume up to 100ml.

20% salt solution: Dissolve 20gm salt in water make volume upto 100ml.

10% $FeCl_3$: Add 120gm Fe in 20ml HCl and mix. Then add distill water and make volume up to 1000ml.

Ice salt mixture: To prepare freezing mixtureadd some salt in ice than mix it in melting ice. This mixture shows freezing point dispersion.

1% Iodine solution: 6gm Potassium iodide (KI) dissolve in 200ml dist. Water than add 3gm I_2 and mix properly to form homogenous solution and make volume 1000ml by adding distil water.

10% NaOH Solution:10gm of NaOH pellets dissolve in a small quantity of distill water. Wait for cooling the solution. When cooled, add distil water upto 100ml in measuring flask.

Nessler's reagent: It is a mercury iodide solution(HgI_2) in potassium iodide(KI) and potassium hydroxide(KOH). It is used as a confirmatory test for ammonium ion (NH_4^+).

Neutralizer: It is a substance which is used to neutralise acidity eg. sodium carbonate / bicarbonates are mainly used to avoid the spoilage of milk by neutralizing the natural as well as developed acidity of milk by bacteria. 2% use of sodium bicarbonate generally recognize as a safe category

P-Dimethyl amino benzaldehyde solution (DAMB): DAMB solution is prepared by dissolving 0.4gm p-dimethyl amino benzaldehyde powder in 250ml of alcohol and 23ml of concentrated HCl.

Potassium iodide solution: Dissolve 83g of potassium iodide in water and dilute up to 100ml.

Potessiumpermagnet and Mercuric acid solution (1:1): That means no. of moles of potassium permagnet and mercuric acid solution is always in same ratio.

10% Potassium chromate solution: Place 10gm potassium chromate in measuring flask. Now slowly add distil water with constant stirring up to the mark of 100ml of measuring flask.

0.1% Rosalic acid solution: 100mg Rosalic acid powder mix with 30ml ethyl alcohol and make up the volume 100ml by adding distill water.

0.5% Resorcinol solution-Add 0.5gm resorcinol in to 20ml distil water than add this solution in to 35ml conc. HCl and now volume make up to 100ml by adding distill water**.**

1% Resorcinol solution-Add 1gm resorcinol in to 20 ml distil water than add this solution in to 35ml conc.HCl and make volume up to 100ml by adding distill water.

0.1 N Silver nitrate solution: Place 17gm silver nitrate ($AgNO_3$) in measuring flask. Now slowly add distil water with constant stirring up to the mark of 100ml of measuring flask.

1% Sodium carbonate solution- Place 2.5gm of sodium carbonate in measuring flask. Now slowly add distil water with constant stirring up to mark of 100ml of measuring flask.

20% Salt solution: Dissolve completely 20gm common salt in 10ml distil water than add distil water up to 100ml.

Tincture iodine: 7gm of iodine powder mix with 3gm of potassium iodine. Than 100ml ethyl alcohol add in the mixture for preparing tincture iodine solution.

Tri chloroacetic solution: Chromotropic solution is prepared by dissolving 1,8 dihydroxynepthalene-3,6 di sulphonic acid in 72% sulfuric acid.(for preparing 72%

sulphuricacid add 72ml of absolute acid in 28ml of water slowly. Never add water in acid because adding of water in acid is highly exothermic.

Trypsin solution: It is made up of trypsin powder- A mix of protease (protein digesting enzyme).

Turmeric paper strip: It is a paper strip form by dip in turmeric solution. It is use as an indicator. If it touched with a basic/alkaline solution changes from yellow to orange red.

15.6 CHEMICALS (FORMULA) LIST

- Potash Alum- $K_2SO_4.Al_2(SO_4)_3.24H_2O$
- Chloroform-$CHCl_3$
- Nitric Acid- HNO_3
- Hydrochloric Acid- HCl
- Ethyl Alcohol-C_2H_5OH
- Calcium Hydroxide- $Ca(OH)_2$
- Ferric Chloride-$FeCl_3$
- Sulphuric Acid- H_2SO_4

15.7 EQUIPMENT USED IN EXPERIMENT:

Watch glass: It is a circular concave piece of glass to hold sample and staining. Some time it is also use as lid.

Test tube: Also known as sample tube. It is figure like tubular structure and made up of clear plastic or glass.

Burner: It is an equipment to produce open flame for heating.

Water Bath: It is an equipment that is used to incubate samples at a constant temperature over a long period of time.

Beaker: It is a cylindrical container with flat bottom, may be graduated and made up of plastic or glass.

Filter Paper: Filter paper is a semi-permeable paper barrier placed perpendicular to a liquid/ air for separating fine solid particle from liquid or gas.

Stirrer: It is an equipment to agitate the liquid for speeding up the reactions or improving mixtures.

Glass plate: It is made up of glass in different size and use for various function like cover, mixing surface.

Centrifuge: It is an equipment, driven by a motor and spin liquid sample at high speed.

Pipette: It is an equipment used to transport a measured volume of liquid. It is graduated, manual or automatic and made up of glass or plastic.

Measuring Cylinder: It is a graduated cylinder used to measure the volume of a liquid

Blotting paper: It is a paper with high absorbance. It is used to absorb excess liquid.

Sprit lamp: It is a lamp used in laboratory to produce open flame by using sprit as fuel.

Measuring cylinder: It is used to measure chemical solutions.

Weighing machine: It is used to measure weight of compounds.

15.8 PRECAUTIONS DURING EXPERIMENTS

1. One should be careful while handling concentrated HCl, sulphuric acid (H_2SO_4) and other basic and acidic chemical which have corrosive properties.

2. Do not touch or taste any unknown substance or chemical.

3. Do not work alone in the laboratory.

4. While heating keeps the face away from the mouth test tube or flask.

5. The apparatus to be used in an experiment should be arranged properly before beginning an experiment.

6. Waste material should bediscarded through appropriate procedure.

7. Avoid adding solid material in warm liquids.

8. Always wash expose area of body before leaving the laboratory.

9. Gloves and apron should wear while working with chemical in laboratory.

10. While diluting acids, pour acid slowly in to the water. Not water into the acid.

11. Labeled the date of manufacture on the chemical solution.

15.9 SUMMARY

Food adulteration is aheinous socio-economic crimethat iscommitted when a food product meant to be consumed by humans has been contaminated in any way. Food adulteration is a common practice in various parts of the world including India. It is not only hazardous to health but also pose a threat to life. The main challenge of food adulteration is lack of acceptance in market due to distrusting its originality. To avoid this negative impact and harmful effects of food adulteration, people prefer organic food but it is not the perfect solution of this problem and not whole population of world including India opt this option due to its high coast and low availability. Therefore, following recommendations are forwarded-

1. Adequate law, funding and staffing should be appeared.In order to prevent this adulteration of food, the Indian government has enacted a consumer legislation called "the prevention of food adulteration act, 1954.

2. Different agencies also play a great role to reduce adulteration to develop appropriate sampling programs based on statistical validity.

3. An effective analytical technique helps to overcome adulteration problem predominately.

4. Create awareness in society against adulteration by different programs and education

15.10 GLOSSARY

Abortion: Condition of pregnancy termination.

Argemone seeds: Seeds of *Argimonemaxicana***.** Use in several disease treatment. Its adulteration in mustered oil cause oxidative stress and death of R.B.Cs.

Azo dye: Azo dyes are organic compounds having functional group R-N=N-R'. It is generally use to dye textile, leathers etc. But use as a colouring agent in food is harmful for heath (may induce cancer)

Boric acid: White crystalline acid obtained from its salt $B(OH)_3$.

Calcium carbide: It is a chemical compound with the chemical formula CaC_2 and also known as calcium acetylide. In industry it is used in the production of acetylene and calcium cynamide. Use as an artificial ripening agent in food industry which is strictly prohibited and illegal.

Cancer: A state when normal cell loose the capacity of regulated cell division, divides continuously in random manner and form tumor.

Chalk: Limestone (Calcium Carbonate, $CaCO_3$).

Chicory powder: Powderobtained from the flower of exotic plant *Cichorium intybus*.

Coal tar dye: A dye made from coal-tar derivatives.

Common salt: White crystalline substance of sodium chloride (NaCl).

Detergent: It is a surfactant having cleansing properties.

Deteriorate: To make inferior or to disintegrate.

Diarrhea: It is a condition when liquid stools are frequently discharge from bowl. It is of 2 types: i. Acute, ii. Chronic.

Dysentery: Bloody diarrhea condition.

Ergot: It is a fungal disease of cereals caused by many species of fungus *Clavicep*mostly*clavicep purpurea.* Black colour fruiting bodies grows in the ears of cereals, have toxic alkaloid.

Food: It is any nutritious substance that human/ animal intake to maintain, repair body and help in growth.

Formaline: it is 40% formaldehyde solution.

Frothing: Overflow mass of small bubbles.

FSSAI: Food safety and standards authority of India.

Insomnia: Poor sleeping condition/ Habitual sleeplessness.

Jaggery: It is an unrefined sugar made for sugar cane or palm.

Khesari dal: This dal obtaind from *Lathyrus sativus* or grass pea. Use as common adulterant in besan.

Laboratory: A well equipment place for experiments.

Lathyrism: It is chronic toxic nutritional neurological disease cause by long term intake of certain legumes of genus *Lathyrus* containing neurotoxin Oxalyldiaminopropionoic acid (ODAP).

Malachite green: It is a hazardous dye and common adulterant in green vegetables.

Petroleum ether: It is the petroleum fraction consisting of aliphatic hydrocarbons that boils in the range of 35-60^{0}C, and commonly used as a laboratory solvent.

Red lead A red form of lead oxide (PbO).

Resin: A mixture of organic compounds, secreted by some plants for protection.

Rhodamine B(RhB): It is water- soluble dye. Carcinogenic and illegal colourents to food product.

Sabudana: It is a starch extracted from the stem of many tropical palm trees.

Sella rice: Rice obtained after steamed paddy.

Solution: In chemistry solution is the homogeneous mixture of two or more than two components. One component is solute (less is amount) and other is solvent (more in amount). Solution may exist in any phase.

Soya milk: A plant base drink produced by soaking and grinding of soyabeans.

Sugar: A crystalline substance of sucrose.

Talcum powder: It is a soft mineral of hydrated magnesium silicate.

Urea: It iscolourless, weakly basic, crystalline compound. NH_2CON.

15.11 SELF ASSESSMENT QUESTIONS

1. What is mean by food adulteration?

2. What are adulterants?

3. Name some common adulterant in food?

4. Give the name of the different types of food adulteration?

5. How you check starch adulteration in milk?

6. What is the effect of food adulteration?

7. How you test the presence of malachite green adulteration in green vegetable?

8. How you test presence of talcum powder in sabudana?

9. How you test presence of prohibited colour in edible oil?

10. How you test presence of iron filling in tea leaves?

11. How you test presence of lead chromate in turmeric?

12. How you test for extracted clove bud?

13. How you test to the presence of chalk powder in sugar/jaggery?

14. Name three foods that are generally adulterated withmetanil yellow?

15. Can adulterated food produce mineral deficiency?

16 Fill in the blank-

i. Adulteration cause ……………. as well as ……………….disease.

ii. Sella rice is…………………….

iii. Nessler Reagent use in………………………..adulteration.

IV. Initially added substance that affects nature and quality of food is known as…………………

V. ……………….reading shows the presence of water or removal of fat from milk in laboratory.

VI. Sabudana is a………………………… from the stem of many tropical palm tree.

17. Which stage does adulteration take place in food?

a) Producer b) Distributor

c) Retailer d) All

18. Which of the following is an adulterant?

a) Pesticide b) Urea

c) Iron filling d) All

19. Why are adulterant added?

a) To increase shelf life of product.

b) To improve appearance and colour.

c) To gain more profit.

d) All the above.

20. Method/s for detection of common adulterant is/ are-

a) Visual tests b) Chemical test

c) Physical tests d) All the above

21. A substance intentionally added that affects the nature and quality of food is called-

a) Food poison b) Food adulterant

c) Food contamination d) Food material

22. Presence of starch in milk can be detected easily by

a) HCl test b) Iodine test
c) Lactometer d) Glucometer

23. Any perishable food which is not to be consumed immediately should be kept outside the danger zone.

a) <5ºC to > 63ºC
b) 5ºC to 63º C
c) 5ºC to > 63ºC

d) <5ºC TO 63ºC

Answers:

1. Adulteration defines as the addition and deletion of any substance (vital/non-vital) to or form food and food products so that the natural composition and quality of food substance become deteriorated.

2. An adulterant is a substance which is use in adulteration process eg. Detergent in milk.

3. Detergent, starch in milk, chalk in flour, sugar in honey, argemone oil in mustered oil etc.

4. There are 4 types of food adulteration- i. Intentional adulteration, ii. Non intentional/ Accidental adulteration, iii. Metallic adulteration iv. Packaging/ Handling adulteration v. Natural adulterations.

5. A 5 ml sample milk in test tube and pour 2-3 drops of tincture iodine. If sample turns blue confirm the presence of starch in milk.

6. There are many adverse effects of food adulteration like-cancer, insomnia, neurological problems, liver disorder, diarrhea, stomach disorder, lathyrism, vomiting, dysentery, joint pain, heart diseases, food poisoning, abortion, weaken the immune system, brain damage etc.

7. Take few provided green vegetable in a dish. Take a cotton ball soaked in liquid paraffin. Rub this cotton ball on the surface of provided green vegetable. If cotton ball turns green confirm presence of malachite green on the surface of green vegetable.

8. Take small amount of sabudana on a steel spoon and burn it over the flame. If after burning sample left ash, confirm presence of chalk in sabudana.

9. Take 5 ml sample in test tube and add equal amount of conc. HCl. Shake gently and let it stand for 5 minutes. Separation of colour on the upper layer confirms the presence of prohibited colour in the oil.

10. Take tea/coffee sample on watch glass and magnet come over the sample. If some particles attract toward magnet gives confirmation about the presence of iron filling in tea leaves.

11. Take 5 gm turmeric sample in test tube, add 5 ml water and few drop of conc. HCl. Appearance of pink colour confirms presence of lead chromate in turmeric.

12. Put some clove in a glass of water. If clove will float on the surface of water, it confirms the extraction of essential oil from clove.

13. Take 5 ml of jaggery/ brown sugar in test tube and dissolve it in water. Chalk powder settles down. OR Take a melted jaggery in test tube and add few drops of conc. HCl. Sample show effervescence.

