PLANT PHYSIOLOGY, CELL BIOLOGY & GENETICS
(DSBOT32)
(BSC BOTONY-IV)

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Lesson-1

ENZYMES

1.0 Objective: Enzymes are organic catalysts or biocatalysts present in all living cells. They regulate the rate of biochemical reactions without being used in those reactions. Enzymes have been called the agents of life since they control almost all life processes.

Structure of the lesson:

1.1. Historical Account

1.2. Properties

1.3. Chemical Nature of Enzymes.

1.4. Nomenclature.

1.5. Classification.

1.6. Mechanism of enzyme action.

1.7. Factors affecting the rate of enzyme activity.

1.8 SYNOPSIS

1.9 Technical terms:

1.10 Self Assessment Questions:

1.1 Historical account: - The study of enzymes in called Enzymology.

- In 1857 *Louis Pasteur* proposed that the process of fermentation occurs only in living cells.
- In 1878 *Freidrich W.Kuhne Coined* the word enzyme *(en=in. zyme = yeast)* for ‘ferments’ proposed by earlier investigators.
- In 1887 *Edward Buchner* discovered a substance in the yeast extract which fermented the sugar solution. He termed it as ‘Zymase’.
- In 1926 *J.B. Sumner* isolated the enzyme Urease in a crystal form from jack bean seeds, for which he was awarded Nobel Prize.
- In 1930 *John Northrop*. Isolated pepsin and trypsin in crystallized form and convincingly proved that all enzymes are proteins.

1.2 PROPERTIES OF ENZYMES

Enzymes are characterized by the following properties.

1) Catalytic property: - All enzymes are catalysts and as such that they accelerate the speed of reaction. But it remains unchanged during the reaction.

2) Nature of Enzymes: - Enzymes are basically proteins. Hence they exhibit protein properties like high molecular weight, slow-diffusion, colloidal nature, movement in response to electric current, inability to diffuse through living membranes.

3) Enzyme Specificity: - Enzymes are highly specific. A particular enzyme catalyzes only a particular kind of a reaction. e.**g.** Malic dehydrogenase acts on malic acid, urease acts only on urea.

4) Reversibility of action:- Enzyme reaction proceeds in both directions.
e.g. The enzyme aldolase catalyses the conversion of fructose bis-phosphate (Hexose) into two trioses. The same enzyme also catalyses the formation of hexoses.

5) Turnover Number: - It is the number of substrate molecules converted into products by a molecule of enzyme in one minute. It is also known as molar activity or molecular activity. The turnover number for different enzymes varies from 100-3,00,000 molecules.
e.**g.** The turnover number of sucrose is 10^{6}

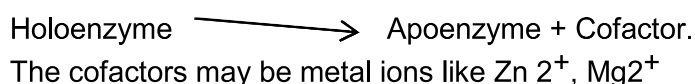
6) Thermo labile: - Enzymes are heat sensitive. At low temperatures they are temporarily inactivated. At high temperature they get denatured. At optimum temperatures (25^0 – 30^0C) the enzymes are active.

1.3 Chemical nature of Enzymes:-

All enzymes are proteins. But all proteins are not enzymes. Based on chemical composition, enzymes are classified into simple and conjugated enzymes.

(I) Simple Enzymes: - These enzymes contain only protein part.
e.g.:- Trypsin, Pepsin, Amylase etc.,

(II) Conjugated Enzymes;- They contain protein and non protein part. The protein part is called apoenzyme and non protein part is called cofactor. Together they constitute to form Holoenzymes.

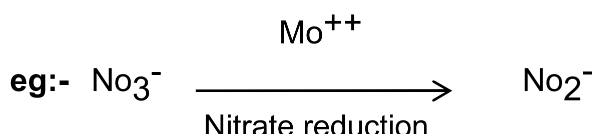


The cofactors may be metal ions like Zn^{2+}, Mg^{2+}

The non protein part may be some times an organic compound called Coenzyme like NAD,TPP and FAD. Coenzyme can be separated from its apoenzyme by dialysis.

The cofactors which are permanently associated with their protein are known as Prosthetic group. e.g.: The heme prosthetic group of hemoglobin.

Some enzymes require activators to come into active form and catalyses a reaction, these are called active cofactors. They are not an integrated part of enzyme.



1.4 Nomenclature:- Name of enzyme generally ends with the suffix.'ase'. Names of some of the enzymes are derived from the substrate on which they act.

eg:- Cellulase, Sucrase, Lipase etc.,

Some enzymes are named after the biochemical reactions which they catalyse.

eg:- Dehydrogenase, decarboxylase etc.,

Some other enzymes are named after the substrate as well as type of biochemical reaction.

eg:- Pyruvic decarboxylase.

1.5 Classification of Enzymes:- International Union of Biochemists (I U B) appointed a commission on enzymes in 1961. Salient features of the classification recommended by the I U B are as follows :

- All enzymes are grouped into six major classes.
- Each enzyme is a given a code number with four digits
- In enzyme code First digit indicates class, second digit indicates Sub-Class, third digit indicates Sub-Sub class and fourth digit indicates the Serial number of the class.

eg:- Enzyme code 4.1.2.7 for ketose 1-phosphate aldehyde lyase is 4.1.2.7.

4	1	2	7
Class	Sub Class	Sub-Sub class	Enzyme number

- The Six major classes are as follows :-

I) Oxidoreductases IV) Isomerases

II) Transferases V) Ligases

III) Lyases VI) Hydrolases

Class I : Oxidoreductases:- The enzyme catalyses oxidation reduction reaction. Oxido reductases are again divided into further Sub class like Oxidases, Dehydrogenases, Reductases etc., **eg:-** Alcohol N A D Oxidoreductase Enz. Code - 1 1 1 1

Acetaldehyde + NADH $\longrightarrow$ Ethyl alcohol + N A D^+

Class II : Transferases :- It catalyses the transfer of groups from one substrate to another group. There are sub classes in it. They are transketolase, transaminase, transaldolase, transphosphorylase, hexokinase etc.,

eg:- ATP glucose - 6 - phosphosphotransferase Enzy. Code - 2.7.1.1

Glucose +ATP $\longrightarrow$ Glucose - 6 - phosphate + ADP.

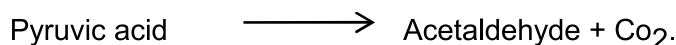
Class III : Hydrolyses :- This group of enzymes is involved in the cleavage of bonds by the action of water. These include carbohydrases, Lipases, Phosphotases etc.,

e.g.:- Glucose - 6 - phosphate phosphohydrolase. Enzy.Code 3.1.3.9.

Glucose - 6 - Phosphate + H_2O $\longrightarrow$ Glucose + Phosphoric acid.

Class IV : Lyases :-These enzyme catalyse the removal of groups from the substrates without the addition of water.

e.g.:- Pyruvic acid decarboxylase Enzy. Code - 4.1.1.1



Pyruvic acid $\longrightarrow$ Acetaldehyde + Co_2.

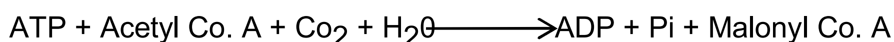
Class V : Isomerase :- These enzymes bring about isomeric changes.

e.g.:- Glyceraldehyde 3 - Phosphate Ketol Isomerase Enzy.Code - 5.3.1.1

Glyceraldehyde - 3- Phosphate $\longrightarrow$ Dihydrorxy actone Phosphate.

Class VI : Ligases or Synthetases :- They help in the synthesis of a new Compound generally with the cleavage ATP.

e.g.:- Acetyl Co. A. Co_2 Ligase (ADP) Enzy. Code - 6.4.1.2.



ATP + Acetyl Co. A + Co_2 + H_20 $\longrightarrow$ ADP + Pi + Malonyl Co. A

1.6 Mechanism of Enzyme Action:-

Two main theories were proposed to explain the mechanism of enzyme action.

They are **I) Lock and Key Model II) Induced Fit Theory.**

I) Lock and Key Model:-

This theory originally proposed by Fischer (1898). According to him the enzyme and the substrate fit into each other structurally like the two pieces of the lock and key. Just as only a particular key can fit into a particular lock, enzymes have specific active sites, where the substrate molecules can fit in. No other molecule can fit into this slot.

II) Induced Fit Theory:-

This theory was proposed by Koshland. According to it, a substrate induces some structural change in the enzyme. As a result, the active site of the enzyme becomes flexible and changes its shape according to the necessity of the substrate molecule.

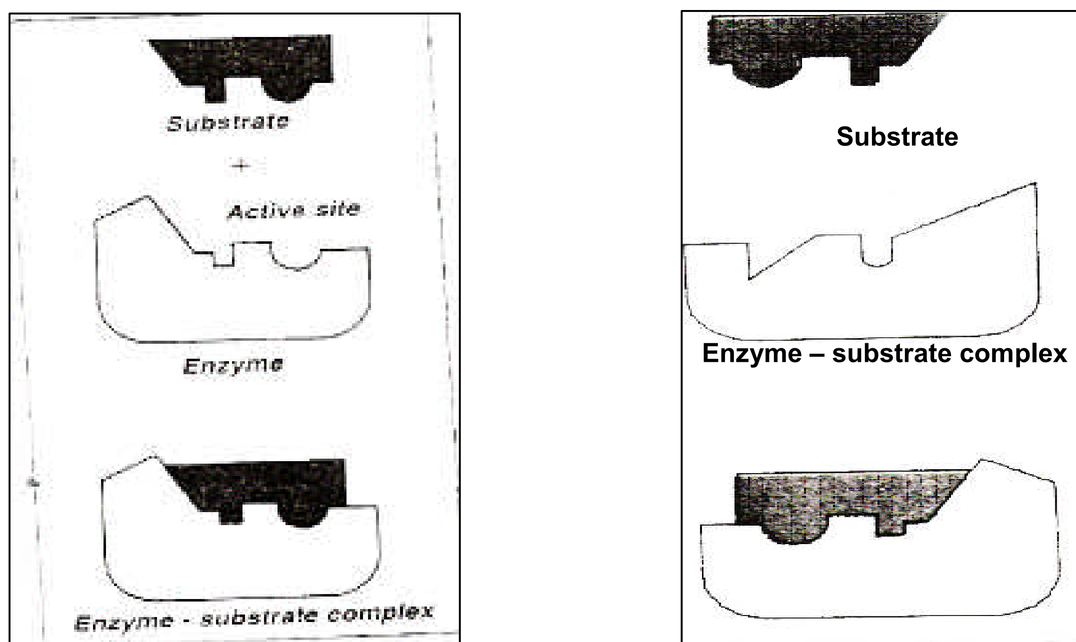
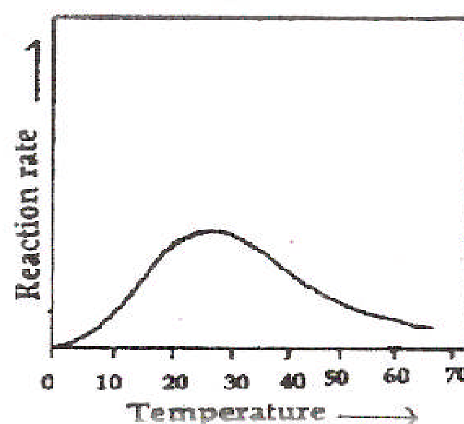


Fig: 2.0 Mechanism of Action

1.7. Factors effecting enzyme action:

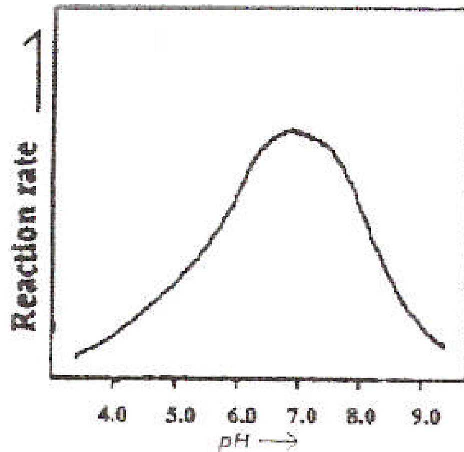
(I) TEMPERATURE: Enzyme cease to function at O^0C at which they remain inacive. As the temperature increases, rate of enzyme action also increases. For most enzymatic reactions, rate of the reaction doubles for every 10^0C rise in temperature (Q_{10}) between 5^0C - 35^0C. An *Optimum temperature range for most enzymes is 25^0C - 30^0C.*



A

Above 30^0C, the rate decreases. At 60^0C or more the enzymes are denatured (permanently damaged). Temperature sensitivity of an enzyme depends on the water content of the tissue. Enzymes present in dry seeds remain functional even if the seeds are subjected to high temperature.

(II) pH: Each enzyme will have an optimum pH at which the rate of enzyme activity will be

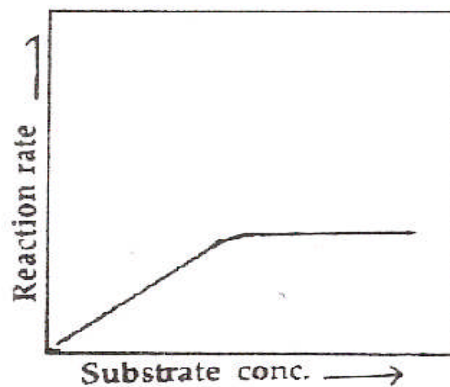


maximum. If pH is below or above the optimum, the rate of reaction decreases. *Optimum pH for many enzymes is around seven,* However some enzymes function activiely at very acidic and very alkaline pH.

e.g. Pepsin - 2 pH

Trypsin - 8 pH

(III) SUBSTRATE CONCENTARTION: Rate of enzymatic activity increases with an increase in the concentration substrate. Later, the rate of enzyme activity does not depend on substrate concentration. If the substrate concentration reaches the turnover capacity of the enzyme, rate of the reaction remains limited to that concentration only. It is of no use to raise the substrate concentration beyond the turnover capacity of the enzyme.



Concentration of substrate decreases with time with a proportionate increase in the concentration of products.

(IV) ENZYME CONCENTRATION: Increase in concentration of enzyme results in an increase in the rate of a reaction provided the substrate is abundant. Increasing the enzyme concentration without increasing the concentration of substrate is of no use.