14. Turmeric, Yellow pulses and Sella rice.

15. Yes.

16. i) Chronic as well as Acute; ii) parboiled rice; iii) Ammonium salt; iv) Adulterant; v) Lactometer; vi) Starch extract

17(d); 18 (d); 19 (d); 20 (d); 21(b); 22(b); 23 (a)

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BLOCK-4-MOLECULAR BIOLOGYAND BIOTECHNOLOGY

UNIT-16: ISOLATION OF PLANT GENOMIC DNA AND ITS QUANTIFICATION BY UV-SPECTROPHOTOMETRIC METHOD

16.1 Objectives
16.2 Introduction
16.3 Experimental
- 16.3.1 Materials and reagents
- 16.3.2 Equipments required
- 16.3.3 Preparation of reagents
- 16.3.4 Procedure
- 16.3.5 Agarose Gel Electrophoresis
- 16.3.6 Quantitative estimation of DNA
- 16.3.7 Observation and Result
- 16.3.8 Interpretation (expected)
- 16.3.9 Trouble shooting guide

16.4 Summary
16.5 Glossary
16.6 Self Assessment Question
16.7 References
16.8 Suggested Readings
16.9 Terminal Questions

16.1 OBJECTIVES

After reading this unit students will be able-

- to understand about Agarose Gel Electrophoresis
- to know Quantitative estimation of DNA

DNA extraction from plant tissue can vary depending on the material used. Mostly the DNA extraction protocols recommend fresh leaf sample for genomic DNA isolation. Several researchers have attempted to eliminate to the use of hazardous chemicals, expensive kits, equipments, and labour intensive steps for high throughput DNA extraction. Essentially any mechanical means of breaking down the cell wall and membranes to allow access to nuclear material, without its degradation is required. The objective of this study was to develop a simple method to isolate DNA in an open laboratory environment, a method that eliminates the need to use liquid nitrogen and toxic phenol. The resulting optimized CTAB (Cetyl trimethylammonium bromide) protocol enables the isolation of high quality genomic DNA amenable to RAPD (Random amplified Polymorphic DNA), restriction digestion, and amplification of plant barcode genes with reduced cost and health concerns. In order to measure the purity of DNA content from different plant tissues, U.V/Visib. Spectrophotometery used.

16.2 INTRODUCTION

Plants are multicellular eukaryotic organisms with rigid cell walls composed of multiple layers of cellulose and many other polysaccharides. Cell wall provides the shape and rigidity to the plant cells. Even some plant species such as conifers and fruit trees contain high amount of polyphenolic compounds. These polysaccharides and polyphenolic compounds co-precipitated with DNA during isolation; therefore their removal is essential before DNA precipitated. To isolate the DNA, plant cells usually grounded using a pestle and mortar in liquid nitrogen. The powdered plant cells is then transferred to an extraction buffer that contains detergent to disrupt the membranes. Cetyltrimethyl ammonium bromide (CTAB) is commonly used for this purpose. The extraction buffer also contains a reducing agent (β- mercaptoethanol) and a chelating agent (ethylenediamine tetraacetic acid, EDTA).This helps to inactivate nucleases that are released from the plant cell and can cause serious degradation of the genomic DNA. Keeping the reactions cold, when possible can minimize their effects. In order to purify DNA, insoluble particulates are removed through centrifugation, while soluble proteins and other material are separated by mixing with chloroform: Octanol followed by centrifugation. Phenolic compounds may also be released on disruption of plant tissues and these may also interfere with subsequent uses of the DNA (*e.g.* if it is to be used in the PCR). Polyvinyl pyrolidone (PVP) can be added to the extraction buffer to remove phenolic compounds.

Phenol extraction can be used to remove any traces of proteins and the genomic DNA can be precipitated using either ethanol or isopropanol. Precipitated DNA can be hooked out of the solution or collected by centrifugation. It is important that DNA is not sheared, for this reason

the DNA should not be vortexed or pipetted repeatedly using a fine tipped pipette and all manipulations should be as gentle as possible. The purified DNA is then resuspended and stored in Tris-EDTA buffer or sterile distilled water. This method has been shown to give intact genomic DNA from plant tissue. To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualized under UV light.

16.3 EXPERIMENTAL

16.3.1 Materials and reagents

The following chemicals and reagents are used: 2–Mercaptoethanol, CTAB Extraction solution, CTAB / NaCL Solution, CTAB Precipitation solution, High Salt TE Buffer, TE Buffer, Ethanol, Isopropranol, Chloroform / Isoamyl alcohol solution, 50X TAE, Diphenylamine, Chloroform, Octanol and Agarose.

16.3.2. Equipments required

1. High speed centrifuge
2. Microfuge tubes
3. Auto-pipettes 2-20µl, 20-200µl, 200-1000µl
4. Water bath
5. -20°C Deep freezer.
6. Preheat the water bath or heating block to 65°C.
7. UV/Vis-Spectrophotometer
8. DNA-gel casting tray and electrophoretic apparatus

16.3.3 Preparation of reagents

1. Preparation of CTAB Extraction Buffer: Just before starting the experiment, add 90 µl of β-mercaptoethanol and 90 mg of CTAB powder to 9 ml of CTAB Extraction Buffer in a 15 ml centrifuge tube. Preheat the solution to 65°C.

2. Preparation of CTAB Wash Buffer: To 2 ml of CTAB Wash Buffer add 6 ml of Ethanol (96-100%).

3. Chloroform: Octanol (24:1) preparations: To 4.8 ml of chloroform, add 200 µl of octanol. Store in dark at room temperature.

4. Preparation of 70% Ethanol: Add 5.6 ml of ethanol (96-100%) to 2.4 ml of distilled water.

5. Prechill the CTAB Wash Buffer and 70% Ethanol prior to use.

6. Clean the mortar and pestle with distilled water before use.

16.3.4. Procedure

DNA extraction has three main steps:

1. Lysis of cell walls and membranes to release DNA into solution.

2. Purification of DNA by precipitating proteins and polysaccharides.

3. Precipitation of DNA and resuspension in a buffer.

A number of published methods are available for the extraction of genomic DNA, general method is discussed here.

Take young and tender leaves (for e.g. mint, spinach, tulsi, ginger etc) and wash them with distilled water. Finely cut the leaf material. Midrib and petiole should be removed from the leaf material before grinding, as they are a major source of carbohydrate contamination.

Note: DNA is a large molecule that can be broken down by shear forces, care should be taken to mix the samples gently, never vortex the DNA.

1. Grind 350 mg of freshly cut leaves in a mortar and pestle by adding 4 ml of pre-warmed CTAB Extraction Buffer. Transfer the mixture to 15 ml centrifuge tube using a clean spatula.
2. **Lysis:** Transfer the mixture to 15 ml centrifuge tube containing 5 ml pre-warmed CTAB Extraction Buffer using a spatula. Mix gently by inversion.
3. Incubate the sample at 65°C for 60 minutes, with occasional inversion of the tube.
4. Allow the sample to cool down by keeping the tubes at room temperature (15-25°C) for 5 minutes.
5. **Phase Separation:** Add 5 ml of Chloroform: Octanol (24:1) and mix by rocking the tube gently for 5 minutes.
6. Centrifuge the samples at 2,300 rpm for 2 minutes at room temperature (15-25°C).
7. Transfer the top aqueous layer into a fresh 15 ml centrifuge tube and add 25 ml of RNase A. Mix the sample gently by inversion and incubate for 30 minutes at room temperature (15-25°C).
8. **Precipitation of DNA:** Add 6 ml of isopropanol and mix the samples gently by inversion until a white fluffy DNA precipitate appears (it should appear within 1 minute after addition of isopropanol).
9. Centrifuge the samples at 2,300 rpm for 5 minutes at room temperature (15-25°C). Discard the supernatant.
10. **Wash:** Add 8 ml of cold CTAB Wash Buffer to the sample and mix by pipetting. Incubate at room temperature (15-25°C) for 20 minutes. Do not vortex as it may result in shearing of DNA.
11. Centrifuge the samples at 2,300 rpm for 5 minutes at room temperature (15-25°C). Discard the supernatant.
12. Add 8 ml of cold 70% ethanol to the tube containing the DNA and mix by pipetting. Centrifuge at 2,300 rpm for 5 minutes. Discard the supernatant.
13. Air dry the pellet for 10-15 minutes for the ethanol to evaporate.

14. Elution: Add 1ml of Elution Buffer and resuspend the above pellet.

15. Storage of the DNA: For short-term storage (24-48 hours) of the DNA, 2-8ºC is recommended. For longterm storage, -20ºC or lower temperature (-80ºC) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

16.3.5. Agarose Gel Electrophoresis

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water. Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 0.8 % agarose gel, add 0.4 g agarose to 50 ml of 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate or burner, swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool to about 55-60ºC. Add 0.5µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

NOTE: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken by wearing latex gloves; however, use of nitrile gloves is recommended.

Loading of the DNA samples: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10µl of DNA sample. Mix well by pipetting and load the sample onto the well. Load the Control DNA after extracting the DNA sample.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

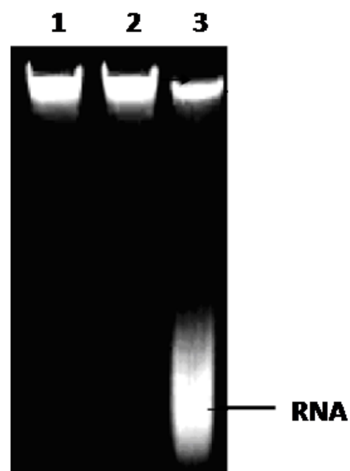
NOTE: Molecular biology grade water is recommended.

16.3.6. Quantitative estimation of DNA

100 mg of calf thymus DNA was dissolved in 100ml distilled water (1mg/ml Primary stock solution) then pipette out 1ml Primary stock solution of and make up the volume up to 10ml with distilled water. Now prepared different dilution ranging from 20-100 µg/ml. The absorbance was measured at 260 nm by using UV-Spectrophotometer. In this method, the absorbance of the unknown sample in a 1-cm cuvette was measured at 260 and 280 nm. The A260\A280 values were determined, as shown in table 16.1.

For a good and clean preparation of nucleic acid, the A260/280 ratio, which represents protein contamination, should be between 1.8 to 2.0 while the A260/230 ratio, which represents carbohydrate contamination, should be more than 2.0.

16.3.7. Observation and Result



Lane 1: control DNA
Lane 2: Extracted plant DNA
Lane 3: Plant DNA with RNA contamination

Fig.16.1 Expected result of gel electrophoresis

Table 16.1 Absorbance of the extracted genomic DNA at 260 nm and 280 nm

Sample	Dilution factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration(µg/ml)
1					
2					
3					
4					
5					

Calculate the concentration of isolated DNA using following formula.

Concentration of DNA sample (µg/ml) = $50 \times A_{260} \times$ dilution factor.

16.3.8. Interpretation (expected)

The lanes 1 and 2 demonstrate that highly purified DNA has been obtained with no visible RNA contamination when electrophoresed on agarose gel. If RNA contamination is present, one would see a faint and smeary RNA band below the genomic DNA as shown in lane 3. RNA being of lower molecular weight than DNA runs faster than the genomic DNA. RNA contamination is observed when the RNase treartment has either been skipped or not been carried out properly.

16.3.9. Troubleshooting guide

S.N.	Problem	Probable cause	Solution
1	Carbohydrate contamination in the sample	Grinding of the midrib along with the leaf material	Remove the midrib from the leaf before grinding, mainly in case of maize and lettuce, as these plant species have pronounced midribs. Removal of the midrib is not important in case of very young leaves or in small grain cereals
2	DNA appears degraded (as a smear running down the gel)	DNA appears fragmented or broken	DNA being a large molecule can be broken by shear forces if treated vigorously. Therefore mix the samples gently, never vortex the DNA. To minimize shearing always use a wide bore pipette tip for mixing
3	Difficult to dissolve DNA in Elution Buffer	This is due to over-drying of DNA pellet	The DNA should not be allowed to over-dry at any stage during the preparation as it hinders the resuspension and solubilization in Elution Buffer

16.4 SUMMARY

Current exercise describes the general and routine method of plant genomic DNA extraction. The current protocol based on commonly used CTAB (Cetyl trimethyl ammonium bromide) detergent that is used to break plant cells and solubilize its contents. The extraction process involves breaking or digestion of cell wall in order to release the cellular constituents. This is followed by disruption of the cell membrane to release the DNA into the extraction buffer. The released DNA should be protected from endogenous nucleases. Some time, EDTA is included in the extraction buffer to chelate magnesium ions, a necessary co-factor for nucleases. The initial DNA extracts often contain a large amount of RNA, proteins, polysaccharides, tannins and pigments which may interfere with the extracted DNA and difficult to separate. Most proteins are removed by denaturation and precipitation from the extract using chloroform and octanol. RNAs on the other hand are normally removed by treatment of the extract with RNase A. The DNA is precipitated and washed in organic solvents before re-dissolving in aqueous solution.

16.5 GLOSSARY

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

CTAB: Cetyltrimethyl ammonium bromide is a detergent used to break plant cells and to solubilize its contents and used in plant genomic DNA extraction.

β-mercaptoethanol: It's a reducing agent used to reduce disulfide bonds present in proteins.

PVP: Polyvinyl pyrolidone a chemical used to remove polyphenolic compounds from plant cells during plant genomic DNA extraction.

EDTA: Ethylenediamine tetraacetic acid is a chelating agent used to precipitate divalent ions and inactivates nucleases that are released from the plant cell and can cause serious degradation of the genomic DNA during plant genomic DNA extraction.

PCR: Polymerase chain reaction used to amplify DNA fragment.

UV/Vis-Spectrophotometer: Spectrophotometer is an instrument used for quantitative and qualitative measurement and based on Lambert & Beer's law.

EtBr: Ethidium bromide is a dye used to stain DNA fragments on agarose gel.

Agarose gel: Agarose gel is used in DNA electrophoresis. Agarose is a polysaccharide extracted from red algae

16.6 SELF ASSESSMENT QUESTION

16.6.1 Multiple choice questions:

1. For isolating DNA from plants, the most suitable method is

a) CTAB method b) SDS-phenol extraction

c) SDS-proteinase K treatment d) all of these

2. Which of the following reagent is commonly used for bacterial cell wall lysis

a) CTAB b) phenol extraction

c) lysozyme d) penicillin

3. DNA extraction from plant tissues are difficult due to

a) Presence of large amount of DNA,

b) Presence of large amount of RNA along with DNA

c) Both a and b

d) Presence of secondary metabolites and polysaccharides

4. The action of Cetyl Trimethyl Ammonium Bromide (CTAB) in DNA extraction from plant tissue is

a) CTAB complex with nucleic acids and form precipitate

b) CTAB complex with proteins and form precipitate

c) CTAB complex with polysaccharides and form precipitate

d) CTAB complex with secondary metabolites and form precipitate

5. Which of the following reagents are used for precipitating DNA

a) isopropanol b) ethanol

c) both a and b d) none of these

6. The action of EDTA in EDTA-lysozyme treatment in bacterial DNA extraction is

a) Removes Mg+ ions essential for maintenance of bacterial cell wall structure

b) Inhibits degradation of DNA by cellular enzymes

c) Removes outer lipopolusaccharide layer of Gram negative bacteria

d) all of these

7. Phenol used in DNA extraction

a) Precipitates DNA and leave proteins in aqueous solution,

b) precipitates RNA-protein complex and leave DNA in aqueous solution,

c) precipitates cell debris and leave nucleic acids-protein complex in aqueous solution,

d) precipitates proteins and leave nucleic acids in aqueous solution

8. In cell extracts with high protein content, before phenol treatment

a) Chloroform is used to break polypeptides to small fragments

b) SDS is used to break polypeptides to small fragments

c) Proteases are used to break polypeptides to small fragments

d) all of these

9. Which of the following reagent is used for quantifying DNA

a) Chloroform b) CTAB

c) Diphenylamine d) dansyl chloride

10. Which of the following is in the correct order regarding DNA extraction

a) RNAase treatment->Protease treatment->Cell lysis-> ethanol precipitation

b) Cell lysis->phenol treatment->RNAase treatment-> ethanol precipitation

c) Cell lysis-> RNAase treatment ->protease treatment-> ethanol precipitation

d) Cell lysis-> phenol treatment ->protease treatment-> ethanol precipitation

16.6.1. Answers: 1.(b), 2.(c), 3.(d), 4.(c), 5.(c), 6.(b), 7.(d), 8.(c), 9.(c), 10.(b)

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16.9 TERMINAL QUESTIONS

Q1. Describe the UV/Visib. Spectrophotometric method for quantitative estimation of DNA.

Q2: Describe the DNA-gel electrophoresis process to confirm the successfulness of genomic DNA isolation protocol from plant tissues.

Q3: Describe the basic steps of genomic DNA isolation from plant tissues.

Q4: Why are young plants are best source of DNA.

Q5: Why is it more difficult to isolate DNA from plant cell compared to animal cell and bacterial cell?

UNIT-17: RESTRICTION DIGESTION OF DNA AND ITS ANALYSIS BY AGAROSE GEL ELECTROPHORESIS

17.1 Objectives
17.2 Introduction
17.3 Types of restriction endonucleases and their modification system
17.3.1 Nomenclature
17.3.2 Restriction Enzyme cleavage
17.3.3 Factors affecting Restriction Enzyme Activity
17.4 Instruments Used
17.5 Procedure
17.6 Summary
17.7 Glossary
17.8 Self Assessment Questions
17.9 References
17.10 Suggested Readings
17.11 Terminal Questions

17.1 OBJECTIVES

After reading this unit student will be able to understand-

- how to use restriction enzymes to digest DNA
- Detailed procedure of restriction digestion of different types of DNA
- Detailed procedure of gel electrophoresis and separation of digested DNA bands

17.2 INTRODUCTION

Restriction endonucleases also known as molecule scissors are the type of enzymes used to cut the DNA at specific sites (restriction sites). Naturally, these enzymes present inside the bacterial cells to provide the protection against invasion of the bacterial cell by foreign DNA-especially bacteriophage DNA but the cell's own DNA is not cleaved by these Restriction enzymes. This self protection is achieved by the help of the specific DNA methyl transferase enzyme which will methylates the specific DNA sequence for its respective restriction enzymes by transferring methyl groups to adenine or cytosine residues to produce N^6-methyladenine or 5-methylcytosine. These enzymes specifically break the phosophodiester bond at restriction sites. Since the discovery of restriction endonucleases by Werner Arbor, Daniel Nathan and Hamilton Smith (won the the nobel prize of physiology and medicine in 1878), a number of applications of these enzymes have been explored. Three main applications of restriction enzymes are:

- Construction of restriction map
- DNA-Fingerprinting
- Recombinant DNA-Technology

17.3 TYPES OF RESTRICTION ENDONUCLEASES AND THEIR MODIFICATION SYSTEM

Type I enzymes: Type I restriction enzymes exhibit both restriction and DNA modification activities. They require the cofactors such as Mg^{2+} ions, S-adenosylmethionine (SAM) and ATP for their activity. The recognition sequences are quite long with no recognizable features such as symmetry. Type I restriction endo nucleases cleaves DNA at nonspecific sites and that can be 1000 base pair or more from recognition sequence. However, because the methylation reaction is performed by the same enzyme which mediates cleavage, the target DNA may be modified before it is cut. Because of these features, the type I systems are of little value for gene manipulation.

Type III enzymes: Like Class I enzymes, Type III enzymes possess both restriction and modification activities. They recognize specific sequences and cleave 25-27 base pairs outside of the recognition sequence, in a 3´ direction. They too require Mg^{2+} ions for their activity.

Type II enzymes: Type II enzymes and their corresponding modification methyl transferases act as separate proteins. They have a number of advantages over type I and III systems. First,

restriction and modification are mediated by separate enzymes so it is possible to cleave DNA in the absence of modification. Secondly, the restriction activities do not require cofactors such as ATP or S-adenosylmethionine, making them easier to use. They require only Mg^{2+} ions as cofactors. These enzymes are site-specific as they hydrolyze specific phosphodiester bonds in both DNA strands. Class II restriction endonucleases are generally used as the key material in molecular biology and recombinant DNA techniques, including genome mapping, RFLP analysis, DNA sequencing, and cloning.

17.3.1 Nomenclature

The first three letters of the restriction enzyme refer to the organism from which the restriction enzyme was originally isolated, the fourth letter (if present) refers to the strain, and the Roman numerals serve as indices if the same organism contains several different restriction enzymes.

Example: **EcoR I** and **EcoR V** are both from *Escherichia coli*, strain R; I and V are the order in which they were discovered.

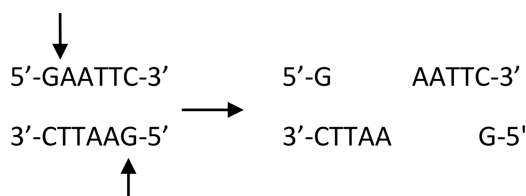
Table 17.1 Some restriction enzymes and their details

Restriction Enzyme Nomenclature			
Enzyme name	**Enzyme source (Bacteria)**	**Strain**	**Discovery order**
Eco RI	*Escherichia coli*	R	Ist
Bam HI	*Bacillus amyloliquefaciens*	H	Ist
Hin dIII	*Haemophilus influenza*	d	IIIrd
Hae III	*Haemophilus aegyptius*	e	IIIrd
Sma I	*Serratia marcescens*	a	Ist

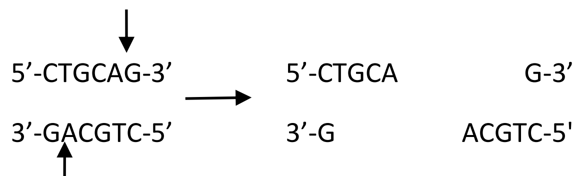
17.3.2 Restriction Enzyme cleavage

Class II restriction enzymes generate three types of DNA ends, all possessing 5´-phosphate and 3´-hydroxyl groups:

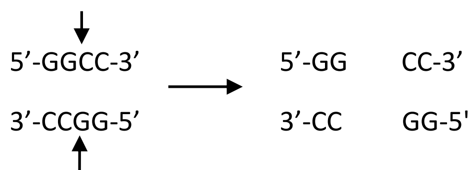
a) Cohesive 5´ ends: For example, ends generated by *Eco*R I:



b) Cohesive 3´ ends:- For example, ends generated by *Pst* I:



c) Blunt ends:- For example, ends generated by *Hae* III



Sticky ends (Blunt ends) are produced by cutting the DNA in a staggered manner within the recognition site producing single stranded DNA ends. These ends have identical nucleotide sequence and are sticky because they can bind to complementary tails of other DNA fragments cut by the same Restriction enzyme.

17.3.3 Factors affecting Restriction Enzyme Activity

Temperature: Most digestions are carried out at 37°C. However, there are a few exceptions e.g., digestion with *Sma*I is carried out at lower temperatures (~25°C), while with *Taq*I at higher temperature i.e., 65°C.

Buffer Systems: Tris-HCl is the most commonly used buffering agent in incubation mixtures, which is temperature dependent. Most restriction enzymes are active in the pH range 7.0-8.0.

Ionic Conditions: Mg^{2+} is an absolute requirement for all restriction endonucleases, but the requirement of other ions (Na^{+}/K^{+}) varies with different enzymes.

Methylation of DNA: Methylation of specific adenine or cytidine residues within the recognition sequence of the restriction enzyme affects the digestion of DNA.

17.4 INSTRUMENTS USED

Materials Required

- Microcentrifuge tubes
- Vial stand
- 10µl pipette
- Pipette tips

- Beakers
- Table top mini centrifuge
- Reagents

Reagents	**Supplies & Equipment**
0.2 µg/µl DNA	Electrophoresis box with
Restriction Enzymes:	Gel tray, rubber dams
Example: *Eco*RI, *Bam*HI, *Hin*dIII	8 well comb, spacer
10X restriction buffers	Gel box lid with leads
Water (molecular grade)	Power supply
10 or 6 X gel loading buffer (GLB)	Incubator
Agarose (analytical grade)	
Ethidium bromide stain	
1X TBE buffer	

DNA staining dyes

Staining with ethidium bromide (EtBr) is a rapid, sensitive, and highly reliable method for visualizing DNA in gels. The stained gel is illuminated from below ('transillumination') with short- or medium-wavelength of UV light causing the EtBr, bound to DNA, to fluoresce brightly. As a molecule that binds DNA, however, EtBr is a mutagen and likely carcinogen. EtBr should be handled with appropriate caution, and its use restricted to limited areas. Gloves and a lab coat should always be worn when using EtBr or handling items that might be contaminated with EtBr (such as gel staining containers). To limit the generation of waste, the EtBr staining solution can be reused repeatedly until staining in gels begins to fade. Stained gels and the first rinse from destaining will be collected as hazardous waste.

Another alternative safest approach is to use SYBR Safe DNA Stain; SYBR Safe is a cyan based, non-mutagenic dye `form of SYBR Green dye. The dye absorbs in the blue range, fluoresces only when complexes with DNA and then emits in the green (lmax 520 nm). The dye is purchased as a highly concentrated stock (often 10,000X). Add to cooling agarose gel before pouring into a casting stand.

TBE electrophoresis buffer (10X)

Reagent	**Quantity (1L)**	**Final concentration**
Tris base	121.60 g	1 M
Boric acid	61.80 g	1 M
EDTA (disodium salt)	7.40 g	0.02 M

Prepare with RNase-free H_2O. Dilute 100 mL to 1 L to make gel running buffer. Store for up to 6 months at room temperature.

Gel loading buffer (6X GLB)

The dyes xylene cyanol FF (XC) and bromophenol blue plus 30% glycerol in water are present in the "6X GLB" provided. The glycerol makes the final solutions dense so they sink to the bottom of the wells. The 'tracking dyes' are used to follow the progress of the electrophoresis. Under the conditions we use, BB migrates at about the same rate as a linear double-stranded 400 bp DNA fragment whereas XC migrates at about the same rate as a 8000 bp DNA fragment. See table below for the approximate migration of these dyes in other concentrations of agarose gels (using 1X TBE running buffer).

% Agarose	Xylene cyanol blue FF	Bromophenol blue
0.5	20-40 kb	4,000 bp
0.8	8,000 bp	400 bp
1.0	4,000 bp	300 bp
1.3	1,800 bp	150 bp
1.5	1,200 bp	100 bp
2.0	700 bp	65 bp

Before we move on procedure of restriction digestion following important points must be keep in mind:

Note:

1. Under incorrect buffer conditions, or in the presence of >5% glycerol, RE's can display altered DNA cleavage specificity, known as "star activity." Under such conditions, the enzyme may recognize, for example, a 4 base pair subset of its normal 6 bp recognition site, and therefore will cut the DNA at many more sites than expected. For example, the familiar enzyme *Eco*RI is notorious for its star activity in low ionic strength solutions.

2. Keeping with the ratio of enzyme to substrate, many researchers use a rule of thumb that 10 units of RE is enough to overcome variability in DNA quality and purity. NEB suggests that 1 µg of purified DNA in a final volume of 50 µl (of course using the appropriate buffer) is enough to cut most, or all of the DNA in one hour at xx temperature.

3. Most enzymes are supplied in a 50% glycerol solution and thus the volume of enzyme added should not exceed 10% of the total reaction volume.

4. It is vital to ensure your mixture is mixed and all of the components are "at the bottom of the tube". The reaction must be thoroughly mixed to achieve complete digestion. Pipette the final mixture "up and down" and flick the tube followed by a very brief microcentrifuge (just a touch of a spin) to bring the now homogeneous mixture to the bottom of the tube and avoid error.

17.5 PROCEDURE

17.5.1 Part I. Preparation for restriction digests

Mark the tops of five 1.5 ml eppendorf tubes indicating the reactions, such as:

1 = *Enzyme 1*, 2 = *Enzyme 2,* 3 & 4 = *Enzyme 1 & 2,* 0 = *no enzyme*

Tube no.	DNA*	10X buffer	Water	RE
1	4 µl	1 µl plasmid	4 µl	1 µl (RE-I)
2	4 µl	1 µl plasmid	4 µl	1 µl (RE-II)
3	4 µl	1 µl plasmid	4 µl	1 µl (RE-I & II)
4	4 µl	1 µl plasmid	3 µl	1 µl (RE-I & II)
0	4 µl	1 µl plasmid	5 µl	-

Gel casting

First step of procedure is casting of gel. DNA molecules migrate at different rates, depending on the concentration of agarose in the gel. Higher percentage gels are better for resolving small fragments; lower percentage gels are better for resolving large fragments. Please see the figure-17.1 for gel casting, sample loading and running of gel.