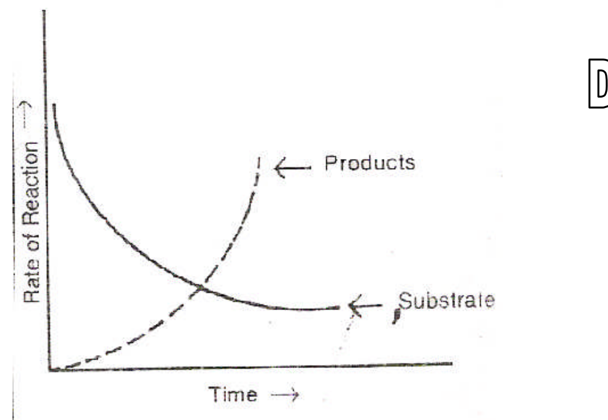


Fig:2.1 Factors Affecting Enzyme Activity

A. Temp.
B. pH
C. Substrate Conc.
D. End Products

(V). END PRODUCTS: Accumulation of end products of a metabolic path results in a decrease of enzymatic activity. Such an inhibition is called *allosteric inhibition* or *feedback inhibition.* The accumulated end product molecules act as modulate substances and bind with a specific site of the enzyme (allosteric site), different from its substrate binding site and inhibit the enzyme action.

VI. Competitive and non competitive inhibition: Enzyme inhibitors are two types competitive and non competitive inhibitors. Substances which are structurally analogous to the substrate molecules occupy active sites on an enzyme and inhibit the enzyme action. Substances that act in this manner are called competitive inhibitors.

e.g.: succinic acid dehydrogenase which is an enzyme for succinic acid is inhibited by malonic acid, which is therefore, competitive inhibitor.

The non competitive inhibitors do not affect the formation of the enzyme substrate complex. It may prevent the breakdown of the complex completely or may reduce the rate of its breakdown.

1.8 SYNOPSIS

The enzymes are mostly proteins whose biological function is to catalyze chemical reactions in living system. Most enzymes are conjugated proteins which consist of the protein component the apoenzyme and the non protein part the cofactor. International Union of

Biochemists classified the enzymes and gave the enzyme code with 4 digits. Accordingly enzymes are classified into 6 major classes. Regarding the enzyme action mechanism two major theories are proposed.

1.9 Technical terms:

Enzymology, Turnover number, Apoenzyme, Cofactor, Prosthetic group, Coenzyme, Dialysis, Allosteric inhibition.

1.10 Self Assessment Questions:

Essay Questions:

1. Write a question on enzyme classification?
2. What are enzymes? Explain the enzyme mechanism and factors?

Short Questions:

1. Enzyme properties.
2. Feed back inhibition.
3. Nomenclature.
4. Enzyme Mechanism
5. Competitive inhibition.

Further reading :

1. S. Mukherji A.K. Ghosh, 2005, Plant Physiology, New Central Book Agencie, Kolkatta
2. Taiz & Zeiger, 2005, Plant Physiology, Sinauer Associates Publishers, U.S.A.
3. Devlin & Witham, 2000, Plant Physiology, CBS Publishers, New Delhi.

- K.V.S.Durga Prasad

LESSON - 2

PLANT WATER RELATIONS

2.0 Objective: This is an important topic because water plays a crucial role in the life of the plant. For every gram of organic mater made by the plant, approximately 500g of water is absorbed by the roots, transported through the plant body and lost to the atmosphere.

Structure of the Lesson:

2.1. Diffusion

2.2. Osmosis

2.3. Water potential

2.4. Imbibition

2.5. Plasmolysis

2.6. Absorption of water by plants

2.7. Transpiration

2.8. Stress physiology

2.9. Synopsis.

2.10. Self Assessment Questions

2.11. Reference Books

2.1 Diffusion:-

Movement of ions or molecules from a region of higher concentration to a region of lower concentration until the molecules are uniformly distributed throughout the available space is known as diffusion. If a bottle of perfume or either is opened, the characteristic odour can be detected within a very short time. This is due to molecules of the volatile substance diffusing into air. When a crystal of copper sulphate is placed in a beaker containing water, the crystal gradually dissolves and the particles start migrating in all directions and water turns into green because of diffusion. The above examples clearly illustrate the phenomenon of the diffusion. The rate of diffusion of a substance determined by concentration gradient i.e., the concentration difference per unit distance. The diffusion can occur only when the concentration of the diffusing substance is not uniform throughout the system and the process continues only as long as the difference between the concentrations is maintained. The rate of diffusion of gases is generally fast while the liquids and solutes have a slow rate of diffusion.

Diffusion is involved in many plant processes such as gas exchange and the movement of nutrients towards root surfaces. For instance Co_2 from air reaches the chloroplast through the stomatal pores of the leaf by diffusion. Similarly the water vapour accumulated in the intercellular spaces of the leaf tissue escapes out by means of diffusion.

2.2 Osmosis:-

The diffusion of solvent molecules from the region of their higher concentration to the region of their low concentration through a selectively permeable membrane is called osmosis " When two solutions of different concentration are separated by means of a semi permeable

membrane, the diffusion of water or the solvent from the solution of lower concentration to the solution of higher concentration occurs until a state of dynamic equilibrium is attained. This is known as osmosis.

Demonstration of Osmosis:- A thistle funnel is taken and its mouth is tightly tied with a sheet of cellophane (Semi permeable membrane). Sucrose solution is taken in the thistle funnel. The initial level of the solution is noted. It is immersed in a beaker containing pure water. There will be a spontaneous movement of water from the beaker through the membrane into the sucrose solution. Due to this movement the level of sucrose solution in the thistle tube increases. Pure water is the strongest solvent. These solvent molecules from their region of higher concentration diffused into the funnel where its concentration is low through the selectively permeable membrane.

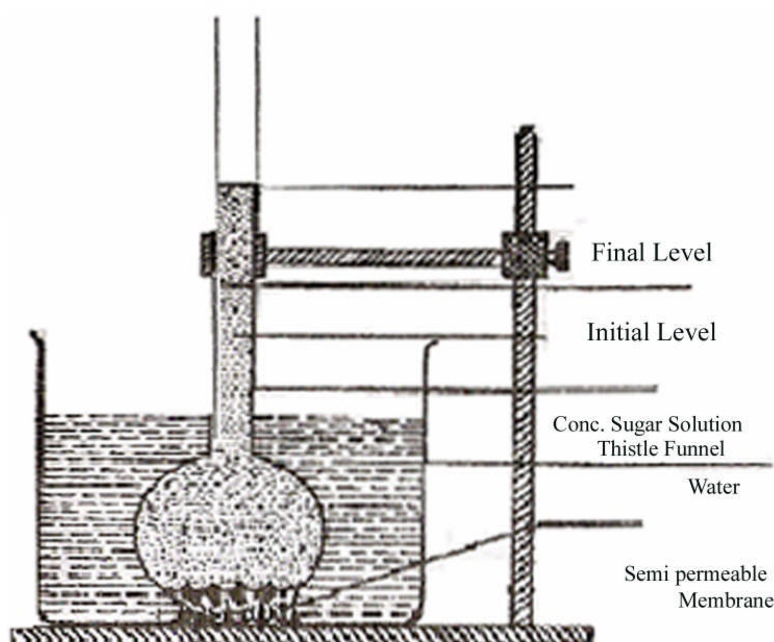


Fig. 1.1 Demonstration of osmosis

Three types of solution are recognized with reference to concentration of solutes.

Hypertonic: High concentrated solution i.e., more solutes and less solvent. If a cell is placed in it, water in the cell diffuses out of it and becomes flaccid called exosmosis.

Hypotonic solution:- A low concentrated solution with less solutes and more solvent. When a cell is placed in it, water enters into it and the cell becomes turgid called endosmosis.

Isotonic Solution: When two solutions are having same concentration of solutes the concentration of the solution is such that it neither gains nor loses water in an osmotic system.

2.3 Water potential: -

The energy liberated from a substance when its potential energy is converted to kinetic energy under constant temperature and atmospheric pressure is called 'free energy'. The amount of free energy present in one mole of substance is called its 'Chemical potential'. Slayter and Taylor (1960) defined that the chemical potential of water is called' Water Potential'. It is the difference between the free energy of water molecules in any system or solution and the free energy of water molecules. It is expressed in terms of bars or megapascals Mpa = 10 bars. It is denoted by symbol ψ (psi). The water potential of pure water is maximum and it is zero (0). When a solute is added to the pure water the water potential decreases and becomes negative. Water always moves from a system of higher water potential (less negative) into a system of lower water potential (more negative). The lower the potential, the greater is the capacity to absorb water. In plant tissues water potential is always less than zero. Leaves and aerial parts of plants in soils with adequate moisture will normally have water potential between 2 and 8 bars. With decreasing supply of water the values become more negative and at 15 bars plant tissues cease to grow.

$$\psi_W = \psi_S + \psi_P$$

This equation states that water potential in a plant cell (ψ_W) is equal to the algebraicsum of the solute potential (ψ_S) and pressure potential (ψ_P) All these components are expressed in units of bars or Mpa.

I) Osmotic potential: - The amount by which water potential is reduced as a result of the presence of solute particles is called osmotic potential or solute potential. The Osmotic potential is a measure of the actual pressure that can develop in a cell to stop the diffusion of water by osmosis. The sign for osmotic potential is ψ_S. The osmotic potential has a negative value. As the solute concentration increases then negative value of ψ_S increases.

II) Pressure Potential:- The actual pressure exerted by the contents of the cell on the cell wall during endosmosis is known as turgor pressure. The cell wall exerts an opposite and equal force to counter the turgor pressure. It is called wall pressure. The quantum of change in water potential owing to pressure is termed as pressure potential. The sign for pressure potential is P(ψ_P). The pressure potential in plant cell is usually positive.

2.4 Imbibition:- The adsorption of water by hydrophilic colloids is defined as imbibition. When solid substances are kept in liquid, they get soaked and swell in size. If seeds are soaked in water. They increase in their size due to intake of water, similarly wooden plugs, velamen roots etc., this process of intake of water and subsequent increase in size noticed in solids such as wood is due to process called imbibition.

During imbibition, there is both absorption as well as adsorption. The water gets into the cell, there by increasing the volume and this is called absorption. Some water molecules however are held over the surface and this is called adsorption.

The basic cause of imbibition is a differences in the diffusion pressure. In a dry piece of wood, the turgor pressure is almost nil and when it is placed in water it readily absorbs water

due to a pressure gradient. The imbibition of water continues until the equilibrium i.e., the pressure of water inside of the imbibant equals the pressure of water outside. Another condition necessary for imbibition is affinity between the colloids present in the imbibants surface and the substance to be imbibed. For instance, a piece of wood placed in oil doesn't imbibe, so also a piece of rubber placed in water.

2.5 Plasmolysis:-

(Plasmo - Cytoplasm, lysis = breakdown) shrinkage of protoplasm of a cell due to exosmosis is called plasmolysis. When a cell is placed in higher concentrated solution as the water potential of the cell is high than that of the outside solution. As we know that water always moves from a region of higher water potential to region of lower water potential, water from cell sap flows out due to water potential gradient and the protoplast shrinks. In such a cell the space between the protoplast and cell wall is occupied by the external solution. If the conditions persist for a long time the cell will die. Wilting of plants noticed under conditions of water scarcity is an indication of plasmolysis of cells. There are three stages in plasmolysis. Viz. incipient plasmolysis, evident plasmolysis and final plasmolysis.

I. Incipient Plasmolysis:- When turgid cell is kept in hypertonic solution water starts moving out of the cell. The plasma membrane, which is in contact with the cell wall around due to its elasitc capacity, separates from the cell wall at the corner, while maintain contact at other regions. This is the first stage of plasmolysis. It is called incipient plasmolysis.

II. Evident Plasmolysis:- The cell under hypertonic condition continues to lose water to the external medium and shrinks further in volume. The plasma membrane gets detached from the cell wall and goes on contracting. This is the second stage of plasmolysis.

III. Final plasmolysis: - As exosmosis continues, cytoplasm will be completely free from the cell wall and remains in the centre of the cell and such a cell is called plasmolysed cell.

When a plasmolysed cell is placed in water the cell gains its turgidity.

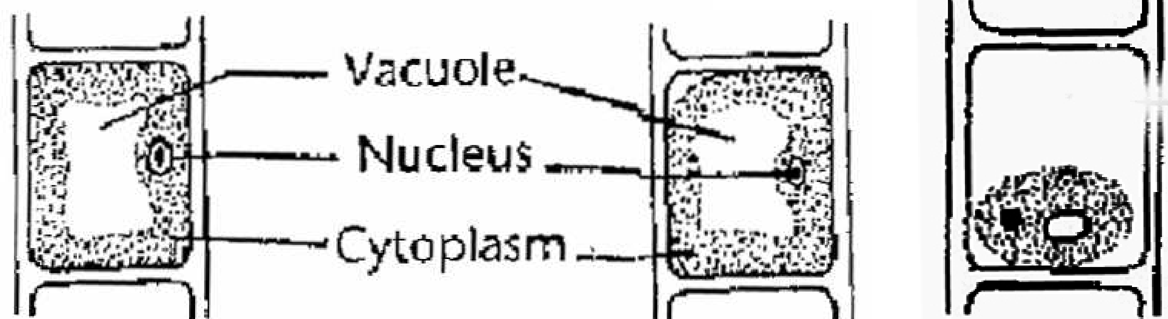


Fig. 1.2 Turgid Cell **Incipient Plasmolysis** **Plasmolysed cell**

2.6 ABSORPTION OF WATER BY PLANTS

ROLE OF WATER IN PLANTS:

- Water is one of the most important compounds that profoundly influences life. Nearly

80% of the fresh weight of the plant tissue is comprised of water. All the cell organelles maintain their structure and function in the presence of adequate quantity of water.

- It is an excellent solvent for the various molecules transported with in the plant.
- Breakdown of various chemical compounds takes place only in the medium of water.
- Water stream flowing throughout the plant body acts as a coolant and maintains the temperature.
- Water acts as an electron and proton donor in the photosynthesis.
- Cell turgidity is maintained by water.

Water Absorbing System in Plants :- It is common knowledge, that the root system of the plant mainly responsible for the absorption of water. Kramer (1956) observed that the maximum intake of water occurs in the root hair zone. Root hair is a delicate tubular prolongation of the epidermal cell. The presence of large number of elongated root hairs on a root increase considerably the exposed surface for the absorption of water.