Table 1 Gel percentage and range of DNA bands separation

Agarose concentration in gel (%, w/v)	Efficient range of separation of linear DNA molecules (kb)
0.3	5 - 60
0.5	1 - 20
0.7	0.8 - 10
0.9	0.5 - 7
1.2	0.4 - 6
1.5	0.2 - 3
2.0	0.1 - 2

Example: casting 0.8% Agarose gel

1. Make sure your gel tray, rubber dams and comb are clean. If not, rinse with deionized water. Remove any dried-on agarose with a moist Kimwipe.

2. Using the appropriate tools (rubber damns or tape) carefully seal the gel tray.

3. Rest the comb holder with 8 well combs down into the end slot of the gel tray. (There are two possible orientations of the comb. Place it in the configuration where the teeth of the comb are further away from the rubber dam. Face the comb into the gel tray to ensure enough space between the edge of the gel and the wells.

4. To ensure the proper spacing between the bottom of the tray and the comb, loosen the comb and place one of the white comb height spacers on the bottom of the tray and rest the comb on the thick part of the spacer. Then, holding the comb firmly in place, tighten the comb-holding screw (the comb can become skewed during this process if you don't hold on to it).

5. Place the gel box and tray away from the edge of the lab bench where it can be easily bumped. When the gel is poured, it should not be disturbed while it is setting.

6. Now calculate the mass of agarose to make 50 ml of a 0.8% agarose in TBE buffer.

Note: The percentage gel is measured as a mass to mass ratio; in the case of water or buffer, we can use mass to volume, where 1 ml = 1 g. Thus a 1% agarose gel would contain 1 g of agarose per 100 ml of total gel volume.

Examples: 2% agarose gel with volume of gel 60 ml (approx mass = 60 g) 2% of 60 g = 0.02 x 60 g = 1.2 g -- Add 1.2 g agarose to 60 ml gel buffer.

Add the correct volume ml of 1X TBE gel running buffer to the flask and swirl. Calculate the weight of the combined flask, TBE and gel. Record in your notebook. Heat up the flask in microwaving, using short 1-2 min times to avoid overheating.

NOTE: The gel is molten when a small amount of boiling has occurred and the gel is clear without any undissolved agarose remaining. Mostly water will have evaporated or boiled off in the process leaving the Tris and other compounds behind. Bringing the mass back to the initial weight with TBE will result in a higher concentration of running buffer than necessary and errors with the gel.

1. Pour the gel into the sealed gel tray. Once the gel is set (it will become somewhat opaque), add a little 1X TBE to the top of the gel on the comb to lubricate it. Gently pull the comb straight out with no back and forth. Remove the rubber dams (starting at the center, not the ends) and place the gel tray in the gel box, with the wells on the – (negative/black) end. Immediately fill the gel box with 1X TBE to cover the gel completely so that the tops of the wells are under the fluid (Never let a gel sit with dry wells since they will deform quickly).

Loading sample and Gel-electrophoresis

1. Before loading, be sure your gel box is situated close enough to plug into one of the power supplies. You should not move the gel after it has been loaded. Once the DNA has been run into the gel for a few minutes, the apparatus can be moved if necessary.

2. Add 2 µl of gel loading buffer/dye (labeled 6X GLB) and mix with the reaction.

3. Leave the outermost wells empty. Load in the following order (left to right):

Tube 1	Tube 2	Tube 3	Tube 4	Molecular ladder	Optional
Enzyme 1	Enzyme 2	Enzyme 1 & Enzyme 2	Uncut		

4. Load the full 12 µl of each reaction + GLB into the well of the gel.

5. Run the gel at 125 V (constant voltage) until the leading dye has reached 2/3 – 3/4 the length of the gel.

Note: when you are done that the 1X TBE running buffer is reusable. Return buffer in the gel box to the 1X TBE container. (Such as, while you are staining your gel in the next section.)

Staining Gel with Ethidium Bromide and Visualization of DNA bands

1. Slide your gel from the gel tray to a small plastic container in the hood.

2. Add enough ethidium bromide (EtBr) staining solution to cover the gel.

3. Close the container and allow your gel to stain for 10-15 min in the hood.

4. Gentle agitation of the gel can improve staining (and destaining) but is not essential.

5. Use a lab rotator at a very slow speed.

6. Pour the EtBr stain back into the stock container, holding your gel with a plastic spatula.

7. Add deionized water to cover the gel and allow the gel to destain for 5- 10 min in the hood.

8. Pour the wash into the EtBr waste container in the hood.

9. Take your gel to photodocumentation system in the equipment room and get a good image. (Using the system will be demonstrated. There are also detailed instructions there.) Print out a copy (mainly as a backup), and save a copy of the image (exported to TIFF) to the computer, then to your own flashdrive or email to yourself. Once you have a good image, dispose of your gel in the EtBr waste container in the hood.

10. By convention, gels are displayed with the wells at the top and are read left to right. The region extending down from a well in a vertical column is called a lane. (If lanes are numbered, the leftmost lane will be '1'.)

The digestion of DNA helps in generation of restriction map of plasmid and genomic DNA. Modern techniques like DNA fingerprinting and r-DNA technologies are based on DNA digestion method. In DNA-fingerprinting, one can confirm the DNA sample whom it is belong therefore having a major application in criminal science. DNA-fingerprinting can also be used in paternity and maternity test. The r-DNA technology is mainly used in species identification through phylogeny test.

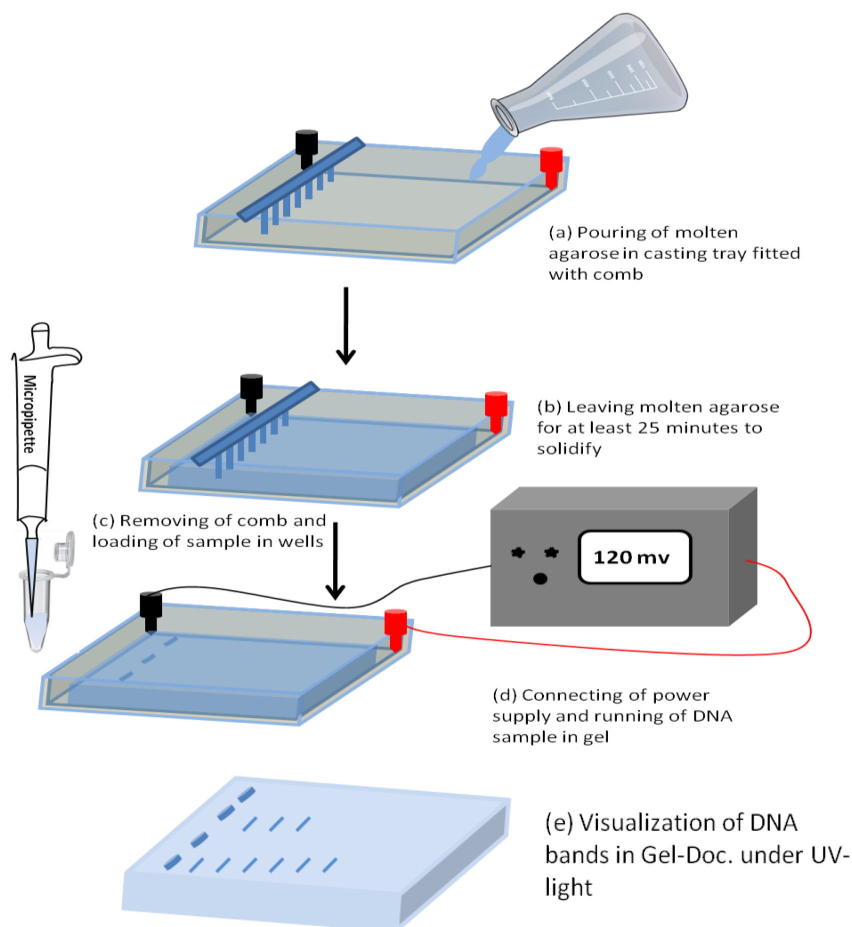


Fig.17.1 Flow chart for the restriction digestion of DNA

17.6 SUMMARY

The current exercise explains about how to digest the DNA sample that may be plasmid or genomic DNA using restriction endonuclease enzymes. To make the exercise for better understanding of students, the process is mentioned in steps. Each requirement and recipe is clearly mentioned such as buffer preparation eg; sample loading buffer. For optimisation of process, different factors affecting to the digestion process are also discussed.

After learning the restriction digestion process one can implement the same in different applications such as DNA-fingerprinting, r-DNA technology etc.

17.7 GLOSSARY

Restriction endonucleases: These are the enzymes having specificity to cut double stranded DNA (ds DNA) at specific sites.

Restriction sites: These are the short (approx 4-6 bps) recognition sites present on ds DNA where restriction enzymes make the cut.

Bacteriophage: These are the viruses survive on bacterial host system.

Phosophodiester bond: These are the phosphate bonds between nucleotides in DNA.

S-adenosylmethionine (SAM): S-Adenosyl methionine is a common cosubstrate involved in methyl group transfers, transsulfuration, and aminopropylation.

RFLP: Restriction fragment length polymorphisms, a technique that is based on restriction digestion of DNA followed by analysis of fragments.

Cloning: It's a technique, where a foreign DNA fragment is inserted into vector and followed by transformation of vector in to host**.**

Sticky ends: Restriction enzymes those make the cohesive cut or staggered cut into DNA.

Blunt ends: Restriction enzymes those make the sharp or blunt cut into DNA.

Ethidium bromide (EtBr): It is a dye that used to stain the DNA fragments.

SYBR Green: Its is a dye that used to stain the DNA fragments but it is less carcinogenic compared to EtBr

Bromophenol blue: A blue color dye that is most commonly used during DNA-gel electrophoresis**.**

Agarose: A carbohydrate derivative polysaccharide used to make gel for DNA-gel electrophoresis.

DNA-fingerprinting: DNA profiling is a forensic technique in criminal investigations, where the genomic DNA is cut using specific restriction enzymes and pattern of fragments compared.

r-DNA technology: Recombinant DNA technology is the joining together of DNAmolecules from two different species. The recombined DNA molecule is inserted into a host organism to produce new genetic combinations.

17.8 SELF ASSESSMENT QUESTIONS

17.8.1 Multiple choice Questions:

1. Which of the following is the most important discovery that leads to the development of r-DNA (recombinant DNA) technology?

a) Discovery of DNA as a genetic material

b) Discovery of restriction enzymes

c) Discovery of double helix model by Watson and crick

d) All of these

2. Who discovered restriction enzymes?

a) watson and crick b) Jacob and monad

c) Nathan, Arber and Smith d) Boyer and cohen

3. Restriction enzymes are

a) capable of cutting DNA molecule

b) capable of adding nucleotides to the 3'OH end

c) capable of restriction protein synthesis

d) capable of joining DNA molecules

4. Restriction enzymes capable of making internal cuts in a DNA molecule is called

a) Restriction endonucleases b) Restriction exonucleases

c) Both a) and b) d) S1-nucleases

5. Restriction enzymes also called

a) Molecular scissor b) Molecular knives

c) Molecular scalpels d) all of these

6. Which of the following is are true about restriction enzyme

a) Restriction enzymes used to cut DNA molecule

b) Restriction enzymes used to construct restriction maps

c) Restriction enzymes used in RFLP

d) all of these

7. Type of restriction enzymes used in r-DNA technology

a) Type I b) Type II

c) Type III d) all of these

8. Which of the following statements are true regarding restriction enzymes

a) Type I and II enzymes cut far away from the restriction sites

b) Type II cuts DNA within restriction sites

c) Eco RI is a type II restriction enzyme

d) b and c are true

9. Restriction sites for type II

a) generally are palindrome sequences b) consist of 4-6 bps

c) generally are not palindromic d) a and b are true

10. Which ion is required for the activity of restriction enzyme type II

a) Mg^{2+} b) Mn^{2+}

c) Ca^{2+} d) Na^{+}

11. Restriction enzymes

a) are present in bacteria and are involved in host restriction system

b) cleave viral DNA inside bacterium

c) are enzymes involved in defence against bacteriophages

d) all of these

12. The first type II restriction enzyme isolated was

a) ECO R1 b) Hind II

c) Bam HI d) Sal I

17.8.1 Answers Key: Q1. b), Q2. c), Q3. a), Q4. a), Q5. a), Q6. d), Q7. b), Q8. d), Q9. d), Q10. a), Q11. d) and Q12. b)

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17.11 TERMINAL QUESTIONS

Q.1. what are the restriction enzymes? Discuss the different types of restriction enzymes

Q.2. Discuss mechanism of DNA digestion for type II restriction enzyme

Q.3. Discuss the procedure to interpret restriction digestion results

Q.4. How the restriction digestion methodology will help to understand DNA fingerprinting and r-DNA technology

Q.5. Discuss the role of DNA restriction digestion in paternity

UNIT-18: SDS-PAGE ANALYSIS OF SEED STORAGE PROTEINS (GLOBULINS) FROM LEGUMES

18.1 Objectives

18.2 Introduction

18.3 Experimental

18.3.1 Material required

18.3.2 Protein extraction procedure

18.3.2.1 TCA-Acetone method

18.3.2.2 Phenol-acetone method

18.3.2.3 Multi-detergent method

18.3.3 Protein concentration measurement

18.3.3.1 Materials and Reagents required

18.3.3.2 Procedure of standard Bradford assay

18.3.4 Sample preparation

18.3.5 Casting Polyacrylamide Gels

18.3.5.1 Assemble the gel casting apparatus

18.3.5.2 Preparation of resolving gels

18.3.5.3 Preparation of stacking gels

18.3.5.4 Chemistry of acrylamide polymerization

18.3.6 Running SDS-PAGE gels

18.3.6.1 Set up the electrophoresis apparatus

18.3.6 .2 Load and run samples on the SDS-PAGE gel

18.3.7 Staining SDS-PAGE gels

18.4 Summary

18.5 Glossary

18.6 Self Assessment Questions

18.7 References

18.8 Suggested Readings

18.9 Terminal Questions

18.1 OBJECTIVE

After studying this unit student will be able to-

- learn the different protocols of protein extraction from seeds.
- Merits and demerits of different protocols of protein extraction.
- Determination of protein concentration in the extract using standard Bradford assay.
- Characterization of proteins using SDS-PAGE.
- Protein Sample preparation
- Preparation and casting of gels
- Assembling the set-up of electrophoresis apparatus
- Loading of sample and running of electrophoresis
- Staining and destining of protein gel
- Documentation or record of gel pictures

18.2 INTRODUCTION

Legume seeds are rich in proteins and traditionally valuable source of proteins for human use. Grain legumes contribute 33% of daily protein intake of humans. Different data indicate that this major storage protein (MSP) attains 60-75 % of the total seed proteins.The use of different methods of isolation and different bean varieties can explain some variations of characteristic data for MSP concerning its molecular weight (MW), subunit pattern, aminoacid composition and percentage from the total seed proteins. The most common legume models are peanut, *Lotus*, *Medicago*, soybean, scarletrunner bean, common bean, pea and broadbean.

SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) is very common method for separating proteins by electrophoresis uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins. This discontinuous electrophoretic system developed by Ulrich K. Laemmli allows a good, who was the first to publish a paper entitled; "Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4" employing SDS-PAGE in a scientific study. SDS (sodium dodecyl sulfate) is an anionic detergent (provides negatively charged ionic molecules) binds with polypeptide chain in proportion to its relative molecular mass. The complex structure of protein is destroyed by the negative charge of SDS, converting complex 3-D structure to linear structure, and is strongly attracted toward an anode (positively-charged electrode) in an electric field.

In polyacrylamide gel electrophoresis, an electric field is used to move negatively charged protein molecules through a matrix of a polymerized polyacrylamide called polyacrylamide-gel . Polyacrylamide gels restrain larger molecules from migrating as fast as smaller molecules. Because the charge-to-mass ratio is nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides. The mobility of a molecule is also affected by the buffer system and the strength of the electrophoretic field used for the separation. In a gel of uniform density the

relative migration distance of a protein (R_f) is negatively proportional to the log of its mass. If proteins of known mass are run simultaneously with the unknowns, the relationship between R_f and mass can be plotted, and the masses of unknown proteins estimated.

Protein separation by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. The purity of protein samples can be assessed and the progress of a fractionation or purification procedure can be followed. Different staining methods can be used to detect rare proteins and to learn something about their biochemical properties. Specialized techniques such as Western blotting, two-dimensional electrophoresis, and peptide mapping can be used to detect extremely scarce gene products, to find similarities among them, and to detect and separate isoenzymes of proteins.

18.3 EXPERIMENTAL

18.3.1 Material required

Different kinds of seeds can be used for the extraction of proteins from legumes. Generally used legume seeds are horsegram (*Macrotyloma uniflorum*) were rajmah (*Phaseolus vulgaris*), mah (*Vigna mungo*), chickpea (*Cicer arietinum*), masoor (*Lens culinaris*) and mungbean (*Vigna radiata*). Among non-legumes rice (*Oryza sativa*), wheat (*Triticum aestivum*), bajra (*Pennisitum glaucum*) and mustard (*Brassica juncea*) were taken for the study. However the method used to extract the protein also depends upon the type of material or legume seed selected.

Deionized water , Acrylamide, Bis-acrylamide, Tris-HCl, SDS, ammonium persulfate (catalyst) TEMED (catalyst), Acetone, Trichloroacetoe, β-Mercaptoethanol (β-ME), Urea, thiourea, DTT and CHAPS, Sucrose, Sodium chloride, Protease inhibitor cocktail, Ammonium acetatedibasic dibasic potassium phosphate, Triton X-100 gel tracking dye (such as bromophenol blue), protein standard markers, liquid nitrogen,gel casting apparatus, Power supply, Micropipette, Eppendorf, cooling Centrifuge, refrigerator (-20° C).

18.3.2 Protein extraction procedure

Different methods are in use with minor modifications, some common methods are listed below. Prior to each type of extraction, 3 g of seeds were soaked in DI water for 24 hrs. Afterward seeds were ground to a fine paste in liquid nitrogen using a prechilled mortar and pestle, transferred to a 50-mL prechilled Falcon tube. Flowchart 18.1 shows a simplified flow-chart comparing the three different extraction methods.

18.3.2.1 TCA-Acetone method

The protocols are developed following the earlier used protocols (Cilia *et al*., 2009). The modifications made in the developed protocol are as follows. The first protocol used for extraction of the proteins was using TCA and acetone. The 100 mg of seed paste was homogenized in 10% TCA containing 2% β -Mercaptoethanol (β -ME) using liquid nitrogen. It

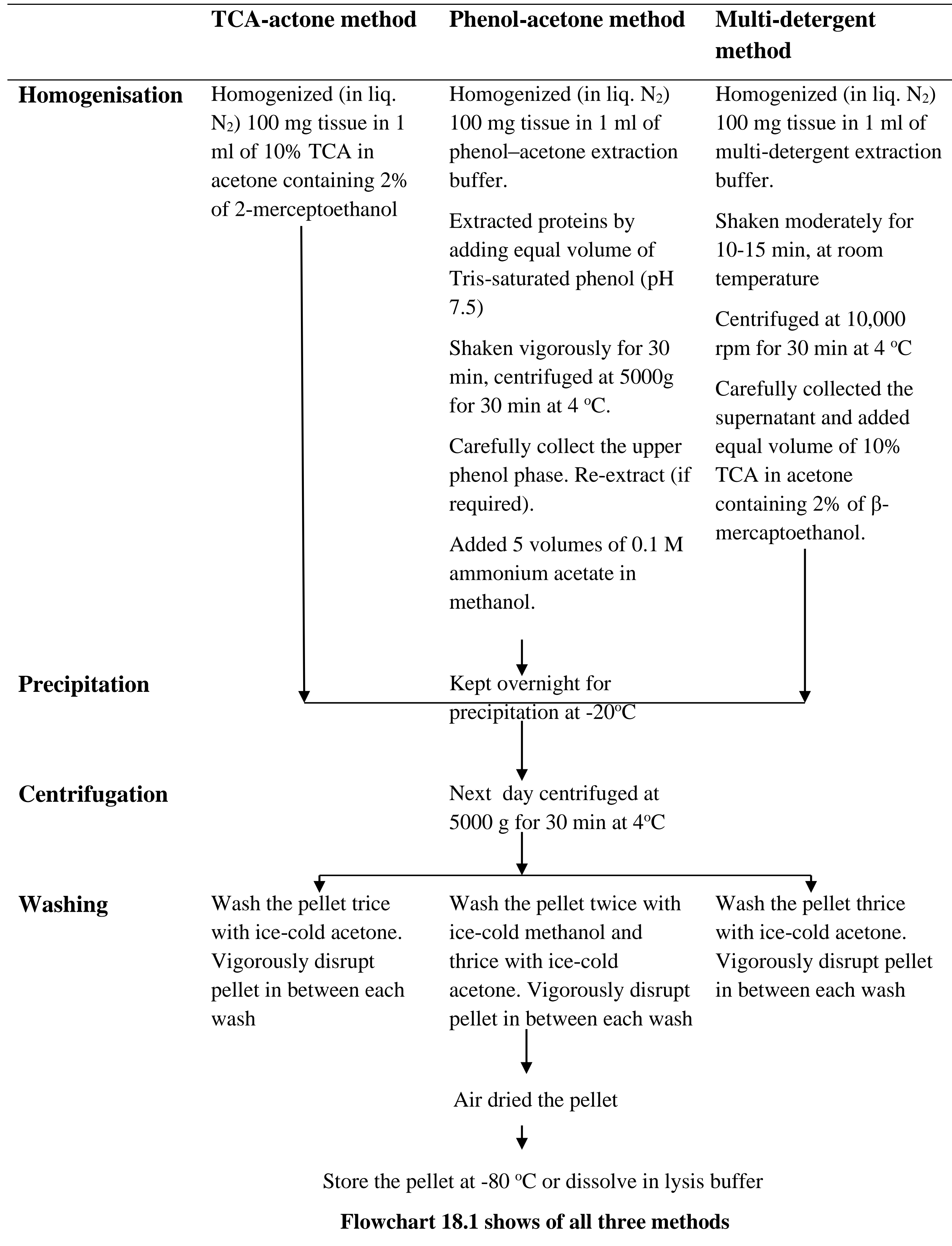
was kept overnight for precipitation at -20°C. Next day, the mixture was centrifuged at 5000 g for 30 min at 4°C. The supernatant was discarded and precipitates were washed thrice with ice cold acetone. The precipitates were agitated vigorously in between each wash. The precipitates were then air dried till they appeared to be damp but not dried or cracked. These precipitates were dissolved in 1 ml of the modified lysis buffer (9 M urea, 2 M thiourea, 1% DTT and 4% CHAPS).

18.3.2.2 Phenol-acetone method

The second protocol involved the extraction of proteins using phenol. The 100 mg of seed paste was homogenized well in the modified phenol extraction buffer (500 mM Tris (pH 7.5), 2% non-idet P 40 (NP- 40), 2% β-ME, 0.7 M sucrose, 0.5 M sodium chlorideand protease inhibitor cocktail). To this, equal volume of cold Tris-saturated phenol (pH 7.5) was added. This mixture was shaken vigorously for 30 min at 4°C and then centrifuged at 5000 g for 30 min at 4°C. The upper phenol phase containing the proteins was collected very carefully. Ammonium acetate (0.1 M) was added five times the volume of the phenol phase. Mixed well and kept for precipitation overnight at -20°C. Next day, the mixture was centrifuged at 5000 g for 30 min at 4°C. The supernatant was discarded and the precipitates were washed twice in methanol and thrice in ice-cold acetone as described for TCA-acetone protocol till their dissolution in the same lysis buffer.

18.3.2.3 Multi-detergent method

Homogenization of 100mg of seed paste was done in the modified multi-detergent extraction buffer (100 mM dibasic potassium phosphate (pH 7.6), 8 M urea, 1% tritonX-100, 20%glycerol, 0.5 M sodium chloride and protease inhibitor cocktail). This was shaken at room temperature for 10-15 min and then centrifuged at 9500 g for 30 min at 4°C. To the supernatant equal volume of 10% TCA containing 2% β-ME was added and kept overnight for precipitation at -20°C. Next day, the mixture was centrifuged at 5000 g for 30 min at 4°C. The supernatant was discarded and the precipitates were washed thrice in ice-cold acetone as described for TCA-acetone protocol till their dissolution in the same lysis buffer.



Flowchart 18.1 shows of all three methods

Although any one of the methods can be used to extract protein, though all three methods have their own pros and cons like; TCA-acetone method often results in the co-extraction of polymeric contaminants. These contaminants precipitate with proteins cannot be removed by the final washing steps. Phenol-acetone method easy to handle although it also results in the co-precipitation of phenolic compounds and give good yield of protein comparison to TCA-acetone method. All the methods were reproducible. The TCA-acetone protocol did not give very clear and high intensity bands in DS-PAGE. The phenol-acetone protocol gave better results, bands were very sharp and distinct. Multi-detergent protocol although gave high intensity bands but failed in clarity and sharpness of 1-DE gel-banding pattern.

18.3.3 Protein concentration measurement

Protein content of air dried or frozen dried pellet is essential before to proceed SDS-PAGE. Generally two classical methods, Bradford is a classical method and in use for long time. but other methods such as Biuret protein assay, Lowry protein assay can also be used. As an example, only Brad ford assay explained.

18.3.3.1 Materials and Reagents required

a. Coomassie Brilliant Blue G-250 (Sigma-Aldrich, catalog number: 27815)

b. Methanol

c. Phosphoric acid (H_3PO_4)

Equipment:

a. Spectrophotometer (UV-Visible)

b.Vortex mixer

c.Weighing balance

Reagents:

a. Bradford reagent

b. Ovalbumin (Protein standard)

Glassware and plasticware

a. Pipettes

b. Pipette tips

c. A 5 *ml* glass pipette

d. Pipette aid

e. 100 *ml* measuring cylinder

f. Test tubes (for standard assay)

g. 1.5 *ml* microfuge tubes (for microassay)

h. Plastic cuvettes

Preparation of reagents

Bradford reagent**:** Bradford reagent is prepared as follows:

1. Weigh 200 *mg* of Coomassie Blue G250 dye and dissolve it in 50 *ml* of 95% ethanol.
2. Mix this solution with 100 *ml* of concentrated (85%) phosphoric acid.
3. Make the final volume of the solution to 1 *litre* by adding distilled water.
4. Filter the reagent through Whatman No. 1 filter paper.
5. Transfer the filtrate in an amber colored bottle and store at room temperature.

Protein standard: Ovalbumin; the standard solution is prepared as follows:

1. Weigh accurately 5*mg* ovalbumin.
2. Dissolve it in 5 *ml* distilled water; this gives a protein stock solution of 1 *mg/ml* concentration.
3. Store the protein standard at –20°C.

18.3.3.2 Procedure of standard Bradford assay

1. Take out the frozen protein standard and allow it to come to room temperature.
2. As the concentration of the unknown protein sample can be anything, the assay will be performed with a range of dilutions (1, 1:10, 1:100, and 1:1000). Prepare 100 *μl* of each of the dilutions.
3. Take 15 test tubes and label them from 1 to 15.
4. Pipette out 10 *μl*, 20 *μl*, 30 *μl*, …….., 100 *μl* ovalbumin standard in the glass tubes labeled 1-10; leave blank the tube no. 11.
5. Add distilled water to make the final volume 100*μl* in each of the tubes (including blank).
6. Take 100*μl* of each of the unknown protein dilutions in the tubes labeled 12 – 15.
7. Add 5 *ml* of Bradford reagent in each of the tubes and mix well by inversion or gentle vortex mixing (avoid frothing).
8. Within 5 – 60 *min*, measure the absorbance of tubes 1 – 10 and 12 – 15 at 595 nm in the quartz/glass cuvette against the reagent blank (tube 11).
9. Record the readings in the suggested observation table-1 below:

Table 18.1 Observation table for Bradford assay

Tube no.	Volume (µl)	Mass (µg)	Distilled water (µl)	Bradford reagent (ml)	A_{595}
Standard Ovalbumin					
1.	10	10	90	5	
2.	20	20	80	5	
3.	30	30	70	5	
4.	40	40	60	5	
5.	50	50	50	5	
6.	60	60	40	5	
7.	70	70	30	5	
8.	80	80	20	5	
9.	90	90	10	5	
10.	100	100	0	5	
11.	Blank (0)	0	100	5	
Unknown sample					
12.	100 (1:1000 dil.)	Unknown	0	5	
13.	100 (1:100 dil.)	Unknown	0	5	
14.	100 (1:10 dil.)	Unknown	0	5	
15.	100 (undiluted)	Unknown	0	5	

18.3.4 Sample preparation

To resolve the proteins in a sample according to their size, investigators must convert the proteins to a uniform geometry and impart a uniform charge/mass ratio to the proteins. In SDSPAGE, the solution is to denature the proteins by boiling them with the anionic detergent, sodium dodecyl sulfate (SDS) and 2-mercaptoethanol. The combination of heat and detergent is sufficient to break the many noncovalent bonds that stabilize protein folds, and 2-mercaptoethanol breaks any covalent bonds between cysteine residues. Denatured proteins bind quite a lot of SDS, amounting to ~1.4 g SDS/g protein, or ~one SDS molecule for every two amino acids.