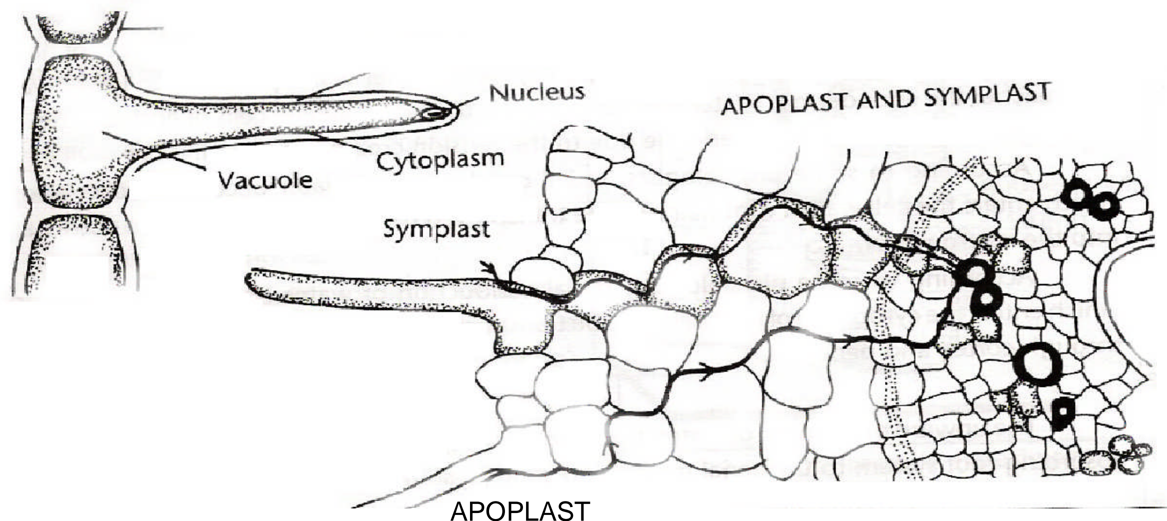


Fig 1.3 Absorption of water.

Mechanism of Absorption of Water : Kramer (1949) recognized two mechanisms for absorption of water. They are active absorption and passive absorption.

Active Absorption : "Absorption of water due to the forces developed in the root cell is called active absorption." There are two major theories to explain the active absorption.

i) Osmotic theory of active absorption.

ii) Non-Osmotic theory of active absorption.

Osmotic theories of active absorption :- Atkins (1916) and Priestly (1922) postulated that, the difference in the concentration gradient between the cell sap and soil solution is responsible for the entry of water into the roots. As the water potential of cell sap is very low i.e., with a higher negative value than the soil solution, osmotic migration of the solvent takes place into the cell.

Non - Osmotic Theory: - Many physiologists believe that water absorption occurs with the utilization of metabolic energy. Absorption of water has been observed against the concentration gradient. The following are the evidences to suggest the involvement of respiratory energy in water absorption.

a) There is a correlation between the rate of water absorption and respiration.
b) Water absorption rate is lowered by low temperature, oxygen tension, and respiratory inhibitors.
c) Respiratory promoters increased the rate of absorption.

Objection to Non-Osmotic Theory:-

- Low temperature and lack of O_2 apparently increases the viscosity of cytoplasm and reduce the permeability. This doesn't indicate involvement of respiratory energy.
- Lack of O_2 inhibits water absorption by passive mechanisms brought about by concentration gradient.
- If respiration is inhibited, generally it upsets all the physiological systems. Naturally a weakened cell cannot carry on water absorption.

In view of this koziwski (1964) opined that the bulk of water intake in plants is by the passive mechanisms and active uptake is negligible.

The Osmotic potential of the cell sap of the root hairs of mesophytic plants is generally as high as 0.3 to 0.8 Mpa. Osmotic potential of soil solution is, much lower than that of cell sap and it is less than 0.1 Mpa Due to this difference in Osmotic potential water enters into the root hair from the soil solution. When an actively water absorbing plant is decapitated, sap exudes out of the cut end of the stem indicating active absorption of water. This is due to root pressure. When water enters into the root, a positive pressure is developed in the xylem. Which is known as Root pressure.

A number of plant physiologists have supported the Osmotic absorption. Some of those scientists are Lundegardh (1950), Broyer (1951), Hoagland (1944) etc., According to Atkins, the xylem sap always has a high concentration of solutes due to the release of Sugars into them by the adjoining parenchyma cells.

Objections to Osmotic Theory: - The main objections are
1) The sap concentration in xylem is not always high. As a result a gradient may not exist between cortical cells and xylem.
2) Root pressure is not universal in plants.

Passive Absorption: - Absorption of water by the root cells due to forces developing in the shoot is called passive absorption. The roots do not play any role in the absorption of water in the sense, the force or forces responsible for water absorption are generated else where in the plant body. It is believed that transpiration has a direct influence on the rate of water absorption. A tension is created in the leaf cells due to transpiration. As a result leaf cells absorb water from xylem elements and the tension is passed on to the xylem elements of the root. Thus a tension gradient is established between the leaf cells and the root cells. As a result of this water,

migrates radially across the root cortex and traveling through the xylem finally reaches the leaf mesophylls cells, from where it escapes (transpiration). According to this theory, rate of water absorption increases the rate of transpiration.

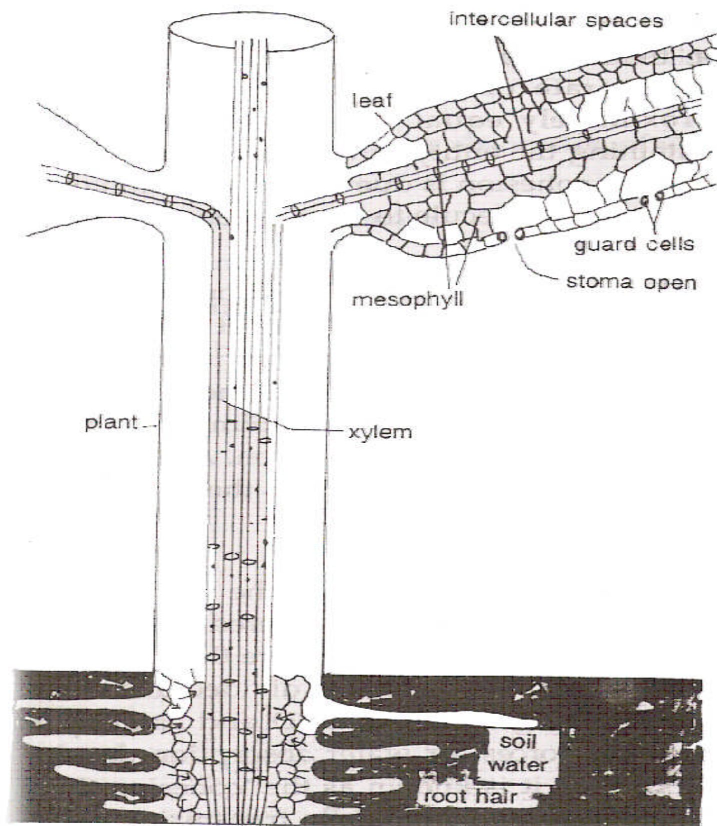


Fig 1.4 Diagrammatic representation of passive absorption of water.

2.7 TRANSPIRATION

Plants lose more than 95% of water absorbed by them and utilize less than 5% for their normal growth and metabolism. Loss of water in the form of vapour from the aerial parts of plants is called transpiration. Basically transpiration is a phenomenon of evaporation of water but differs from it due to the involvement of the biological system.

Magnitude of transpiration :- In order to understand the physiological impact of transpiration, it is necessary to know the quantity of water lost from the plant. The amount of water lost varies from plant to plant. The sunflower plant loses about 56kg of water in about 4-5 months time. A corn plant loses 54 gallons while a tomato loses 34 gallons in a growing season. The above statistics indicate the physiological importance of transpiration in the life of plants.

Types of Transpiration: - All the aerial parts of the plant i.e., stem, leaf & flowers transpire, but their efficiency will vary.

I. Stomatal Transpiration: It is the loss of water vapour through tiny pores called stomata. It accounts for 95 to 98 percent of the total transpiration in plants.

II. Cuticular Transpiration: It is transpiration through the cuticle or evaporation from the general surface of plants. Upto 2% of the total transpiration may takes place through cuticle.

The total transpiration that occurs through leaves is called foliar transpiration.

III. Lenticular Transpiration: It is transpiration through lenticels. In woody stems and fruits negligible amount of water (0.1%) is lost through lenticels.

Structure of a Stomata : The stoma is a minute pore or opening in the epidermis of the leaf. The opening is usually surrounded by two elliptical or kidney shaped cells called guard cells in dicots and they are dumbbell shaped in monocots. The epidermal cells adjacent to the guard cells are called subsidiary cells. The guard cell is a living parenchymatous cell with dense granular cytoplasm, chloroplasts and a central vacuole filled with cell sap. The cell walls are unevenly thick and this plays an important role in the opening and closing of stomata. Generally the wall facing the pore is thicker than the one away from the pore. The outer wall is thin & elastic. The stoma along with guard cells and subsidiary cells constitutes the "Stomatal Apparatus."

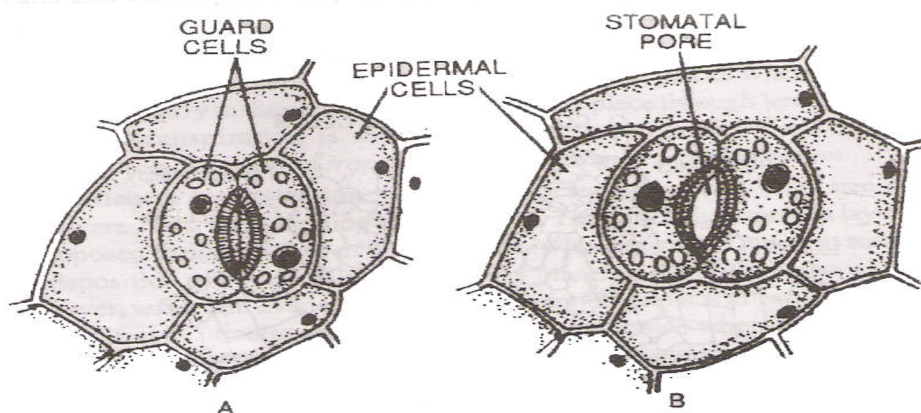


Fig:1.5 Structure of a Stomata

Mechanism of stomatal opening and closing:

Guard cells regulate opening and closing of stomata. When guard cells absorb water, they become turgid and the stoma opens. When guard cells lose water and become flaccid, the stoma closes. Generally stomata open during the day time and closes at night time. These are called. "Photoactive." In succulent plants like Bryophyllum, Stomata close during the day and open at night. These are called 'Scotoactive.'

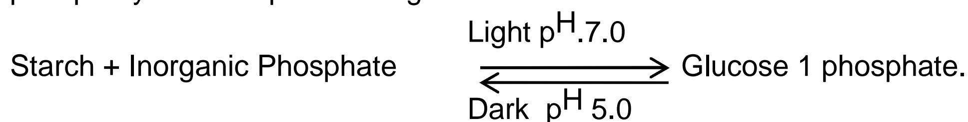
Many theories were proposed to explain the stomatal mechanism. Important among them are:

I. Photosynthetic Theory :- It was Von Mohl (1856), who thought that the photosynthetic activity in the guard cells is responsible for the opening of the stomata. According to him, the chloroplasts in the guard cell during day time synthesizes the sugars. As a result the O.P of guard cells increases. This causes the endosmosis, turgidity and the stomatal aperture opens. During night because of no photosynthesis stomata are closed.

II. Starch-sugar Hypothesis :- Lloyd (1908) observed that the amount of starch in guard cells increased during night and decreased by day. In reverse sugar content is more during day less during night. According to him the starch sugar interconversion is responsible for the opeing and closing of the stomata.

III. p^H :- Sayre (1926) observed that the p^H in the guard cells increased during the day when the stomata were open and decreased at night. According to him utilization of Co_2 during photosynthesis causes the increase in p^H resulting in the conversion of starch to sugar. Scarth (1932) proposed that during night as there is no photosynthesis, Co_2 accumulates in the intercellular spaces and increases the H^+ ion concentration and decreases the p^H, promoting conversion of sugars into starch.

Iv. Phosphorylase Enzyme :- Yin and Tung found that the starch-splitting enzyme phosphorylase was present in guard cells.



Light p^H.7.0

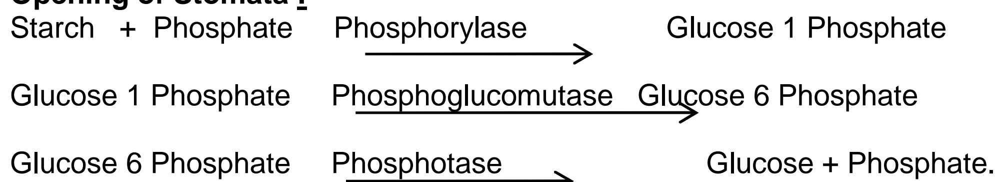
Starch + Inorganic Phosphate ⇄ Glucose 1 phosphate.

Dark p^H 5.0

Starch is hydrolyzed to form Glucose 1 phosphate at p^H 7.0 during day time and at p^H 5.0 glucose 1 phosphate condenses into starch during night time.

V. Free Glucose :- Steward (1964) proposed that it is not the phophorytaled glucose but the free glucose which is osmotically, active and ATP is necessary for the reformation of starch for stomatal closure. Steward's scheme is as follows.

Opening of Stomata :

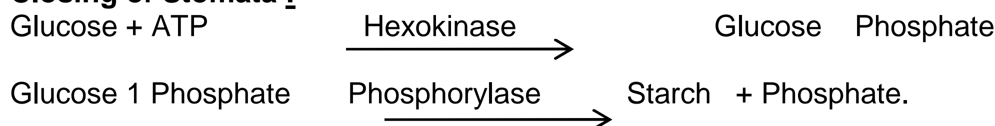


Starch + Phosphate —Phosphorylase→ Glucose 1 Phosphate

Glucose 1 Phosphate —Phosphoglucomutase→ Glucose 6 Phosphate

Glucose 6 Phosphate —Phosphotase→ Glucose + Phosphate.

Closing of Stomata :



Glucose + ATP —Hexokinase→ Glucose Phosphate

Glucose 1 Phosphate —Phosphorylase→ Starch + Phosphate.

Objections : Some guard cells don't have starch. There is no evidence to show the presence of sugar when the stomata opens. It accounts for utilization of energy for closing and for opening doesn't require any energy.

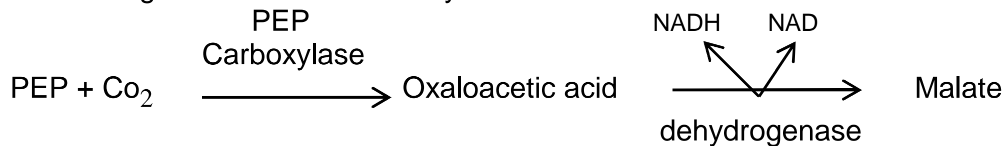
VI. Potassium (K^+) Transport Mechanism:-

Recent studies revealed that the active transport of K^+ plays a critical role in opening and closing of stomata. Imamura (1943) first discussed the role of K^+ in stomatal movement. Later Fujino (1967) supported the same. The theory can be briefly summarized as follows.