Procedure for protein sample preparation for electrophoresis

1- Add 1 part 2X sample buffer to 1 part sample on ice.

2- Mix well.

3- Heat sample at 95°C - 100°C in a boiling water bath for 4 minutes.

4- Place on ice until ready-to-use or store at –20°C for up to 6 months

Table18.2 Composition of sample buffer

2X Tris-Glycine SDS sample buffer	
2X concentrate	Amount to add for 2X concentrate
126 mM Tris-HCl, pH 6.8	2.5 ml of 0.5 M Tris-HCl, pH 6.8
20% Glycerom	2 ml Glycerol
4% SDS	4 ml of 10% SDS
0.005% Bromophenol blue	O.5 ml of 0.1% Bromophenol blue
Adjust volume to 10 ml with distilled water	

(1X = 63mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol blue, 2.5% βME)

18.3.5 Casting Polyacrylamide Gels:

The percentage of polyacrylamide in resolving gel determines the molecular weight ranges of proteins to be separated, see table 2. These instructions are designed for constructing two 12% SDS-PAGE gels with the Bio Rad Mini Protean system.

Table18.3 Protein separation ranges

Polyacrylamide concentration	**Protein separation range**
7.5%	50kDa-200 kDa
10%	25kDa-200 kDa
12%	20kDa-100 kDa
15%	10kDa-50 kDa
4-20%	5kDa-200 kDa
10-20%	5kDa-150 kDa
4-12%	25kDa-250 kDa
8-16%	15kDa-200 kDa

18.3.5.1 Assemble the gel casting apparatus

i. Assemble the components that you will need for casting the gel: a tall glass plate with attached 1 mm spacers, a small glass plate, a green casting frame and a casting stand, see fig. 18.1

ii. Place the green casting frame on the bench with the green “feet” resting firmly against the bench and the clamps open (perpendicular to the frame) and facing you.

iii. Place the two gel plates in the frame. Insert the taller spacer plate with the “UP” arrows up and the spacers facing toward you into the casting frame (the BioRad logo should be facing you). Insert the short glass plate in the front of the casting frame. There should be a space between the plates.

iv. Secure the plates in the casting frame by pushing the two gates of the frame out to the sides.

IMPORTANT: the bottom edges of the two plates should be flush with the lab bench before you clamp the frame closed to ensure a watertight seal. To do this, rest the frame vertically on the bench before closing the gates.

v. Clamp the casting frame with glass plates into the casting stand, with the gates of the casting frame facing you. Repeat steps i-v to prepare a second gel in the casting frame.

vi. Check to see if the assembled plates in the casting stand are sealed properly by pipetting a small amount of deionized water into the gap between the plates. If the glass plates hold water and don’t leak, you are ready to make the gels. Pour the water out by holding the entire casting platform over a liquid waste container or sink. Use paper towels or tissues to absorb any residual water. If the gel leaks, disassemble the frame, dry the plates and go back to step iii.

Large spacer plate with spacer goes in rear, oriented so the arrows point up and the logo is readable. Small plate is placed in front. (Corners of the plates are marked with bracket marks.

Green gates of casting frame are open.

Casting frame “feet” and bottom edges of plates are flush against the benchtop.

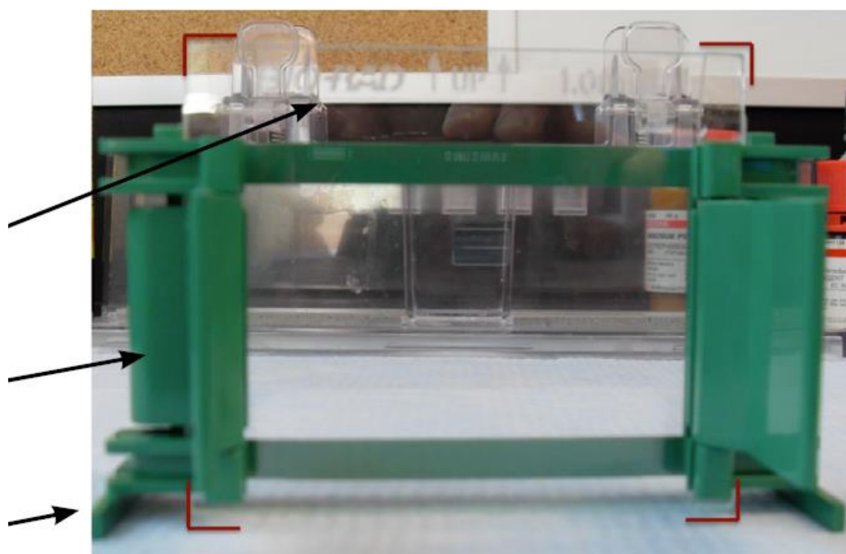


Fig. 18.1 Assembling plates in the casting frame

18.3.5.2 Preparation of resolving gels

Assemble the chemicals that you will need to pour the gels. The table below shows the quantities of each chemical that you will need to pour two gels with the Mini-Protean system. Polymerization occurs rapidly, so be sure to follow the step-by-step instructions below.

***NOTE**: catalysts should NOT be included into the mixture until you are ready to pour the gels!!*

i. Label two 15 mL conical tubes “Resolving gel” and “Stacking gel”.

ii. Prepare ONLY the resolving gels at this time. Mix the acrylamide solution, **pH 8.8** Tris buffer and water, as shown in the table 3. Mix the ingredients gently, trying not to introduce air. Oxygen inhibits polymerization of acrylamide gels.

iii. To the resolving gel mixture, add 100 µL of a 10% ammonium persulfate (APS) solution. Gently mix the solution, trying not to introduce air. Oxygen inhibits acrylamide polymerization.

iv. Add 10 µL of TEMED catalyst. Once again, gently mix in the catalyst trying not to introduce air bubbles.

CAUTION: TEMED has an unpleasant odor. Cover the tube immediately after you aliquot this reagent.

v. Working quickly, use a plastic transfer pipette to fill the space between the two plates until the resolving gel solution reaches a height just above the green clamps on the gel casting frame. Draw up any remaining acrylamide into the transfer pipet. (You will know that the acrylamide has polymerized when you can no longer push the acrylamide out of the pipet.)

vi. Using a transfer pipet, add deionized water so that it gently flows across the surface of the polyacrylamide mixture. The water layer ensures that the polyacrylamide gel will have a level surface once it polymerizes.

vii. Allow the gel to polymerize, which takes ~15-20 minutes, see fig. 18.2. You will note that the interface between the polyacrylamide and water overlay disappears temporarily while the gel polymerizes. A sharp new interface then forms between the two layers, indicating that polymerization is complete. (You can also check the remaining polyacrylamide in the transfer pipette to see if it has polymerized.)

viii. When polymerization is complete, remove the water from the top of the resolving gel by tilting the gel to the side and using a paper towel or Kimwipe to wick out the water.

Casting stand and plates are clamped into the casting stand

Fill plates with running gel solution to the top of the closed green gates of the casting frame.

Bottom edges of plates are flush against the grey pad of the casting stand.

Fig.18.2 Casting the SDS-PAGE gel

Table18.4 Composition of resolving and stacking gel

Reagent	Resolving gel	Stacking gel
Deionized water	3.5 ml	2.1 ml
30% acrylamide:bis-acrylamide (29:1)	4.0 ml	0.63 ml
1.5 M Tris-Hcl, 0.4% SDS, pH 8.8	2.5 ml	---------
0.5 M Tris-Hcl, 0.4% SDS, pH 6.8	--------	1.0 ml
10% ammonium persulfate (catalyst)	100 µl	30 µl
TEMED (catalyst)	10 µl	7.5 µl

18.3.5.3 Preparation of stacking gels

SAFETY NOTE*: Be sure you are wearing goggles when pouring the stacking gels.*

1- Prepare the stacking gels. Mix the acrylamide solution, pH 6.8 Tris buffer and water, as shown in the chart above in table 3.

2- Add 30 µL 10% APS and 7.5 µL TEMED to the stacking gel acrylamide mixture. Mix the contents by gently inverting the tube twice.

3- Use a transfer pipette to pipette the stacking gel on top of the resolving gel between the two glass plates. Add enough stacking solution until it just reaches the top of the small plate.

4- Carefully, but quickly, lower the comb into position, being careful not to introduce air bubbles. (The Bio Rad logo on the comb should be facing you.) Adding the comb will force some solution out of the gel, but this is fine. If air bubbles become trapped below the comb, remove the comb and reposition it.

18.3.5.4 Chemistry of acrylamide polymerization

The polyacrylamide gels used to separate proteins are formed by the chemical polymerization of acrylamide and a cross-linking reagent, N, N'methylenebisacrylamide. Investigators are able to control the size of the pores in the gel by adjusting the concentration of acrylamide, as well as the ratio of acrylamide to bisacrylamide. Raising either the concentration of acrylamide or bisacrylamide, while holding the other concentration constant, will decrease the pore size of the gel. Polymerization occurs because of free oxygen radicals that react with the vinyl groups in acrylamide and bisacrylamide. The oxygen radicals are generated from the catalyst, ammonium persulfate (APS), when it reacts with a second catalyst, N, N, N', N'-tetramethylethylenediamine (TEMED).

18.3.6 Running SDS-PAGE gels

18.3.6.1 Set up the electrophoresis apparatus:

1- Remove the gels from the casting stand and then from their green frames, see fig . 18.3.

2- Carefully remove the comb from the spacer gel.

3- Remove the casting frame from the gel cassette sandwich and place the sandwich against the gasket on one side of the electrode assembly, with the short plate facing inward. Place a second gel cassette or a buffer dam against the gasket in the other side of the electrode assembly.

4- Clamp the green clamps on the sides of the electrode assembly (below).

5- Lower the chamber into the electrophoresis tank.

6- Fill the space between the two gels with Tris-glycine running buffer. This forms the upper chamber for electrophoresis.

7- Add Tris-glycine running buffer to the outer (lower) chamber until the level is high enough to cover the platinum wire in the electrode assembly.

One gel is positioned on each side of the electrode assembly.

Top of short plate fits snugly into notch in the green gasket of the electrode assembly.

Electrode assembly with two gels is lowered into the clamping frame

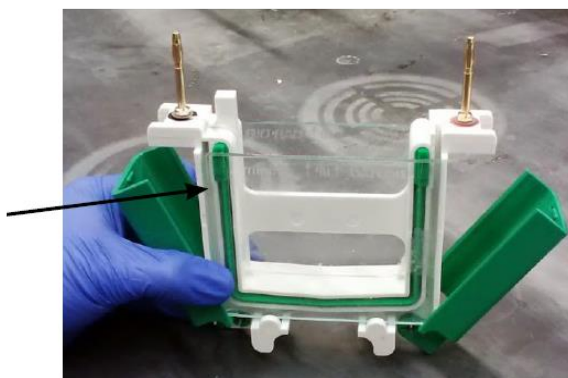


Fig.18.3 Electrode assembly

18.3.6.2 Load and run samples on the SDS-PAGE gel

1- Take out the samples from ice or freezer, those were already mixed with a tracking dye and glycerol. If essential thaw the sample on ice and vortex vigorously for ~ 10 seconds to thoroughly mix the contents.

2- Using gel loading micropipette tips (tips have very long, thin points and fit P20s or P200s), load up to 15 µL of sample into each well. Load 5 µL of a molecular weight standard into one lane of the gel (see the fig 18.4, wells formed in gel). Load samples slowly and allow the samples to settle evenly on the bottom of the well. Be sure that amount of protein loaded in each well must be in range to be detected according to the type of stain used to visualize the protein bands, for Coomassie blue dye-6-10 µg and silver stain in nanogram range.

NOTE: Be sure to record the order of samples loaded onto the gel.

3- Connect the tank to the power supply. Fit the tank cover onto the electrodes protruding up from the electrode assembly. Insert the electrical leads into the power supply outlets (connect black to black and red to red).

4- Turn on the power supply. Run the gel at a constant voltage of 120-150 V. Run the gel until the blue dye front nearly reaches the bottom of the gel. This may take between 45-60 min, see fig. 18.5.

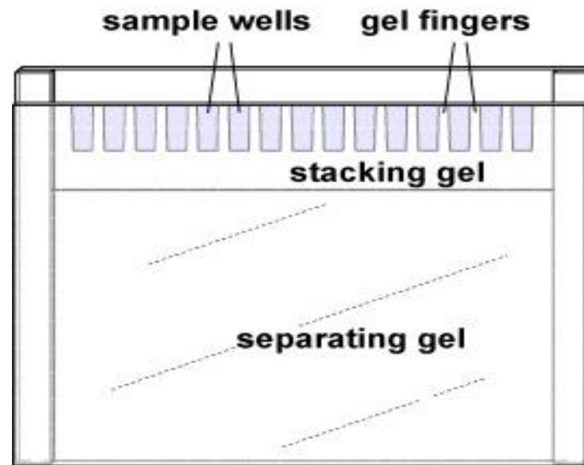


Fig.18.4 Polymerized stacking gel with wells and resolving gel

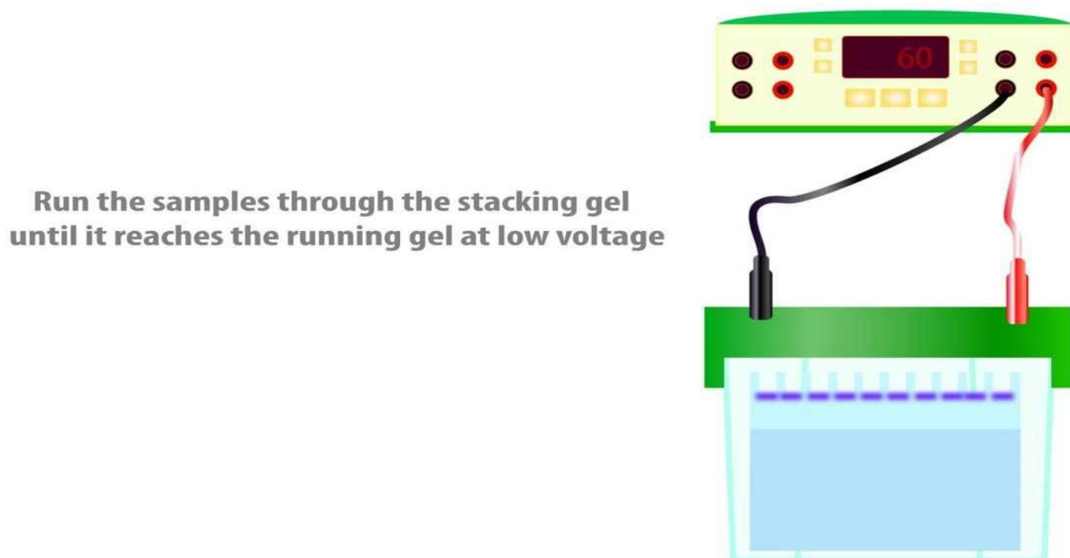


Fig.18.5 Running of gel

18.3.7 Staining SDS-PAGE gels

1- Turn off the power supply.