Day Time :- During the day time malic acid is formed in the guard cells. The formation of malic acid at this stage can be explained by any one of the following.

i) Starch is converted to malic acid.

ii) Willmer (1973) confirmed the presence of PEP carboxylase in the guard cells. The carboxylation of PEP results in the malic acid.



The malate so formed disassociates into malate anion and H^+ cation. By H^+ ion pump. H^+ are expelled from the guard cells, with the result more negative charge develops in the cell. To balance the guard cell's internal negative charge influx of K^+ from the surrounding cells takes place. The uptake of K^+ is an active process i.e., it requires ATP for the accumulation of K^+. As the K^+ ions migrate into guard cells, they are accompanied by Cl^-.

The K^+, Cl^- and malate ions in the guard cells lower the water potential. Thus a water potential gradient is established between the guard cells and the adjacent subsidiary cell. This leads to the movement of water from the surrounding cells into the guard cells resulting in increasing the turgidity and causing the opening of stomata.

Closing of Stomata : In the dark the sequence of events is reverse leading to stomatal closure. The K^+ and Cl^- are transported out of the guard cells passively. The malate is decarboxylated to Co_2 and pyruvate, which through the reversal of glycolysis is converted to sugar. The sugar is converted into starch again. These events lead to lowering of O P and an increase in water potential, with the result exosmosis occurs and the decrease in the turgor causing the closure of the stomata.

Stomatal Movement by Co_2 : Co_2 concentration in the vicinity of the guard cells appears to be the most important key to the stomatal mechanism. When Co_2 concentration in the vicinity of the leaf is lower than the natural atmosphere (during day), K^+ ions are transported into the guard cells, and when Co_2 concentration is high (during night) the reverse process operates.

Stomatal movement by ABA : Abscisic acid (ABA) can cause stomatal closure. It blocks the active excretion of H^+ from guard cells. If ABA is applied externally at very low concentration or if the leaves are subjected to water stress and stomata close. water -stress condition causes increased ABA level in the leaf tissues. ABA causes stomatal closure by rapid acidification in the presence of Co_2. ABA is described as good anti transparent material.

2.8 STRESS PHYSIOLOGY

The term Stress is usually defined as any physiochemical or environmental factor capable of producing an injurious effect in the living organism. Adverse force or influence that tends to inhibit normal system from functioning is called biological stress. Plants encounter the surrounding environmental conditions. Environmental conditions include climatic, soil, and biotic factors. Plants living in adverse conditions such as drought and frost are subjected to stress. The study of the behavior of plants under stress is called stress physiology.

Biotic and abiotic environmental stresses

ABIOTIC		BIOTIC
PHYSICAL	CHEMICAL	
Water deficit	Salinity	Allelopathy
Excess water	Heavy metals	Herbivory
High temperature	Air pollution	Competition
Low temperature	Toxins	Pathogen attack
Light	Pesticide	
Wind	Soil P^H	

(a) Water Stress: - Drought is the condition arising from failure of rainfall throughout the year resulting in water deficit. So this stress is also referred to as drought stress.

Effect of water deficit on growth and metabolism.

- When a plant tissue suffers from water stress, there will be a reduction in turgor pressure, with the result cell expansion will be reduced and cell size will be smaller.
- Water stress affects the structure and function of membranes, which may lead to change in the ultra structure of cell. Chloroplast and mitochondrial structure can be damaged by severe water stress.
- Water deficit cause high ABA concentration low turgor pressure in guard cells. Which may lead to stomatal closure. When stomata are closed. Co_2 in the intercellular space decreases, that causes the inhibition of photosynthesis. This is termed as stomatal inhibition of photosynthesis.

Mechanism to overcome water stress :
The following are the different aspects of plant adaptation to water deficit or drought :

- **Drought Escape :-** Some plants, known as ephemerals, are short lived desert plants. These plants germinate, grow and flower very quickly after rains. Thus they complete their life cycle before the supply of water in soil is depleted and produce dormant seeds before the onset of dry season. These plants are called Drought Escapers.

- **Drought avoidance :-** Some plants are able to withstand periods of water deficit while they still maintain a high tissue water potential. They avoid tissue dehydration by following methods.
  a. Roots grow deeper into the soil to increase water uptake.
  b. Check stomatal transpiration.
  c. Reduces the amount of radiation load on plants by keeping the leaves parallel to the incident radiation.
  d. Production of hairs, surface wax or salt on leaf surface increases reflectance of the leaves.
- **Drought tolerance by osmotic adjustments :-** Turgor pressure of the cells can be maintained by having high solutes. Solute accumulation is an important aspect of plant adaptation to water deficit.

- **Protoplasmic resistance :-** Many species have protoplasm that survives periods of low water content. These desiccation tolerant plants are called Poikilohydric or Resurrection plants. The loss of water from these plants cause dehydration of cytoplasm but the metabolic function can be regained following rehydration.

- **Development of embolism / Xylem cavitation**: - Development of air bubble and breaking the water column in the Xylem vessel is called embolism or xylem cavitation. It reduces the water loss during dry season; water movement to the leaves is reduced which in turn will include stomatal closure and lower transpiration.

II. Excess Water :- Water stress may also arise through an excess of water, which is known flooding stress. The most important detrimental effect of flooding is less oxygen supply to the root zone. The anaerobic conditions caused by flooding are particularly unfavourable for the roots. The arrest in the metabolic conditions of root causes the stomatal closure of leaves, consequently photosynthesis decreases.

Reduction in the aerobic respiration favours the anaerobic respiration in the roots. It demands a utilization of large amount of carbohydrate. As a result, root tissues rapidly deplete carbohydrates and is described as. "Carbohydrate starvation ". It increases the translocation of carbohydrates from leaves to roots.

Adaptive response to flooding:

- Flooding induces the formation of aerenchyma in different plants of the plant body. It enhances the storage of O_2 capacity.
- During flooding, the old roots are replaced by adventitious roots, which act as a survival mechanism.
- In submerged plants at the base of the stem, cell expansion (Hypertrophy) is accelerated which increases the inter cellular spaces. This results in porosity and aeration.
- Deep water rice plants show a unique physiological adaptation with an enhancement of internodal length. As a result the leaves are kept above water level, which promotes the movement of air to the submerged plant parts.

III. High temperature stress : All the living organisms, either plants or animals are adapted to grow within a narrow range of temperature limits. Temperature influences the metabolism by its effect on chemical reactions, which are catalyzed by enzymes. High temperatures denature the proteins and thus destroy the enzymes.

Adaptations to high temperature:
Heat shock proteins: - When seedlings are shifted to temperatures above optimal temperature, in place of normal proteins heat-shock proteins (HSP) are produced. Several types of HSP are described in plants. ubiquinon is one type of HSP, it helps in removal of denatured proteins and recycle them as peptides or amino acids. HSPS contribute, to an organisms ability to survive at high temperature.

IV. Low Temperature stress: - Low temperature stress includes both chilling stress and freezing stress. chilling stress causes injury on plants by temperature above freezing i.e., above O^0C on the other hand freezing stress causes injury to plants at O^0C and below.

Plants growing in cold climate tend to store large portion of their carbohydrate reserves in underground organs or roots. Another advantage of storing carbohydrates reserves in the roots can be related to abnormal cold season when little or no growth occurs. Under such condition, the plant depends on storage carbohydrate to sustain it during stress.

V.Physiology of salt resistance :- Salts accumulate in the soil due to irrigation. Some plants growing in saline soils near sea coasts experience salt stress in such areas.

2.9 SYNOPSIS

In this unit we studied the importance of water and its role in plant life. Later we understood various terms like osmosis water potential, osmotic potential, pressure potential and plasmolysis. We came to know the process of absorption of water. In transpiration the mechanism of stomatal movement and loss water through the stomata has been understood. Stress physiology revealed the various physio chemical factors causing the stress to the plants and how the plants adopt to over come these adverse conditions.

Technical Terms : Osmosis, Diffusion, Water potential, Osmotic potential, Pressure potential, Plasmolysis, Imbibition.

2.10 Self Assessment Questions:

Essay Questions:

1. Write an essay on the mechanism of absorption of water?
2. What is transpiration?
3. Describe the mechanism of closing and opening of stomata?

Short Notes:

1. Water potential.
2. Plasmolysis
3. Osmosis
4. Stomatal movement.

2.11 Reference Books:

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- K.V.S. Durga Prasad

Lesson - 3

MINERAL NUTRITION

3.0 Objective: - Green plants which are autotrophic receive inorganic nutrients from the environment . The source of these inorganic requirements of plants are minerals, hence the inorganic nutrition of plants is also called mineral nutrition. Among the inorganic nutrients oxygen, carbon dioxide are generally absorbed by plants from the atmosphere, while for the rest of the elements soil is the source.

Structure of the Lesson :

3.1. **History of study of Mineral Nutrition**

3.2. **Criteria of essentiality**

3.3. **Detection of Mineral Elements**

3.4. **Role of essential elements and deficiency symptoms.**

3.5. **Mechanism of absorption of mineral ions**

3.6 **Synopsis**

3.7 **Technical Terms**

3.8 **Self Assessment Questions**

3.9 **References**

3.1 History of study of Mineral Nutrition:

Aristotle (300 BC) proposed humus hypothesis according to which, plants absorb nutrients in the organic form from the soil.

Von Helmont for the first time experimentally proved that water is most important for the growth of the plant.

Wood ward (1699) proved that not only water, but materials dissolved in it are also important for plant growth.

De Sassure (1804) explained clearly that the mineral elements found in the plants are also obtained from the soil.

Julius sachs & Knops (1830) grown plants in nutrient solution (Hydroponics) containing minerals. They discovered at least 10 elements essential for the growth of the plant.

Liebeg (1940) clearly demonstrated the necessity of mineral elements in the life of the plant.

3.2 Criteria of Essentiality: -

About 90 elements have been reported from plants by analyzing the ash of different plants. However all these elements are not necessary for normal growth of plants. **Arnon and Stout (1939)** proposed three Criteria of essentiality for mineral elements. They are.

1. The element must be essential for normal growth and reproduction of a plant.
2. The element can not be substituted by any other element.
3. The element should be directly involved in the metabolic process of the plant.

According to the above criteria, sixteen elements were found to be essential for plant growth.

Macro Nutrients :- Among 16 elements, nine are required in large quantities, referred to as macro or major nutrients. ***Carbon, Hydrogen, Oxygen, Nitrogen, Sulphur, Phosphorous, Calcium, Potassium and Magnesium.***

Micro Nutrients: - Among 16 elements apart from the nine macro elements the other seven elements are required in minute or trace quantities are known as micronutrients or trace elements. They are ***Iron, Manganese, Boron, Copper, Zinc, Molybdenum and Chlorine.***

3.3 Detection of Mineral Elements:-

There are three methods to determine the mineral requirements of plants. These are 1) Plant analysis 2) Solution or hydroponics 3) Solid medium culture.

i. Plant Analysis :- The plant material is dried in an oven at a temperature of 70-80°c. The dried sample is then powdered. and is subjected either to wet digestion or ash analysis.

a) Wet Digestion :- A small quantity of concentrated sulphuric acid the powdered sample is taken and heated on a low flame. The materials dissolved and a clear solution is obtained.

b) Ash preparation :- Ash is prepared by keeping the powdered sample to high temperatures (600°C) in a muffle furnace. All the volatile & non-volatile organic compounds are burnt into gases, leaving a white powder called ash. It consists of oxides of metals. The ash content of different plants and tissues varies form 1 percent to 4 percent of fresh weight. The ash is dissolved in warm dilute hydrochloric acid or nitric acid.

The detection of elements in the solution obtained by either of the methods (Wet & ash preparation) are done by chemical, physical and physio-chemical methods.

Hydroponics :- In 1860 Pfeffer, Sachs and Knop grew plants in this way and it is referred as Hydroponics or solution culture. In this method the plants are grown in a nutrient solution that contains all essential nutrients in suitable concentrations. A glass jar (Pyrex maker) or a poly ethylene bottle is taken. It is washed with chromic acid and then with distilled water. The jar is covered with black paper to prevent growth of algae and exposure of roots to light, The mouth

of the jar is fitted with a 2 holed rubber-stopper, The plant is placed in one hole with roots immersed in nutrient solution. The nutrient solution (Hoaglands or knops solution) is used. These solutions contain all essential elements. The second hole of the rubber stopper is meant for air inlet tube through which air can be pumped into the nutrient solution. Thus the roots are well aerated.

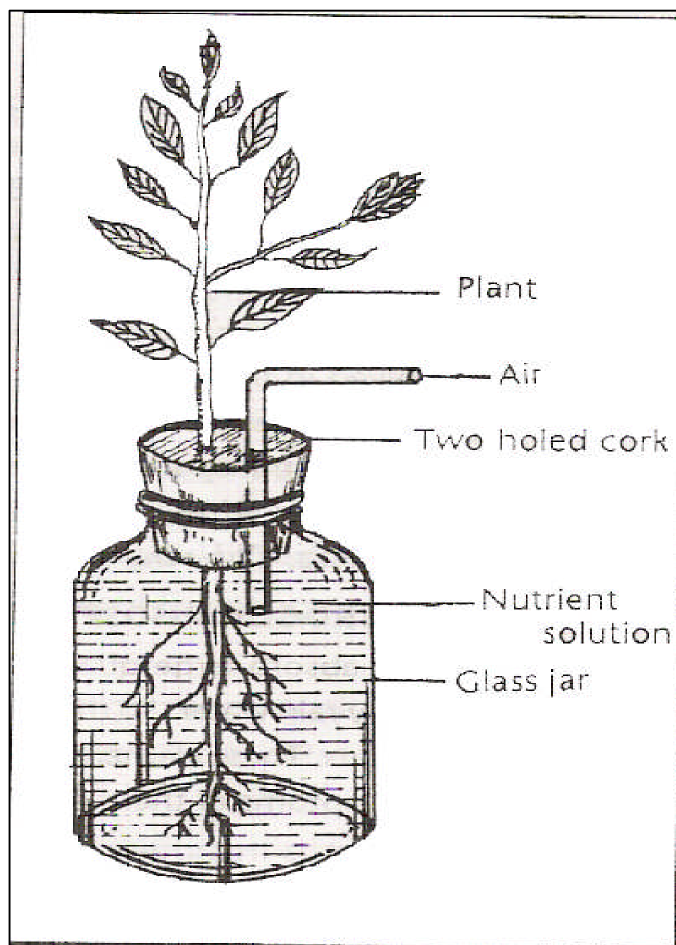


Fig.1.6 Solution cultures

As absorption of nutrients takes place, the pH of the solution alters. So occasionally the pH has to be adjusted and the nutrient solution becomes unavailable by forming precipitate this problem is over come by adding EDTA (Ethylene diamine tetra acetic acid) EDTA forms a soluble complex with iron, thus making it available EDTA is not taken up by the plant. Such compounds which form soluble complex with metal ions are called chelaters.