2- Remove the gel apparatus from the tank. Open the clamping frame and remove the gel cassette sandwich. Carefully, separate the two plates apart with a spatula. With the spatula, remove the lower right or left corner of the gel to serve as an orientation marker.

Be sure to indicate in your lab notebook whether the notched corner corresponds to lane 1 or lane 10 of the gel.

3- You may also remove the stacking gel with the spatula, if you desire.

4- Place the gel in a small plastic tray and label the tray with your initials on a piece of tape. To do this, fill the tray about halfway with deionized water. Gently free the gel from the glass plate, allowing it to slide into the water. The gel should move freely in the water. Place the gel and tray on a rocking platform. Rock the gel for ~2 minutes.

5- Drain the water from the gel and add enough Simply Coomassie stain to cover the gel, while allowing the gel to move freely when the tray is rocked. Cover the gel container with saran-wrap and rock overnight. Make sure that the gel does not stick to the bottom of the tray.

6- In the morning, drain the stain into an appropriately labeled waste container in the hood of the lab room.

7- Destain the gel by filling the container about half full with deionized water. Shake the gel in the water for ~2 minutes. Pour off the water and add new deionized water. Repeat, if necessary, until protein bands become visible.

8- When individual bands are detectable, record your data. You may photograph the gel with your cell phone camera against a white background. Alternatively, place the gel in a clear plastic page protector and scan the gel.

9- After recording the data, dispose of the gel in the Biohazard waste container.

18.4 SUMMARY

The globulins are dominant storage proteins in legume seeds and usually account for 50–90% of the seed proteins. However the protein content in the same legume species may vary according the environmental conditions. In this exercise author describes the different methods of protein extraction from legume seeds. The TCA-acetone method is easy to operate but the maximum protein yield obtained from phenol-acetone method. Characterization of globulin proteins can be done using classical SDS PAGE experiment.

18.5 GLOSSARY

SDS-PAGE: stands for sodium dodecyl sulfate polyacryamide gel electrophoresis.

Cathode: Negative electrode

Anode: Positive electrode

Globular protein: Generally have a more compact and rounded shape and have functional roles

Legume: Legume also called pod fruit of plants in the pea family

SDS: sodium dodecyl sulfate, is an anionic detergent (provides negatively charged ionic molecules) binds with polypeptide chain in proportion to its relative molecular mass.

β-Mercaptoethanol: 2-Mercaptoethanol is one of the most common agents used for disulfide reduction. Sometimes referred to as β-mercaptoethanol, it is a clear, colorless liquid with an extremely strong odor.

Coomassie Brilliant Blue: Coomassie blue dyes (R and G types) have been the most popular stains used in proteomics to date, because of their low cost, ease of use, and good compatibility with MS. Coomassie blue R-250 dye is used to visualize proteins via a regressive staining approach in which gels are saturated with dye and then destained with an aqueous solution containing methanol and acetic acid.

Bradford reagent: A solution of coomassie blue dye in methanol.

Bromophenol blue dye: A tracking dye for nucleic acid or protein electrophoresis in agarose or polyacrylamide gels.

TMEDA: *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine is a bidentate tertiary amine. It is a Lewis base having good solvating properties. It is used as a catalyst to polymerize the acrylamide and bis-acrylamide.

APS: Ammonium persulfate, is also used as a catalyst to polymerize the acrylamide and bis-acrylamide.

18.6 SELF ASSESSMENT QUESTIONS

18.6.1: Multiple Choice Questions

1- In a SDS-PAGE

a) Proteins are denatured by the SDS

b) Proteins have the same charge-to-mass ratio

c) Smaller proteins migrate more rapidly through the gel

d) All of the above

2- Proteins can be visualized directly in gels by

a) staining them with the dye

b) Using electron microscope only

c) Measuring their molecular weight

d) None of these

3- In SDS-PAGE, the protein sample is first

a) Treated with a reducing agent and then with anionic detergent followed by fractionation by electrophoresis.

b) Fractionated by electrophoresis then treated with an oxidizing agent followed by anionic detergent.

c) treated with an oxidizing then with anionic detergent followed by Fractionation by electrophoresis

d) None of the above

4- Electrophoresis of histones and myoglobin under non-denaturing conditions (pH 7.0) results in.

a) Both protein migrate to anode

b) Histone migrates to anode and myoglobin to cathode

c) Myoglobin migrates to anode histone and to cathode

d) Both protein migrate to cathode

18.6.1 Answer Key: 1- (d), 2-(a), 3-(a), 4-(c)

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18.9 TERMINAL QUESTIONS

1- Describe the TCA-acetone method for protein extraction from legume seeds?

2- Describe the Phenol-acetone method for protein extraction from legume seeds?

3- How you should quantitatively measure the protein concentration in a given protein sample?

4- Describe the process of stacking and resolving gel casting?

5- Describe the process of protein band migration in SDS-PAGE?

6- What is the role of SDS and β-Mercaptoethanol in protein sample preparation?

7- Why is it important to wear gloves and eye protection when making gels for SDS-PAGE?

8- What is the purpose of a stacking gel?

9- What is the purpose of a separating gel?

10-What are the differences in the way agarose and acrylamide gels are run?

11-When is native PAGE used?

12-Why are molecular weight standards run in a gel with unknown proteins?

13- What does the size of the band indicate?

UNIT-19: ISOLATION OF RNA AND ITS QUANTIFICATION BY UV - SPECTROPHOTOMETRIC METHOD

19.1 Objectives
19.2 Introduction
19.3 Experimental
19.3.1 Materials and reagents
19.3.2 Instrument required
19.3.3 Procedure
19.3.4 Discussion
19.3.5 Alternative method (simple and rapid method)
19.3.6 Quantitative estimation of RNA by Nanodrop Spectrophotometer
19.4 Summary
19.5 Glossary
19.6 Self Assessment Question
19.7 References
19.8 Suggested Readings
19.9 Terminal Questions

19.1 OBJECTIVES

After reading this unit students will be able -

- To learn the detailed procedure of RNA isolation.
- To understand the critical steps essential in RNA isolation and purification.
- Able to gain the general concept of nucleic acid separation.
- Able to learn the role of pH in nucleic acid precipitation.

19.2 INTRODUCTION

RNA isolation is the primary requisite for the people working on RNA-expression level and RT-PCR. It is the basis of further RNA manipulations. Isolation of high-quality RNA is considered to require too much expertise because after lysis of cells during the course of RNAs isolation, ribonucleases (RNases) become free in solution to digest RNAs molecules. RNases are very active, widespread, stable, and no-cofactor required.

The source of contamination by RNases during RNA extraction can be exogenous or endogenous. Exogenous sources include the reagents, glassware, and plastic ware used in RNA isolation, especially the skin of the investigator. However, these RNases can be eliminated through sensible measures, such as treatment of reagents and plastic utensils with diethyl pyrocarbonate (DEPC); baking the glassware, mortar and pestle; and wearing disposable gloves throughout the whole procedure. However, endogenous RNases are innate to biological tissues and are normally sequestered in organelles and vacuoles.

Most important application of isolated RNAs is to check quantitative changes in gene expression using reverse transcriptase quantitative PCR (RT-qPCR). RT-qPCR is very useful and sensitive technique therefore to ensure significance of the results a significant number of replicates are required, which adds a high throughput component to the RT-qPCR. These requirements impose the need to have simplified protocols to reduce labour and costs without compromising RNA integrity or yield.

Although a number of protocols have been proposed to extract the RNAs with modifications at different levels of extraction. Extraction method also depends on the type of cells or tissues used. Plant tissues are characterized by a variable composition, and some tissues may be recalcitrant to RNA extraction. Purification of nucleic acids from plant tissues is often hindered by contaminating polysaccharides, polyphenolics, and secondary metabolites that, in the last steps of resuspension after precipitation by alcohol and salt, cause the formation of opaque and viscous slurry that is difficult to separate from pelleted nucleic acids. The greater the sugar contamination, the greater is the difficulty in the subsequent use of RNA for Northern blotting, reverse transcription, electrophoresis, poly $(A)^+$–RNA selection or quantification. Purification of RNA from sugar is generally achieved by precipitation with lithium chloride (LiCl), salt–alcohol,

ethylene glycol monobutyl ether (BE), or by cesium chloride (CsCl) centrifugation. These steps are time consuming, involve hazardous chemicals, and may increase RNA degradation. Despite these steps, in some cases, RNA quality and yield are poor.

In recent time, the application of RNAs extraction kits in laboratory experimental courses increased mainly to save time and reduced cost. However, working with kits is not the best choice to learn about RNA techniques as in readymade kits only instructions to follow blindly available, what reagent or chemical added is hidden and basic understanding of principles behind the procedure lacking. Because no special reagents or equipment or complicated techniques are required, proposed method could be performed in a routine plant molecular biology lab with reproducible results by even novice investigators. To save the time the proposed method is modified.

19.3 EXPERIMENTAL

19.3.1 Materials and reagents

RNA extraction buffer (0.2 M Tris, 0.4 M KCl, 0.2 M sucrose, 35 mM $MgCl_2$, 25 mM EGTA), Tris-saturated phenol (pH 8.0), chloroform/isoamyl alcohol (24:1, v/v), 3 M NaAc (pH 5.2), 3 M NaAc (pH 5.6), 0.3 M NaAc (pH 5.6), and DEPC-treated water. NaAc solutions and DEPC-treated water were prepared by treating NaAc solutions and water with 0.1% DEPC overnight at 37 ^{o}C, and then autoclaved at 121 ^{o}C for 20 min. The RNA extraction buffer was prepared with DEPC-treated water. Mortar and pestle, stainless lab spatulas, 1.5 mL Eppendorf tubes, 50-mL centrifuge tubes, pipettes, vortex, disposable tips and gloves.

The RNase-free treatment was performed as follows: all tubes and tips were soaked in 0.1% DEPC overnight at 37 ^{o}C, then autoclaved at 121 ^{o}C for 20 min. The mortars and pestles as well as the stainless lab spatulas were baked at 180 ^{o}C for 12 hr.

The following reagents were required for RNA analysis: agarose, 1 $\times$ TAE, 10 $\times$ RNA loading buffer (1 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol), and 10 mg/mL EtBr. Solutions for RNA electrophoresis were not administered the RNase-free treatment because RNase and RNA bands have different mobility in agarose gels, which means they could be separated once an electric field is applied.

19.3.2 Instrument required

Cooling centrifuge, low-volume spectrophotometer (Nano Drop), electrophoresis facility and gel imaging system (Gel Doc system).

19.3.3 Procedure

The following procedure can be applied to a wide variety of herbaceous plant tissues. For plant tissues rich in polysaccharides and/or polyphenols, specific purification protocols should be adopted, such as the modified cetyltrimethylammonium bromide method.

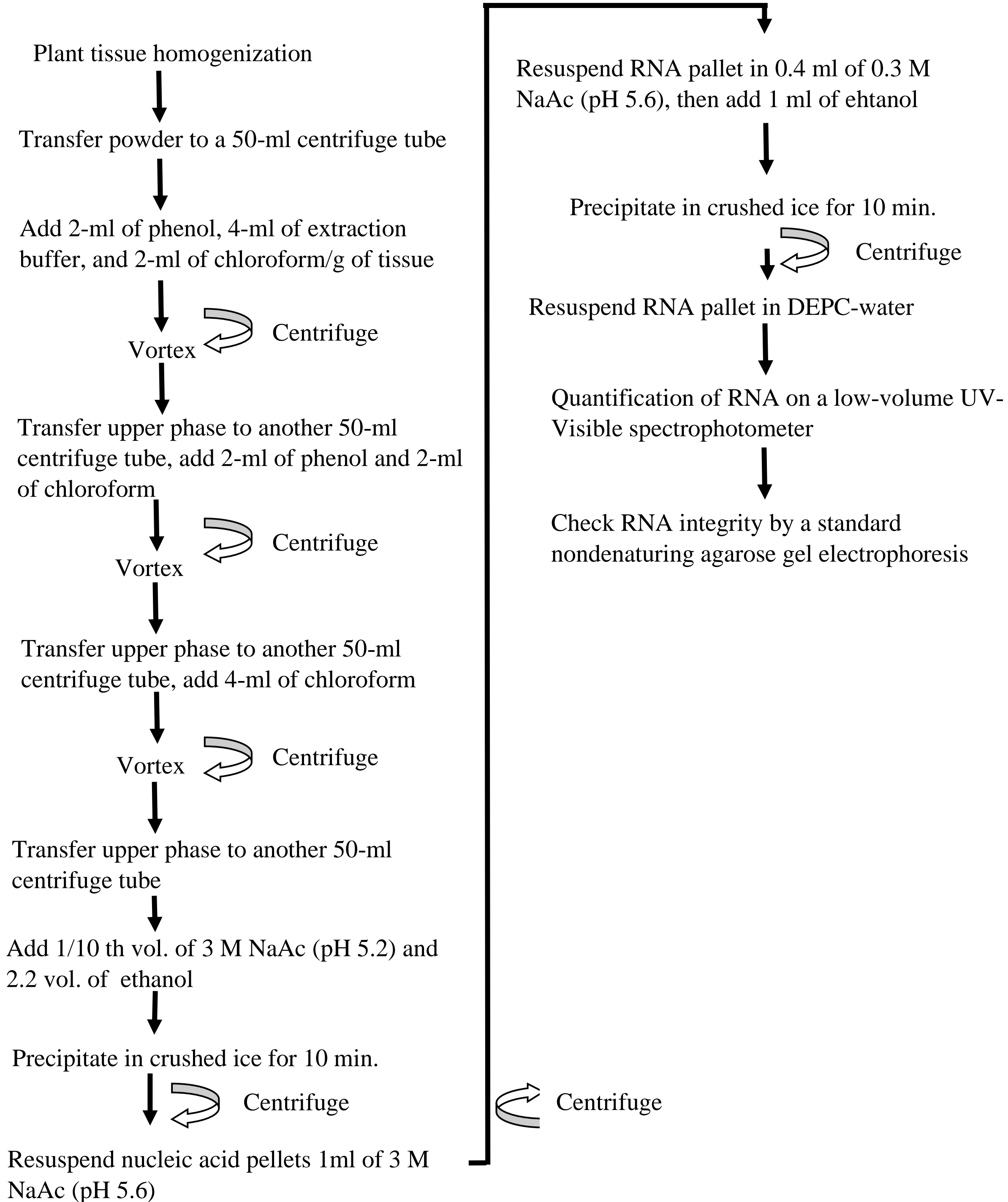

Fig.19.1 Flow chart diagram of RNA isolation from plant tissue

- Collect 1 g of plant young leaves and grind them in liquid nitrogen in a pre-cooled mortar to a fine powder.
- Do not let the tissue thaw and immediately transfer the powder to a 50-mL centrifuge tube, add 2 mL of Tris-saturated phenol (pH 8.0) immediately.
- Then 4 mL of extraction buffer and 2 mL of chloroform/isoamyl alcohol (24:1, v/v) sequentially (phenol should be added first to establish a denaturation environment for the endogenous RNases to be released into).
- Vortex the tube until a complete emulsion was formed. Centrifuge at 8,000 × g for 5 min at 4°C.
- Carefully transfer aqueous phase to another 50-mL centrifuge tube, then add 2 mL of phenol and 2 mL of chloroform/isoamyl alcohol (24:1, v/v), mix vigorously and centrifuge at 8,000 ×g for 5 min at 4°C.
- Take the upper phase and add 4 mL of chloroform/isoamyl alcohol (24:1, v/ v), then mix and centrifuge the sample as before.
- Transfer the upper phase to a 50-mL centrifuge tube and record the volume that was transferred.
- Combinations of ethanol and NaAc (pH 5.6) were used to selectively precipitate the RNA.
- First, add 0.1 volume of NaAc (pH 5.2) and 2.2 volumes of ethanol precooled at -20°C to the transferred aqueous phase and mix it by inversion several times.
- After embedding the tubes in crushed ice for 10 min, centrifuge them at 15,000 3 g for 15 min at 4°C.
- The nucleic acids were spun onto the wall of the centrifuge tube. After centrifugation, decant the liquid and mark the location of nucleic acid sediments on the outer wall of the tube. Invert the tube and place it on a filer paper to allow it to air-dry to remove residual ethanol.
- Resuspend the nucleic acid sediments with 1 mL of 3 M NaAc (pH 5.6) using a pipette, then transfer the resuspended nucleic acid to a 1.5 mL Eppendorf tube.
- Centrifuge the tube at 15,000×g for 10 min at 4 ^{o}C, carefully decant and discard the supernatant, place the tube inverted on a filter paper to air dry the nucleic acids.
- Redissolve the sediment in 400 µL of 0.3 M NaAc (pH 5.6) and add 1 mL of ethanol precooled at -20°C, mix the tube by inversion several times.
- Embed the tube in crushed ice for 10 min, then centrifuge the tube at 15,000 ×g for 15 min at 4°C.

- Wash the RNA pellet twice with 200 µL of 70% ethanol, then air-dry and dissolve the pellet in 50 µL of DEPC-treated water.

19.3.4 Discussion

In this report, we mainly modified the conditions of ethanol precipitation of nucleic acids, and the modified protocol included precipitation with ethanol by incubation tubes in crushed ice (0 ^{o}C) for 10 min, then the tubes were centrifuged for 15 min to collect the insoluble nucleic acids. As a result, the duration of RNA isolation was shortened to 2.5 hr. Furthermore, we also modified the quality control methods: the agarose gel electrophoresis for checking RNA integrity was performed by directly running nondenatured RNAs on a standard agarose gel; and besides UV absorbance measurement, a spectrophotometric spectrum analysis was introduced for checking RNA purity and assessing the presence of possible contaminants in RNAs. The duration for RNA quality control measurements was 1.5 hr. The whole procedure required 4 hr to be carried out, which is only about half of the duration of previously reported protocols.

19.3.5 Alternative method (simple and rapid method)

This method is proposed by Farhad MasoomiAladizgeh et al., and requires very less effort and time to extract RNA from plant species. This method also has an advantage as suitable for the elimination of polysaccharide and polyphenolic compounds which reduce the quality of nucleic acid. In this experiment, which lasted few years only a strategic reagents were used and prohibited the use of unnecessary materials for preparing the extraction solution. Thus, it has developed a high throughput protocol which can be employed for a widespread range of plant tissues that can compete with commercial kits. This protocol was tested with different samples like apple, pear, potato, thyme, wheat, rice, and many other samples. This method is easy and cost efficient than all RNA extraction protocols and commercial kits that have been developed until now. The purpose of introducing this procedure was accelerating nucleic acid extraction from diverse plants which takes within one hour.

19.3.5.1 Reagents

Hexadecyltrimethylammonium bromide (CTAB)

- Tris (hydroxymethyl) aminomethane (Tris base)
- Ethylenediaminetetraacetic acid disodium dehydrate (EDTA)
- Sodium chloride (NaCl)
- 2mercaptoethanol (βME)
- Polyvinylpyrrolidone (PVPP)
- Isopropanol
- Chloroform
- Isoamyl alchohol

19.3.5.2 Procedure

19.3.5.2.1 Extraction buffer preparation:

Add CTAB (0.5 g), EDTA (1 g), Tris base (2.5 g), and NaCl (5 g) as tetrad components of the extraction buffer to 100 ml autoclaved water and then gradually dissolve it by shaking at room temperature.

Note: Add 10 µl βME to 1 ml of the extraction buffer before use to decrease the probably oxidation only for tissues with high polysaccharides and secondary metabolites.

Note: add 15 mg PVPP per 1 ml of extraction buffer only for tissues with high polyphenolic pollution.

19.3.5.2.2 Homogenization of Tissues: TIMING 10 min for 5 samples

1- Ground samples (leaf, shoot, root, and recalcitrant samples, approximately 0.51 g) with 1 ml extraction buffer without liquid nitrogen in mortar and pestle that was sterilized.

CRITICAL STEP- The procedure are carried out at room temperature except centrifugation steps (at 4℃) and after precipitation of nucleic acid using the isopropanol (on ice).

CRITICAL STEP- severely disrupt the tissues to create the glaze mode of samples.

2- Transfer the resulting solution to a sterile centrifuge tube (size=2 ml), and then mix sample by briefly vortexing until the sample is thoroughly resuspended.

19.3.5.2.3 Triple Phase Separation: TIMING 25 min

3- In this step, incubate samples at 65℃ for 10 min for lysing cells completely.

4- Add 600 µl of chloroform: isoamyl alcohols (24:1) to each tube, homogenize them by vortexing.

5- Centrifuge at 13700 g at 4℃ for 10 min.

CRITICAL STEP- Upper phase with extraction buffer at PH=8.59 has both DNA and RNA together, while we reduce the PH to 6-7, DNA precipitates and only RNA remains at upper phase.

19.3.5.2.4 Precipitation of Nucleic Acid TIMING 15 min

6- Transfer the upper aqueous phase to a new tube (size= 1.5 ml).

7- Add 700 µl of cold isopropanol to precipitate RNA or DNA and then invert tubes 3-4 times to mix the solution.

8- Centrifuge tubes at 13700 g at 4℃ for 10 min. The white pellet will be visible on the bottom of the tubes.

CRITICAL STEP Do not disturb down phases of solution when you removing the upper phase.

19.3.5.2.5 Purification of Nucleic Acid: TIMING 5 min

9- Discard the supernatant and wash the precipitate nucleic acid gently with 70% EtOH.

10-After centrifuging at 5400 g at 4˚C for 5 min, remove the supernatant and then air dry the resultant pellet.

19.3.5.2.6 Dissolving and Storage Condition TIMING 5 min

11- Dissolve the pellet in 20-30μl of RNase free water (commercial) or autoclaved water.

12-After incubate at room temperature for a few minutes, put solubilized nucleic acid in −20˚C for the short time storage or in −80˚C for long time storage.

19.3.4 Quantitative estimation of RNA by Nanodrop Spectrophotometer

The RNA was analyzed on a Nano Drop 2000c spectrophotometer according to the manufacturer's instructions. An absorbance spectrum was obtained from 220 to 350 nm, the

RNA concentration was calculated with the equation 1 A_{260}= 40 µg RNA mL^{-1}, and ratios of A260/A280 and A260/A230 were calculated to evaluate the purity of the RNA samples that were extracted**.**

19.3.4.1 Procedure

1- Wash the sample reader with molecular grade water and dry with a KimWipe.

2- Following the software's instructions, load 2 µL of elution water (blank) and initialize the system.

3- Change the computer's setting to RNA and click the blank button.

4- Load 2 µL of sample and click the measure button.

5- After the read is complete, record the A260/A280 and A260/A230 ratios as well as the amount of RNA recovered (in ng/µL).

6- Wipe the sample reader with a clean, dry Kim Wipe between samples and repeat steps 5-6.

19.3.4.2 Interpreting the Results & Troubleshooting

1-As a reference, collagen gels seeded with ~700,000 cells typically yield around 50-150 ng/µL of RNA when eluted in 30-35 µL of water.

2- A lower than expected concentration of RNA indicates low cell numbers in the sample, poor homogenization of samples, or too much volume of water used in the elution step of RNA purification.

3- Very pure RNA will have an A260/A280 ratio of ~2.1. Anything higher than 1.8 is considered to be of acceptable purity, and a ratio of <1.8 indicates potential DNA or protein contamination.

4- A low A260/A280 ratio is likely due to mixing phases when removing the upper aqueous phase of the Trizol separation (if Trizol used to extract RNAs) or is also more common in samples with a very low yield of RNA.

5- The A260/A230 ratio should also be above 2.0. A low A260/230 ratio indicates contamination with the wash solutions, chaotropic salts, phenols or protein.

6-A low A260/A230 ratio is most likely due to contamination of the samples with washing buffers during the Minispin tube washes. Be more careful when handling the tubes, especially when adding washes solution or removing the spin-through. Try to gently pour out the flow-through and then carefully wipe away drops on the outer rim of the collection tube with a KimWipe.

19.3.5 Qualitative estimation of RNA by agarose gel electrophoresis

Dilute 10 µL of RNA samples to 1 µg/µL with DEPC-treated water according to the quantification results, 0.5, 1, 2, and 4 µg of RNA aliquots were taken out and adjusted to 9 µL with DEPC-treated water, then 1 µL of 10 × RNA loading buffer was added. After mixing the samples, all aliquots were loaded onto a 1.5% TAE agarose gel containing 0.5µg/mL EtBr. Electrophoresis was carried out in 1 × TAE at 5 V/cm until the dye front had migrated two-thirds of the way down the gel. The gel was photographed with a Gel Doc XR System (Bio-Rad).

19.4 SUMMARY

The practice of RNA isolation in undergraduate experimental courses is rare because of the existence of robust, ubiquitous and stable ribonucleases. We reported here modifications to our original protocol for RNA isolation from plant tissues, including the recovery of nucleic acids by ethanol precipitation at 0°C for 10 min and the assessment of RNA quality by visualizing the banding profile of the separated RNAs on a standard nondenaturing agarose gel to shorten the duration of the whole procedure and simplify the operation. As a result, the modified procedure, including RNA isolation and quality control analysis could be finished in 4 hr and divided into two sessions. Because endogenous ribonucleases released upon disruption of the organelles and vacuoles were effectively and quickly inactivated, measures were taken to protect RNA integrity throughout the whole procedure so that total RNA with high purity and integrity as well as an appropriate yield could be obtained by students. The RNA isolation protocol described here was simple, efficient, flexible, and low cost. Therefore, it is an ideal approach for undergraduates to learn about RNA techniques. Similarly a more rapid method is also described for the students to speed up the isolation and purification procedure for RNA quantification.

19.5 GLOSSARY

DNA: Deoxy ribonucleic acid contains the whole genetic constitution of a cell.

RNA: Ribonucleic acid present inside the cell such as massanger-RNA transcribing the genetic code into proteins.

PCR & RT-PCR: Polymerase chain reaction (PCR) used to amplify the DNA fragment into multiple copies. Real time PCR has the same function but it works in real time.

RNases: These are the enzymes, degrade to RNA and also present inside the organelles and vacuoles of cell. Therefore during the isolation of RNA their inactivation is very essential.

DEPC: Diethyl pyrocarbonate used to inactivate the RNases

CTAB: Hexadecyltrimethyl ammonium bromide cationic detergent used in lysis buffer to solubilise the membrane in isolation of nucleic acids and other organelles.

Tris base: Tris (hydroxymethyl) aminomethane is a basic buffer used mostly in cell biology and molecular biology laboratories.

EDTA: Ethylenediaminetetraacetic acid is a detergent used to solubilise the membrane lipids

Nanodrop: Nanodrop is a spectrophotometer used in molecular biology laboratories to measure the small volume (1-2 µl) based concentration of nucleic acids.

Agarose gel electrophoresis: It is an apparatus used to separate the DNA or RNA strands on the basis of size in electric field.

Gel Doc: Gel Doc or gel documentation is an imaging machine used to take the photograph of electrophoretically separated DNA or RNA bands.

19.6 SELF ASSESSMENT QUESTIONS

Q.1. What are the factors or considerations essential during isolation and purification of RNA?

Q.2. What is the role of pH in RNA isolation?

Q.3. Why the isolation of RNA is critical compared to DNA?

Q.4. What are the RNases? Why their inactivation is very crucial during RNA isolation?

Q5. Describe the critical step in separation of RNA from DNA in whole procedure of nucleic acid separation.

Q6. Describe why the RNA is less stable compare to DNA and consequently difficulties in its isolation

Q 7. What do you understand by A260/A280 ratio? How does it help in purity of RNA?

Q 8. What do you understand by Gel Doc ? How it is helpful in RNA isolation?

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19.9 TERMINAL QUESTIONS

Q.1. Describe the sample preparation procedure for RNA isolation

Q.2. Describe the Detailed procedure of sample treatment before RNA isolation

Q.3. Describe the rapid RNA isolation procedure and critical steps to isolate RNA in high yield

Q.4. Describe the methods used in RNA quantification.

Q 5. Describe the qualitative method of RNA confirmation in your sample.

Q 6. Describe the method used to separate DNA from RNA in isolation procedure.

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