Solution culture is best suited for identifying the essentiality of mineral elements. Everytime only one element is left out from the solution and the plant is grown in it. If the plant does not show symptoms of deficiency and it can complete its life cycle, then the element is non-essential. On the other hand if the plant shows some symptom of deficiency and the plant can not complete its life cycle, then the missing element is considered to be essential.

Disadvantages :-

i) There is a need for root aeration which is not sufficient in solution.

ii) There is need to replace the solution every day or two for maximum growth because the solution composition changes as certain ions are absorbed more rapidly than others.

iii) This selective uptake also causes p^H changes.

Sand Culture : Plants are grown in crushed sand or quartz taken in a glazed vessel (crock) provided with a bottom drainage hole. The vessel is filled with pure quartz that is thoroughly washed with acid and then with distilled water. The sand provides the plant with mechanical support but does not supply nutrients. So nutrient solution is supplied by pouring (slop culture), by dripping on the surface (drip culture) or forcing upward from the bottom (sub irrigation).

i. Slop Culture: A nutrient solution is periodically supplied to the surface of the sand. It is allowed to drain out before fresh nutrient is added. Slop culture is the easiest way to grow plants under conditions of controlled nutrition.

ii. Drip Culture: The nutrient solution is continuously dripped on to the sand. The rate of dripping and draining is arranged in such a way that the sand is never saturated with solution.

iii. Sub Irrigation: In this method the nutrient solution is pumped from a bottom reservoir up through the sand until it reaches the surface. The pump is then turned off and the nutrient is allowed to drain off again. The pump drain is repeated several times so that the plant is supplied with the nutrients continuously.

Advantages:

1) Aeration of roots is taken care of.
2) A dark natural environment is created for roots for fixation.
3) Sand culture is easy to arrange and maintain.

Disadvantages:

1) pH of the medium can not be easily corrected
2) The exact concentration of different elements is difficult to determine after a certain interval.
3) The study of mineral elements can no be carried out with sand culture because sand contains micro elements. Solution culture is therefore preferable to sand culture for study of micro nutrients.

3.4 Role of essential elements, functions and Deficiency Symptoms :

I. Macro Elements: C H.O.N.P.K.Ca.Mg and S are called macronutrients because they are required by plants in large quantities.

I. Carbon, II.Hydrogen III.Oxygen : These elements are not mineral in origin. They enter into the composition of practically all organic compounds of the plant. Hence they are called structural components.

Carbon is absorbed in the form of Co_2 from air. It accounts to 45% of the dry weight.
Hydrogen is obtained from water, it constitutes 6% of the dry weight.
Oxygen is derived from water as well as air, it accounts to 43% of the dry weight.

IV) Nitrogen: After C,H,O, nitrogen is the most abundant element in plants.

***Occurrence*:** It is found in the large quantities in the atmosphere. But for the majority of plants, source of nitrogen is soil in the form of nitrates and ammonium salts.

***Functions*:** It is a vital component in proteins, amino acids, nucleic acid, enzymes, coenzymes (ATP,NAD) chlorophyll, alkaloids plant hormones and some vitamins.

***Deficiency Symptoms* :** Nitrogen is mobile element, hence the symptoms first appear in the older parts.

a) Cell division and cell enlargement is inhibited.
b) Yellowing starts from older leaves and progress to young leaves.
c) Anthocyanin pigment develops as a result stem, leaves petioles become red or purple colored.
d) Flowering may be delayed and fruits are developed.
e) Plants supplied with excess of nitrogen show vigorous vegetative growth.

V. Phosphorous: (0.05 - 1.0 %)

Occurrence: It is available to the plants in the form of soluble phosphates such as H_3Po_4 and H Po_4

***Functions*:** It is a constituent of nucleic acids, phospholipids, coenzymes like NAD, NADP and ATP. The highest percentage of phosphorus occurs in the meristematic regions.

***Deficiency Symptoms*:**

a) Growth is retarded with the appearance of necrotic patches on leaves and fruits.
b) Leaves fall of prematurely. They also become purple or dark in colour due to the accumulation of anthocyanin.

VI. Potassium: (1%)

***Occurrence*:** Potassium is present in the soil in three forms viz, a non exchangeable form, exchangeable form and a soluble form. Most of the potassium in the soil is non exchangeable and thus non available to the plants. The available form is exchangeable K^+.

***Functions*:** It is not a constituent of major organic compounds. However it is essential for functioning of about 40 enzymes as an activator. The best known function of potassium ions is its role in stomatal opening and closing.

***Deficiency*:** a) Mottled chlorosis in the middle of leaves is first symptom.
b) Necrotic areas develop at the tips and margins of leaves.
c) Growth is retarded and the plants are readily bending i.e., lodged.

VII. Magnesium: (0.05 - 0.7%)

***Occurrence*:** Magnesium is absorbed as Mg^{2+} ions from the soil.

***Functions*:** It is a component of chlorophyll. Magnesium pectate occurs in the middle lamellum of the cell wall. The binding of two subunits of ribosomes during protein synthesis is

controlled by Mg^{2+}. It is an activator of enzymes involved in the synthesis of nucleic acids, ATP molecules. and Carbohydrate metabolism.

Deficiency Symptoms :
a) Interveinal chlorosis appears in old leaves.
b) Anthocyanin pigments develop and lead to the formation of dead spots on the leaves.

VIII. Sulphur: (0.05 - 1.5%)

Occurrence: It is taken up by plants from the soil as divalent sulphate anions So^{2-}_{4}.

Functions: It is an important constituent of certain aminoacids like Cystine, Cysteine and methionine. Vitamins like Biotin, thiamine and coenzymes have sulphur. It also plays an important role in chlorophyll synthesis. Pungent smell to Brassicaceae family members, onion and garlic is because of sulphur compounds.

Deficiency: As most soils contain enough sulphate. Sulphur deficient plants are less common.
1) A general chlorosis throughout the leaf in younger leaves.
2) Rapid leaf fall takes place.
3) Apical bud is inhibited, but axillary buds grow.

IX. Calcium: (0.1 - 3.4%)

Occurrence: It is absorbed as Ca^{2+} ions. It is abundantly present in the soil and soil is seldom deficient in it.

Function: It is important in the synthesis of middle lamella of cell wall. It is associated with phospholipids of cell membrane as Ca^{2+} ions. Mitotic spindle organization is retarded by deficiency of Ca^{2+}. It is an activator of many enzymes.

Deficiency Symptoms: Leaf margins become irregular and become brownish with malformation.

- Meristematic regions are highly affected resulting in stoppage of growth.

- Cholrotic patches appear near the margins of the young leaves.

Micro Nutrients

Mineral element required in small quantities are called 'Trace Elements' or 'Micronutrients'. The function deficient symptom of the micronutrients are discussed below.

1) Iron: (0.01 %) It is mainly absorbed by the plant in the ferrous form, but ferric ion may also be absorbed.

Functions : It is essential for the synthesis of chlorophyll, even though it is not a constituent of chlorophyll. It is found in cytochromes, ferredoxin, flavoprotiens and many respiratory enzymes.

Deficiency Symptoms: Extensive Chlorosis in young leaves. In respiration because of poor formation of cytochromes, Terminal oxidation is effected. Protein synthesis is inhibited.

2) Manganese: (0.005 %) It is absorbed in the form of bivalent cations Mn^{2+}.

Functions **:** Manganese plays an essential role in photolysis of water for liberation of oxygen. It is an activator for several enzymes such as malic dehydrogenase, nitrate reductase etc.,

Deficiency Symptoms :
1. Necrotic spots appear on leaves due to disorganisation of thylakoids.
2. 'Marsh spot' of peas, 'grey speck' disease in oats and 'speckled yellow' disease of sugar beet are caused by deficiency of manganese.

3) Zinc : It is absorbed by plants as Zn^{++} ions. Zinc is generally deficient in soils of Andhra Pradesh.

Functions **:** Zinc is necessary for synthesis of tryptophan which is a precursor of indole 3-acetic acid (IAA). It is an activator for enzymes like alcohol dehydregenase and hexokinase. Zinc is a part of the enzyme carbonic anhydrase which catalyses the hydration of CO_2 to H_2CO_3.

Deficiency Symptoms **:**
1. Zinc deficiency causes 'little leaf' disease in cereals. The plants are characterized by formation of small leaves.
2. Plants show shortening of internodes and rosette type of growth.
3. In apple, citrus & other fruit trees, 'mottle leaf' disease occurs.

4) Boron : Plants absorb it as borate ($B0^-_3$) or tetraborate ($B_4 O_7$) anions.

Functions : Boron is essential for translocation of solutes (i.e., transport of organic substances). Sugar is transported as sugar borate complex. It regulates pentose phosphate pathway of carbohydrate metabolism.

Deficiency Symptoms **:**
1. The tips and storage organs die.
2. Leaves develop thick coppery texture and become brittle.
3. Plants show increased fruit drop and abscission of flowers.
4. In storage organs, internal tissue dis-integrates causing 'heart rot' of sugar beet, 'leaf roll' of potato, 'water core' in turnip etc.

5) Copper : Plants absorb copper as cuprous (Cu^+) or Cupric (Cu^{++}) ions.

Functions **:** It is a structural component of electron carriers called **plastoyanin and plastoquinone** and oxidative enzymes, such as **tyrosinase** and ascorbic acid oxidaze. It is essential for synthesis of ascorbic acid (vitamin C). Copper acts as an activator for enzymes like polyphenol oxidase and lactase.

Deficiency Symptoms **:**
1. Plants show withered appearance due to distortion of young leaves.

2. **'Reclamation disease'** (yellow tip) in cereals and legumes is due to deficiency of copper.

3. **'Exanthema'** in fruit trees and **'die-back'** in **Citrus** are common.

6) Molybdenum : It is absorbed by plants as **molybdate** (MoO_4^-) ions. It is required in least quantity among all essential elements (around 0.1 ppm).

Functions : Molybdenum is essential for formation of root nodules and plays a key role in **nitrogen metabolism.** It forms the prosthetic group of the enzyme **nitrate reductase.**

Deficiency Symptoms :
1. Plants show **mottling** and **necrosis** of old leaves.
2. Inhibition of flower formation is a common symptom.
3. **'Whip tail'** disease in cauliflower is due to deficiency of molybdenum.

7) Chlorine : Plants absorb chlorine as (Cl^-) ions.
Functions : Chlorine is essential for **hydration of tissues.** It is essential for transfer of electrons from water to chlorophyll in pigment system II (PS II)

Deficiency Symptoms :
1. Roots become short, thick and club shaped near the tip.
2. Plants show **wilting.**

3.5 MINERAL ABSORPTION

Soil as a source of Mineral Salts :
Most of the elements required by the plants are absorbed them from the soil. Plants absorb these minerals in the form of either cations or anions. Some Physiologists are of the opinion, that the zone of elongation and the zone of maturation are the principal sites of mineral salt absorption.

Mechanism of salt absorption :
The various views on the mechanism can be grouped under two headings. I. Passive Absorption. II. Active Absorption.

I. Passive Absorption :- The passive uptake theories believe that salt absorption is purely a physical process controlled by concentration gradient. Metabolic energy is not utilized for uptake of ions. This may be due to diffusion, facilitated diffusion, ion exchange, Donnan equilibrium and mass flow.

Diffusion :- When the outer solution is highly concentrated with reference to salts than the interior, salts migrate into the cell on the principle of diffusion. The ions will diffuse into cell as long as an equilibrium is established.

Mass Flow :- Movement of ions into the root along wither the stream of water under the influence of transpiration is called mass flow or bulk flow.

Ion Exchange :- In this process cations or anions inside the cell are exchanged with ions of equal charge from the external medium. Since this involves exchange of ions of similar charge,

there is no net gain or loss either to plant or to the soil. This is of two types. 1. Contact exchange theory 2. carbonic acid exchange.

1. Contact Exchange Theory :- The ions adsorbed to the root and soil particles are oscillating in a small volume of space called 'Oscillation volume'. When the oscillation volume of the two ions adsorbed to root and soil overlap they exchange position.

2. Carbonic Acid Exchange :- Co_2 liberated by the root during respiration dissolves in soil water to form carbonic acid. It dissociates into H^+ and Hco_3^- ions. Exchange of H^+ and Hco_3^- ions with similarly charged ions of soil solution and soil particle is called carbonic acid exchange theory.

Donnan Equilibrium :- It was proposed by F.G.Donnan. According to him, some anions and cations are firmly attached to the inner surface of the plasma membrane. These are called fixed ions or indiffusable ions. So a charge is created in the cell because of these fixed ions. To maintain electrical equilibrium, the cell absorbs ions of opposite charge sometimes even against concentration gradient.

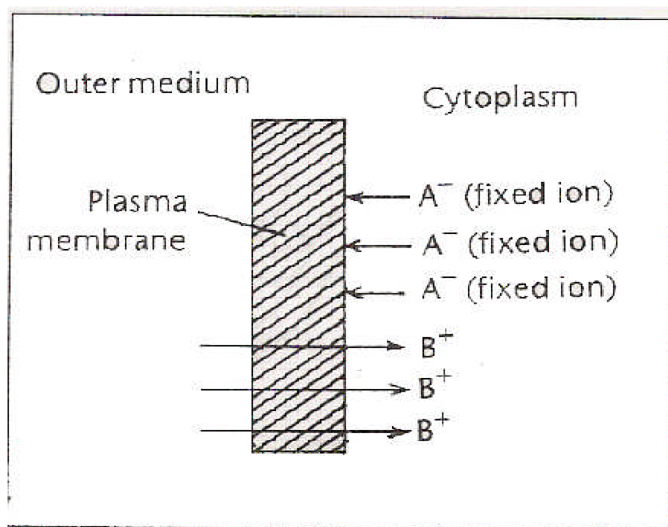


Fig. 1.7 Donnan Equilibrium

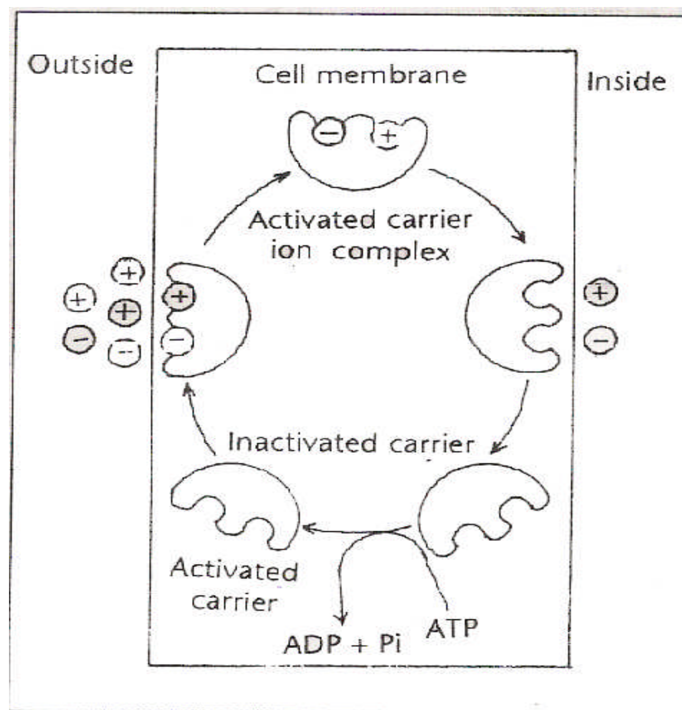
Facilitated Diffusion:- The passive absorption of ions by a carrier is known as Facilitated Diffusion. Like simple diffusion, facilitated diffusion doesn't not require the metabolic energy and it occurs only in the direction of a concentration gradient. The carrier selects out and bind certain molecules and then release them on the other side of the membrane.

Active Absorption :- The ion uptake by plants involving the utilization of metabolic energy is defined as Active Absorption. ATP molecules formed in respiration provide the required energy to drive the process of ion absorption. Active absorption results in more ion concentration in the plants than in external soil solution. for example Nitella (an algal member) absorbs K^+ ions to a concentration several times more than the surrounding water.

The rate of absorption of ions is directly related to metabolic energy. Chemicals such as dinitrophenyl Hydrazane, dichlorophenyl dimethyl ureas which inhibit ATP synthesis also inhibit salt uptake.

To explain the mechanism of active absorption several theories have been proposed.

Carrier concept :- It was proposed by Van den Honert (1937). According to this concept some organic molecules act as carriers for ions and help in transporting the ions across the plasma membrane. The carrier molecule is initially activated by ATP kinase enzyme. The activated carrier molecule picks up ion on the outer surface of the cell membrane to form carrier ion complex. This complex moves across the cell membrane and reaches the inner surface and releases the ion into the cell. The carrier again reaches the outer surface of the cell membrane and repeats the same process.



Cytochrome pump theory :- Lundegardh and Burstorm(1933) proposed this theory. According to this, anions move inward via a cytochrome chain. Several objections have been raised against it. There is a carrier only for anions and not for cataions.Involvement of metabolic energy is not explained properly.

Protein Lecithin Theory :- This theory proposed by Bennet Clark. According this certain phospholipids (Lecithin) compounds act as carriers for both anions and cations. Lecithin is synthesized and hydrolyses in a cyclic manner. In the process the anions and cations taken from the outside are liberated into the cytoplasm of the cell.

Transporters or permeases :- Recent studies revealed that special type of membrane proteins facilitate the transport of ions against concentration gradients. These proteins are called Transporters or carrier proteins. These proteins are fundamentally of two types as uniporters and co-transporter.

i) Uniporters ;- These carriers transport ions from outside to inside or inside to outside called uniporters. e.g.: H^+ - ATPase, Na^+ - ATPase.

ii) Co-transporter:- The carrier portion involves in the transport of an ion coupled with another ion.It is of two types.

a) Symporter ;- These carriers transport two types of ions in the same direction called symporters. e.g.: H^+ coupled with $No3^-$ or Cl^- $Po4^-$ in same direction

b) Antiporters ;- Through these porters movement of ions occurs in opposite directions called antiporters. e.g.: H^+ movement associated with Na^+ in opposite direction.

Primary Active Transport :- Protons from the cytoplasm are expelled out by the H^+ - ATPase pump, present in the plasma membrane. ATP hydrolysis provides energy for this movement. Due to the pumping of protons, a proton concentration gradient is established across the plasma membrane, with more protons outside the membrane.

Secondary Active Transport ;- During this transport the binding of ions to the specific carrier proteins, conformational changes in the protein and finally the release of the substance to the other side. It involves both symporters and antiporters. The proton motive force generated by H^+ transport is used to drive the transport many other ions against their concentration gradient.

Evidences suggest that Cl^- , No_3^-, HPO_4^{--} , sucrose, amino acid enters the cell via specific symporters. Similarly Na^+ is transported out of the cell by Na^+ - H^+ anti porter.

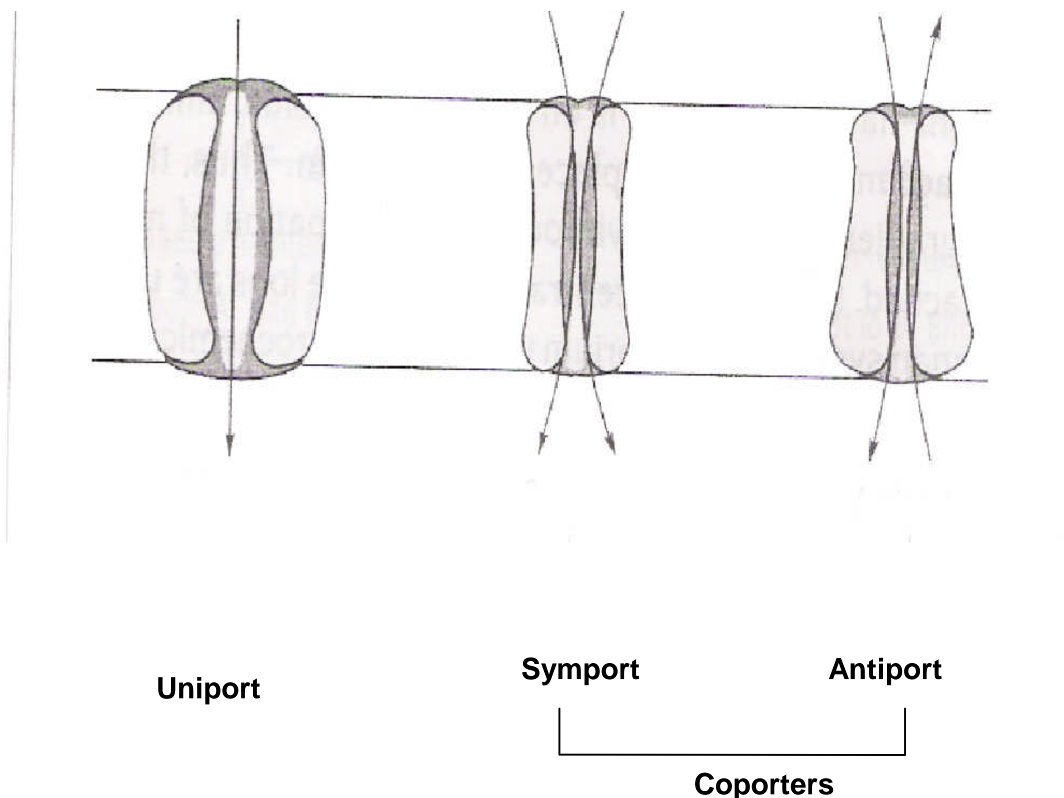


Fig. 1.9 Transporters

Translocation of Na^{+} and K^{-} by primary active transport:

It is an ion pump or cation exchange pump which is driven by energy of one ATP molecule to export three Na^{+} ions outside the cell in exchange of the import of two K^{+} ions inside the cell. The transporter is an antiport which is an integral membrane protein. It has three sites on its extra cytoplasmic surface; two sites for K^{+} ions and one site for inhibitor on its cytosolic side, the larger subunit contains three sites for three Na^{+} ions and also one catalytic site for a ATP molecule. The hydrolysis of ATP molecule brings conformational change in the transporter that allows the pump to transport three Na^{+} ions out and two K^{+} ions inside the cell.

3.6 SYNOPSIS

The living organisms absorb the raw materials from the environment and utilise them in metabolic activities. Such mateirials are called the **nutrients.** The absorption and utilisation of these nutrients is called the **mineral nutrition.** On the basis of ***Arnon's criteria of essentiality,*** 16 elements are found to be essential. Among them 9 are considered as macro nutrients and the rest 7 as micro nutrients. To determine the essentiality and role of element, solution culture or solid culture experiments are carried out.

The plants absorb minerals in the form of ions. Many theories are proposed regarding the absorption of ions. They can be grouped under two headings as *Passive uptake and active uptake,* In many instances, it has been observed that ion absorption is an active process.

3.7 Technical Terms: Uniporter, Coporter, Symporter, Antiproter.

3.8 Self Assessment Question :

Essay Questions:

1. Comment on the role macro elements in plants ?

2. Write an essay on the role of trace elements in plants ?

3. What are essential elements ? Describe the role played by any five major elements ?

4. Write an essay on the active absorption of ions ?

Short Questions :

1. Hydroponics.
2. Sand Culture.
3. Criteria of essentiality.
4. Role of NPK.
5. Donnan Equilibrium.
6. Carrier concept.
7. Transporters.

3.9 Reference Books :

1. Dr. S. Sundara Rajan 2003, Plant Physiology - Anmol Publication Pvt.Ltd. New Delhi
2. Salisbury & Ross, 2000, Plant Physiology - CBS Publishers, N.Delhi.
3. Dr. V. Verma, 2002, Plant Physiology - M.K. Publications, N.Delhi.

- K.V.S. Durga Prasad.

Lesson - 4

PHOTOSYNTHESIS

4.0 Objective :

Photosynthesis is essentially the only mechanism of energy input into the living world. This phenomenon of energy input from the abiological environment to the biological system is always through the green plants. All forms of life are dependent on green plants, directly or indirectly for their sustenance. Photosynthesis not only provides food supply to the heterotrophic organisms but also purifies the atmospheric air.

Structure of Lesson :

4.1. Definition

4.2. Historical Account

4.3. Chloroplast

4.4. Photosynthetic Pigments

4.5. Photo systems

4.6. Nature of Light

4.7. Absorption spectrum and action spectrum

4.8. Emerson Effect

4.9. Light reaction.

4.10. Non Cyclic Electron Transport

4.11. Cyclic Electron Transport

4.12. Dark Phase

4.13. C_4 Cycle

4.14. C_3, C_4 Differences

4.15. CAM

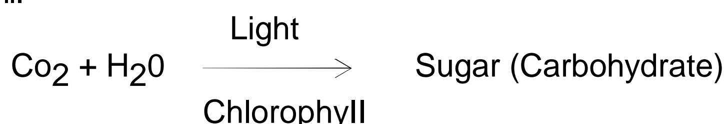
4.16. Photorespiration

4.17. Translocation of Organic Solutes.

4.18. Synopsis
4.19 Self Assessment Questions

4.1 - Definition :

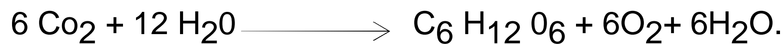
The term “Photosynthesis” means literally synthesis using light. It may be defined as “The process of synthesizing carbohydrates by utiliging Co_2 & H_20 in presence of sunlight and chlorophyll.



In photosynthesis the plant uses solar energy to oxidize water, there by releasing oxygen, protons and electrons. The electrons and protons through intermediary compounds like A T P and N A D P H reduce the Co_2 into organic compound, primarily sugars.

The overall reaction of photosynthesis my be represented as.

$$6\ Co_2 + 12\ H_20 \longrightarrow C_6\ H_{12}\ 0_6 + 6O_2 + 6H_2O.$$



4.2 Historical Account :-

Even though scientific study of photosynthesis is very recent, from time immemorial man has looked upon. The following may be mentioned as significant names in the development of Photosynthetic knowledge.

- **Stephen Hales (1727)** Regarded as the father of physiology. He proposed that green plants obtained their nutrition from the air through the leaves and sunlight may have a role in it.
- **Joseph Pricestley (1771)** Observed that impure air ($C0_2$) would get purified (Vital air - O_2) if kept in contact with the green mint plants.
- **Jan Ingen Houesz (1771)** Demonstrated that plants purify air only in the presence of light.
- **Jean Senebies (1782)** Commented that plants absorb Co_2 from the atmosphere during day time and exhale it during darkness.
- **NT de Saussure(1804)** Identified that water is an active participant in photosynthesis.
- **Mayer (1842)** Observed that during photosynthesis solar energy is stored in the form of Chemical energy.
- **Julius Sachs (1864)** Demonstrated growth of starch grains in the part of leaf exposed to light.
- **BlackMan (1905)** Identified that photosynthesis involves two distinct phases Vi_2 light phase and dark phase.
- **Van Niel (1931)** Demonstrated that bacteria use H_2S instead of H_2O as proton donar with the result oxygen is not released in this process.
- **Robert Hill (1937)** “Photolysis of water “ or “Hill’s reaction”
- **Ruben and Kamen(1941)** Used $^{18}0$ and confirmed that O_2 released in photosynthesis comes from water but not from Co_2
- **Calvin and Bensen(1942)** used $^{14}Co_2$ revealed the path of carbon in the dark reaction.
- **Arnon (1954)** Developed the concept of photophosphorytation (synthesises of A T P from A D P & Pi in presence of sunlight)
- **Emerson (1943)** Indicated the involvement of two pigment systems.
- **Peter Mitchell (1961)** Proposed Chemiosmotic hypothesis for the production of A T P

4.3 CHLOROPLAST :

Within the cell of the green plants are present certain unique organelles called chloroplasts. They are generally spherical or ovoid in higher plants. The chloroplast is

enveloped. by two unit membranes. The matrix of chloroplast is called 'stroma'. Embedded in the stoma are the 'Thylakoids.' The stack of thylakoids is called 'Granum.' The space in the thylakoid is called 'Lumen.' The thylakoid membrane contains pigment molecules.

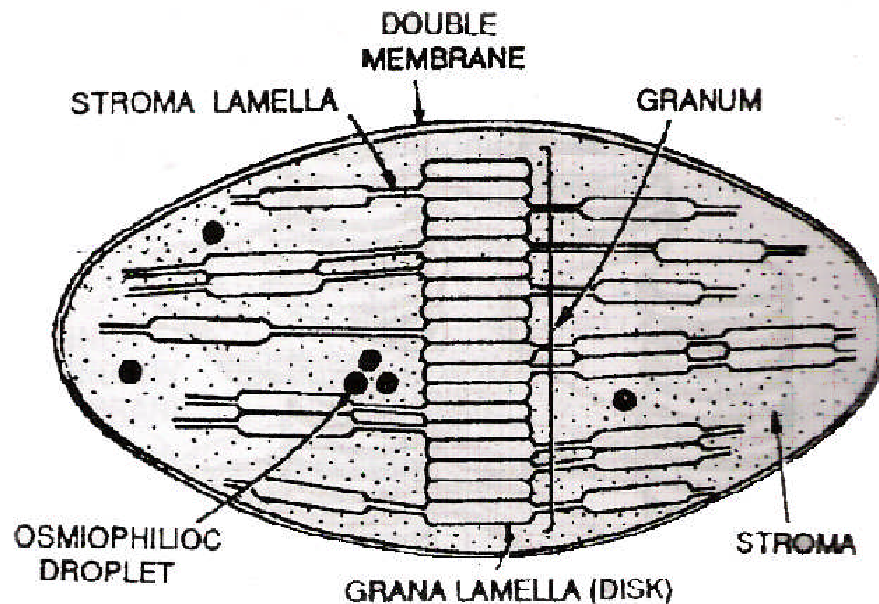


Fig: 2.2 Structure of Choloroplast

4.4 Photosynthetic Pigments :-

Some of the important pigments present in chloroplasts are Chlorophylls, Carotenoids, Cytochromes etc.,

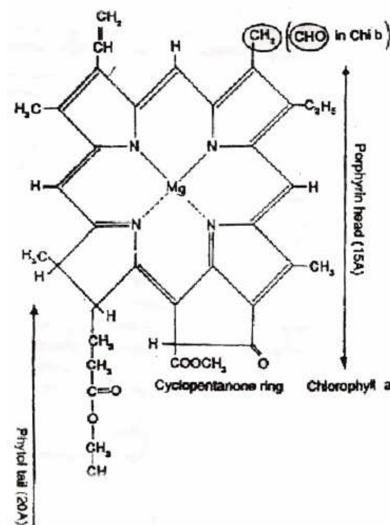


Fig: 2.3 Stucture of Cholorophyll

Chlorophylls :- These are the most important pigments involved in the basic photochemical reaction. The chlorophylls are of nine types as chlorophyll a,b,c,d,and e, bacteriochlorophyll a

and b, chlorobium chlorophyll 650 and 660. Chlorophyll a and b are the best known and most abundant and are found in many autotrophic organisms except pigment Containing bacteria. The Chlorophyll molecule has a cyclic tetrapyrrolic head called “Porphyrin” with a central Magnesium atom. The ‘Phytol’ chain of the chlorophyll molecule extends from one of the pyrrole rings.

Chlorophyll a : It is present in all green plants except in photosynthetic bacteria. Its empirical formula is $C_{55}H_{72}O_5N_4M_{g.}$ It has a methyl group (CH_3) attached to the third carbon.

Chlorophyll b: It is found in all higher plants. The empirical formula is $C_{55}H_{70}O_6N_4M_g$. An aldehyde group (CHo) is attached to the 3rd carbon in the porphyrin head.

Carotenoids:- Carotenoids are lipid compounds that are distributed widely in both animals and plants and range in color from yellow to purple. They are divided into two main categories as.

A) Carotenes ($C_{40}H_{56}$) B) Xanthophylls ($C_{40}H_{56}O$)

Carotenoids protect the chlorophyll from photoxidation and transfer the light energy they absorb to chlorophyll a.

Phycobilins : These are found only in algae. These are of two types : Phycoerthrins (red) and Phycocyanin (blue). These are also involved in transfer of light energy to the chlorophyll.

Light harvesting Complexes: Among the number of pigments residing in the chloroplast, only chlorophyll, a can act directly in photosynthesis. The other pigments like Chlorophyll Carotenoids and phylobilins act as light harvesting or antenna molecules. These antenna molecules transfer their excitation energy to chl a by resonance transfer. These antenna complexes are termed as light harvesting complexes.

4.5 Photo systems:-

The thylakoid membranes contain two structurally distinct photosynthetic units. (Photo systems) as PS - I & PS - II.

Photo system I :- It consists of a reaction centre P700, 3:1ratio of chlorophyll a & b, more carotenes, and also consists of iron containing proteins, Fe - S proteins and cytochromes.

Photo system II :- The reaction centre is P680. The ratio of chlorophyll a & b is 1:1, more Xanthophylls are present.

4.6 NATURE OF LIGHT

Solar radiation is the only source of light for all forms of life. Much of the solar radiation is shedded by the outer reaches of the atmosphere and only visible light together with some amount of U.V. rays reaches the surface of the Earth. The visible light is confined between wavelength of 390nm and 760nm. The spectra analysis of the visible light shows the following rays with their respective wave lengths of given in parameter.

Violet (390 - 430nm)

Indigo (430 - 470nm)

Blue	(470 - 500nm)
Green	(500 - 560nm)
Yellow	(560 - 600nm)
Orange	(600 - 650nm)
Red	(650 - 760nm)

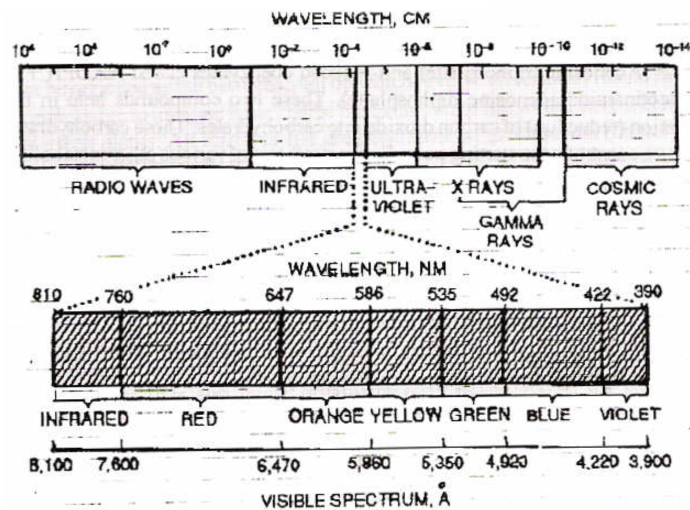


Fig: 2.4 Electro magnetic spectrum

Radiation travels in the form of corpuscles or particles called Quanta or Photon. Each photon represents a unit of energy, the energy quantity of each light depends on its wavelength, shorter the wavelength higher is the energy."

4.7 Absorption Spectrum:-

It is a graph showing the absorption of light by pigment at different wavelengths. It shows that the both chlorophyll a and b absorb very little of green and yellow lights and strongly absorb the blue and red lights.

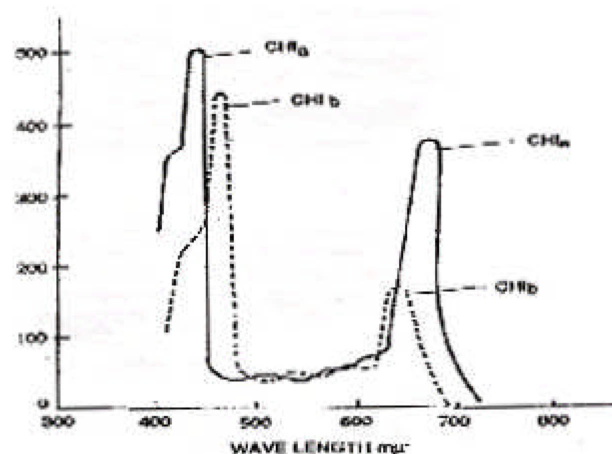
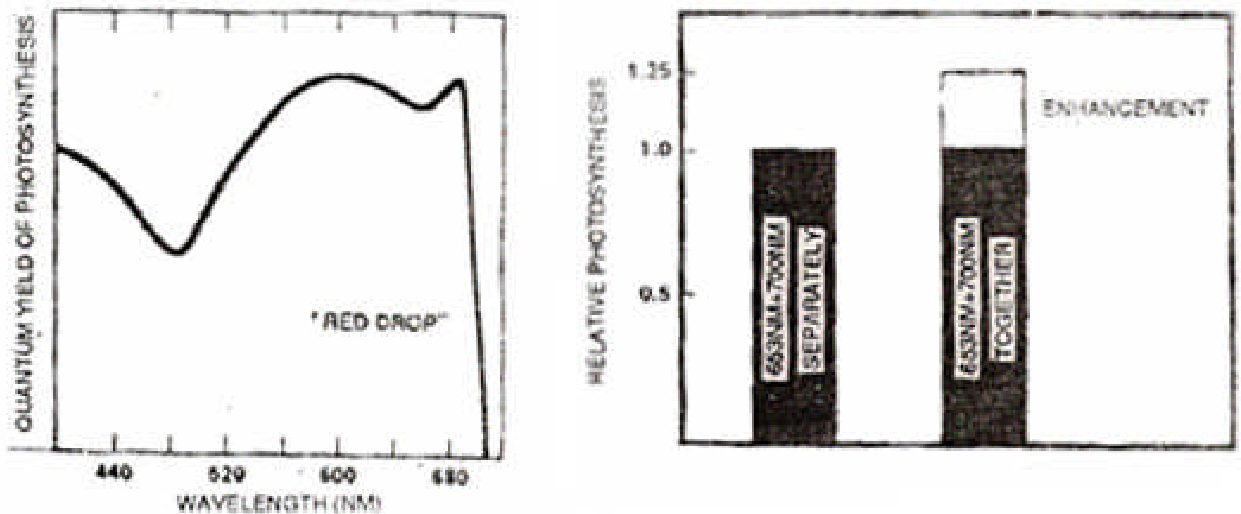


Fig: 2.5 Absorptions Spectrum

Action Spectrum:- It is the measure of the efficiently of a process induced by light of different wavelengths. It is a graph showing the rate of photosynthesis at different wavelengths. All the plants show a major peak in the red region and distinct lower peak in the blue.

4.8 Emerson Effect :-

Emerson (1943) worked on different wave lengths of light as to their quantum yield. They found that the average quantum yield suddenly dropped near the far red. i.e., greater than 680nm. This decline is called "Red Drop'



a. Red Drop. Fig: 2.6 Emerson Effect b. Enhancement Effect

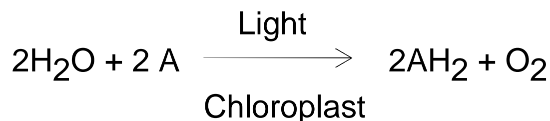
Later on Emerson provided a beam of monochromatic light of shorter wavelength. (650 - 680nm) and longer wavelength (More than 680nm) Simultaneously, the rate of photosynthesis was higher than the sum of the two rates with separate beams. This enhancement of photosynthetic rate under the influence of two wavelengths of light is called 'Emerson Enhancement Effect'. It led to the idea that there must be two different pigment systems one pigment system was more efficient in longer wavelength, the other was efficient in shorter wave length.

4.9 LIGHT REACTION :

Light reaction takes place in the grana region of chloroplast. Due to this photochemical reaction A T P and N A D P H are sythesised, which are called "Assimilatory power." The entire light reaction can be discussed under following aspects.

1. Hill's Reaction **2.** Electron Transport **3.** Mechanism of ATP formation.

I. Hill's Reaction :- Robert Hill isolated chloroplasts and supplied with water and a suitable hydrogen acceptor, O_2 will be released and the H_2 acceptor gets reduced. The various H_2 acceptors used by Hill are called Hill oxidants (Ferric Salts, Benzoquinones, Chromates, Indophenols). This phenomenon is called Photolysis of water or Hill's reaction. Hills reaction attributed that O_2 released during light reaction comes entirely from water.



$$2H_2O + 2\,A \xrightarrow[\text{Chloroplast}]{\text{Light}} 2AH_2 + O_2$$

Electron Transport:- This occurs in the thylakoid membrane. When the Photo system is exited with photons it liberates the energized electrons. These electrons pass through various electron acceptors. This is of two types.

1. Non Cyclic electron transport and
2. Cyclic electron transport.

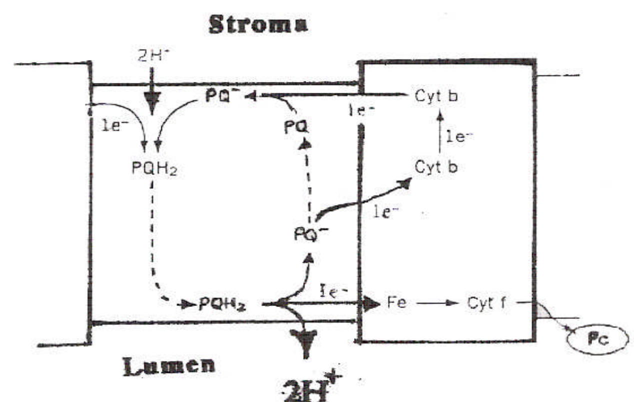
4.10 Non Cyclic electron transport :

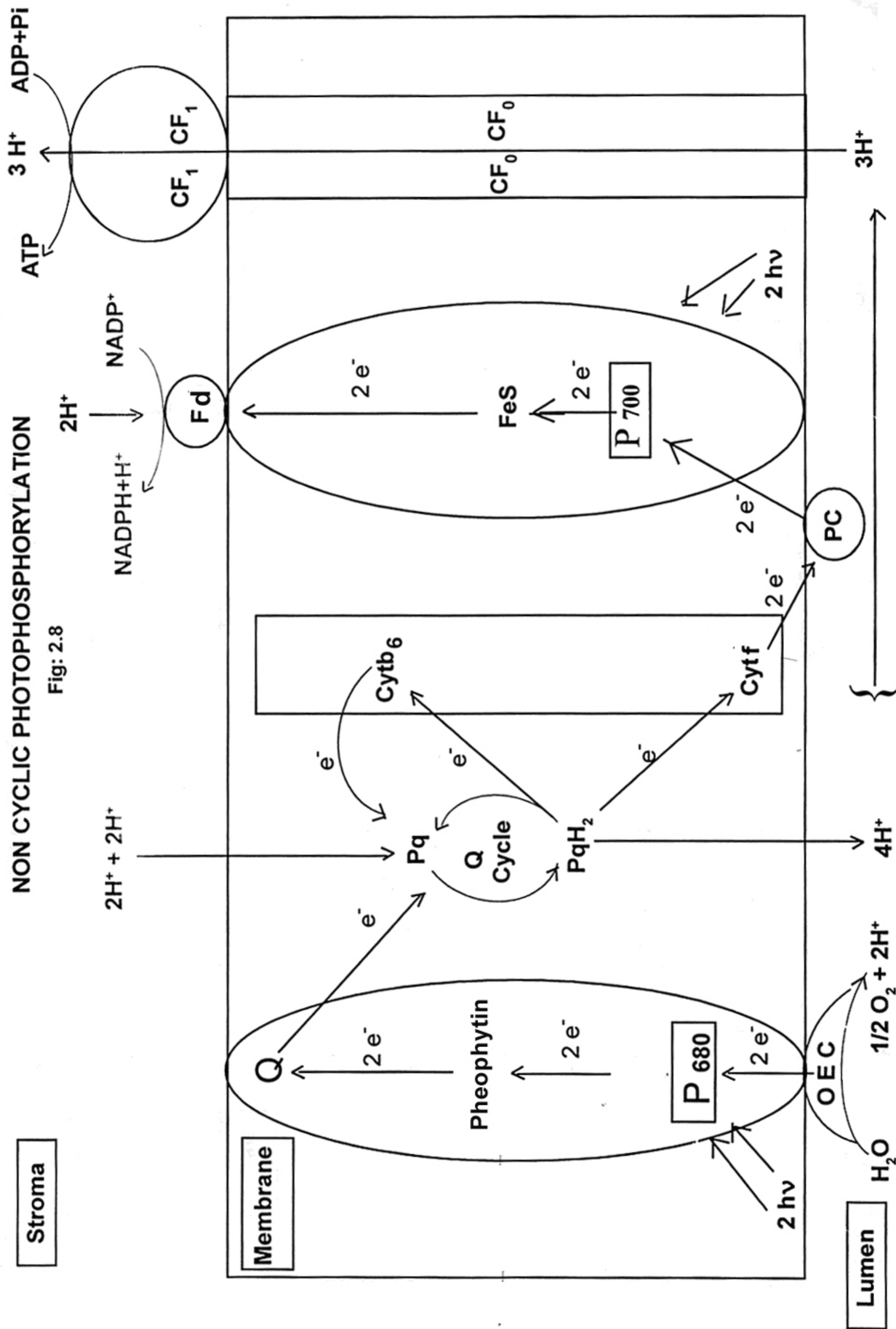
It is also called the 'Z' scheme electron transport. Hill and Bendall proposed this scheme. It involves both photo system I and photo system II. This electron transport system involves the following steps.

- The radiant energy is absorbed by light harvesting complex II (L H C II), its energy is transferred by resonance to the reaction centre P680.
- P680 in Ps II gets excited and ejects one electron which is trapped by pheophytin.
- The electron deficiency at P680 is compensated by the electrons obtained from the photo oxidation of water. Electrons are removed from water by manganese containing oxygen evolving complex (O E C). The protons from water are released into the thylakoid lumen, the O_2 diffuses out across the thylakoid into the stroma.
- Pheophytin is a chlorophyll in which the central magnesium atom has been replaced by two hydrogen atoms. the electrons from pheophytin now enter into a Quinone Cycle (Q.Cycle).

Q.Cycle:- The mechanism of electron and proton flow through the cytochrome b_6f complex is not yet fully understood, but a mechanism known as the Q.Cycle accounts for most of the observations. An electron is transferred from pheophytin to plastoquinone., and it is reduced to plastoquinol (PQH_2) by accepting two protons from the stroma. The PQH_2 released the protons into the lumen and the electron to iron rich cytochrome - b/f complex. Thus for every two electrons passing from Ps II to the Cytohrome b_6f complex, Quinone Cycle transfers four protons from the stroma to the lumen of thylakoid.

Fig: 2.7 Q.Cycle




NON CYCLIC PHOTOPHOSPHORYLATION
Fig: 2.8
Stroma
Membrane
Lumen
2 hν
P 680
Pheophytin
Q
2 e⁻
e⁻
OEC
H2O
1/2 O2 + 2H+
2H+ + 2H+
Pq
Q Cycle
PqH2
4H+
Cytb6
Cytf
PC
P 700
FeS
Fd
2H+
NADP+
NADPH+H+
ATP
ADP+Pi
3 H+
CF1
CF0
3H+

The electrons from Cytochrome f are conveyed to plastocyanin (PC). It is a copper containing mobile protein present along the lumen side of thylakoid membrane. Ultimately this plastocyanin delivers the excited electrons to photo system II.

Reactions at Photo system I : In the meantime the PsI with reaction centre P700 absorbs longer wave length, gets excited and releases two electrons. The two electrons passes through certain electron carriers and finally reduces ferrodoxin. It is an iron-sulphur protien present in the stroma side of thylakoid. The ferrodoxin now transfer its electrons to NADP and it also gets $2H^+$ from photolysis of water with the result $NADPH+H^+$ is synthesised. The electron deficiency at Ps I is compensated by the electron from plastostocyanin of Ps II.

During the process of non-cyclic electron transport for each pair of electrons passing through it, six protons (H^+) are accumulated in the thylakoid lumen. Among them 2 are released from the photolysis of water and four from the stroma to the lumen through Q.Cycle. Thus a proton gradient is generated.

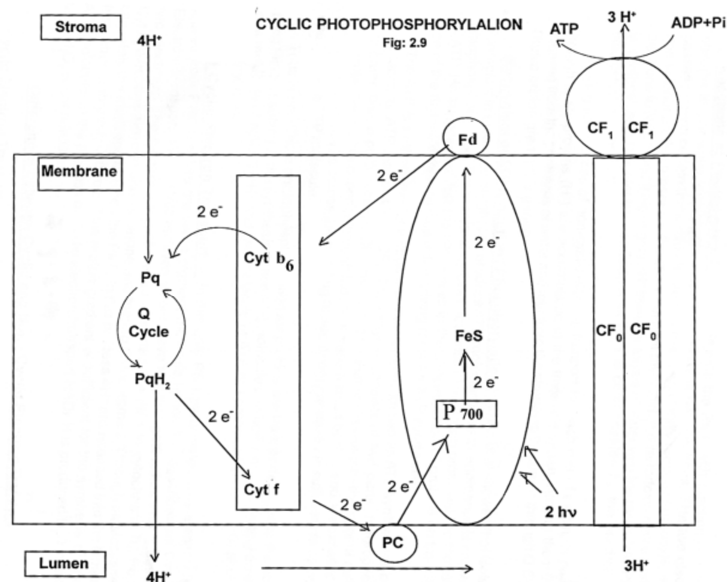
Mechanism of ATP formation / Phosphorylation : Arnon showed that isolated chloroplast in the presence of light can produce ATP molecules. This phenomenon is known as phtophosphorylation. Peter Mitchel by Chemiosmotic mechanism proposed the formation of ATP. He suggested that the differences in the concentration of protons on the either side of the thylakoid membrane is the driving force for ATP synthesis.

The ATP is synthesized by a large enzyme complex known as ATP synthase or coupling factor or ATP ase or CF_O - CF_1. The enzyme consists of two parts, hydrophobic membrane bound portion called CF_O and a portion that sticks out into the stroma called CF_1. Protons enter the channel through the membrane formed by CF_O and are expelled by CF_1. When $3H^+$ moves across the coupling factor, energy released is sufficient to convert one ADP into ATP molecule.

Thus in non Cyclic photophosphorylation six protons are transported across the coupling factor resulting in the synthesis of two ATP molecules. In terms of assimilatory power one molecule of $NADPH+H^+$ and two molecules of ATP are produced in non cyclic photophosphorylation.

4.11 Cyclic Electron Transport

In this only PS I participates. The electron released from P700 reaches the same pigment molecule hence it is described as Cyclic Electron Transport. When P700 receives longer wave length light it gets excited and releases two electrons. The electrons are conveyed to Ferredoxin then to cytochrome b_6, from Cyt.b_6 electrons move to plastoquinone and then to Cytochrome f. Through plastocyanin ultimately the electrons reaches the Ps I . In this process $4H^+$ are released into the lumen from stroma through Q.Cycle. This proton gradient is sufficient for the synthesis of one ATP molecule. In the process of Cyclic electron transport NADPH is produced. H_2O in not utilized. O_2 is not released.



Differences between Cyclic and Non - Cyclic Electron Transport

Cyclic Electron Transport	Non-Cyclic Electron Transport
1. Only photo system I participates	1. Both PS I and PS II Participate.
2. Electrons liberated from chlorophyll return to the same chlorophyll	2. Electrons liberated from chlorophyll molecule do not return to the same chlorophyll.
3. One ATP molecule is formed	3. Two ATP are formed.
4. NADPH is not produced.	4. $NADP^+$ is reduced to $NADPH+H^+$
5. Photolysis of water does not occur, so O_2 is not evolved.	5. Photolysis of water occurs and O_2 is evolved.

4.12 DARK REACTION :

Dark reaction of photosynthesis was discovered by *Blackman (1905*)and so known as Blackman's reaction of CO_2 fixation and carbon assimilation. It occurs in the stroma of chloroplast. Dark reaction takes place during the light period also, but it is independent of light. Dark phase occurs during both light and dark periods. The assimilatory powers (ATP & NADPH) formed during light phase are utilised to reduce CO_2 to carbohydrate during dark phase.

Melvin Calvin and his associates *Benson* and *Basham* traced the carbon reduction pathway in *Chlorella* and *Scenedesmus* by using radioactive isotope of carbon (C^{14}). They used the techniques like chromatography and autoradiography and discovered the path of Co_2

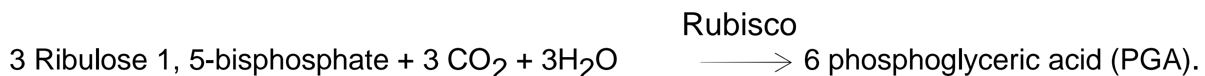
in dark phase. Hence it is called Calvin cycle or Calvin Benson cycle. It is called C_3 cycle because the first stable product is a 3-carbon compound, phosphoglyceric acid (PGA). It is also called reductive *pentose phosphate* pathway because the pentose phosphate RuBP is reduced to triose phosphates.

Calvin and his associates reported that the primary acceptor of carbon dioxide is Ribulose bisphosphate (RuBP), a Pentose phosphate. They reported that RuBP reacts with CO_2 and forms two trioses. They proved that one-sixth of the trioses are utilised in the formation of a carbohydrate and five -sixth of the trioses are utilised in the regeneration of RuBP.

The Calvin cycle includes three phases.
They are: **1.** Carboxylation phase **2.** Reduction phase **3.** Regeneration phase.

Carboxylation phase : In C_3 plants CO_2 is accepted by a five carbon compound the ribulose bisphosphate (RuBP). RuBP carboxylase (RuBP case or Rubisco, previously called carboxy dismutase) catalyses this reaction.

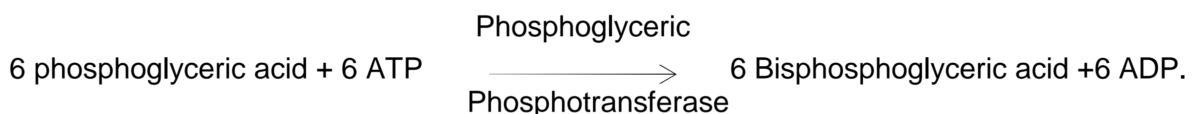
1. $3\ CO_2$ molecules combine with 3 molecules of RuBP in the presence of RuBP carboxylase and water to from 3 molecules of unstable hexose. They are immediately converted into 6 molecules of 3-phosphoglyceric acid (PGA). Thus the product of carboxylation is PGA which is a 3-carbon compound. This is the first stable product.
2.



$$3 \text{ Ribulose 1, 5-bisphosphate} + 3\ CO_2 + 3H_2O \xrightarrow{\text{Rubisco}} 6 \text{ phosphoglyceric acid (PGA).}$$

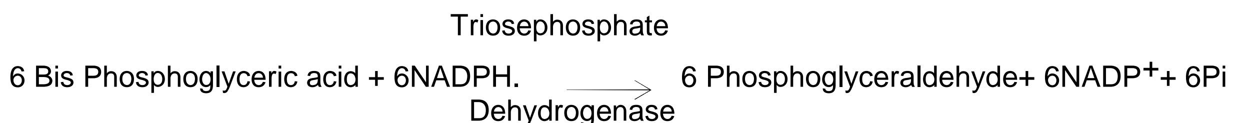
Reduction phase : During this phase PGA is reduced to phosphoglyceraldehyde (PGAL) or glyceraldehyde 3-phosphate (G3P). Both ATP and NADPH are utilised during the formation of G3P.

2. Before reduction carbon fixed in PGA should be chemically activated. PGA utilises ATP synthesized in light reaction and forms bisphosophoglyceric acid (BPGA). The enzyme phosphoglyceric phosphotransferase kinose catalyses this reaction.



$$6 \text{ phosphoglyceric acid} + 6 \text{ ATP} \xrightarrow[\text{Phosphotransferase}]{\text{Phosphoglyceric}} 6 \text{ Bisphosphoglyceric acid} + 6 \text{ ADP.}$$

3. The activated BPGA undergoes reduction easily. These 6 BPGA are reduced to 6 molecules of phosphoglyceraldehyde (PGAL) or glyceraldehyde 3 phosphate (G3P) by utilising 6 NADPH. Triose phophate dehydrogenase catalyses this reaction.



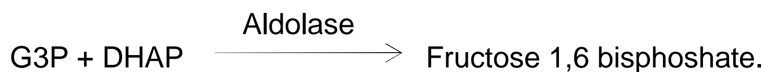
$$6 \text{ Bis Phosphoglyceric acid} + 6\text{NADPH.} \xrightarrow[\text{Dehydrogenase}]{\text{Triosephosphate}} 6 \text{ Phosphoglyceraldehyde} + 6\text{NADP}^{+} + 6\text{Pi}$$

PGA is generally considered as the primary product or first stable product and sugar as the end-product of photosynthesis.

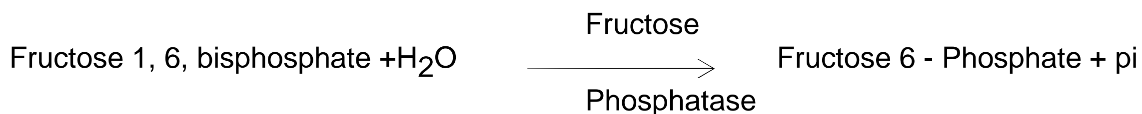
In the formation of PGA, fixation of carbon is completed but it is not reduced. Both the fixation of carbon and its reduction are completed with the formation of G3P. The synthesis of sugars and regeneration of RuBP are only conversion reactions from G3P. G3P is the precursor of strach. Depending upon the necessity G3P may be converted into sugars and starch or may be directly utilised in biochemical reactions.

4. Of the 6 G3P molecules formed 3 G3P molecules are converted to dihydroxy acetone phosphate (DHAP). The enzyme glyceraldehyde keto-isomerase (triose phosphate isomerase) catalyses the reaction. G3P and DHAP are isomers and they exist in dynamic equilibrium. These are known as triose phosphates.

5. One molecules of DHAP condense with one molecules of G3P in the presence of aldolase to fom one molecules of fructose 1, 6-bisphosphate.

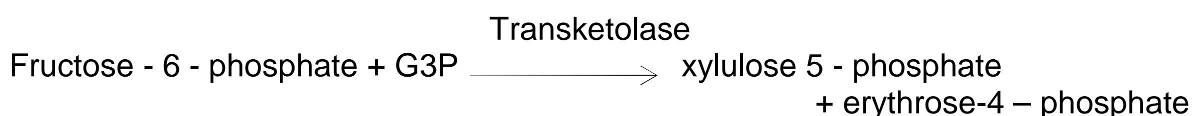


6. The fructose 1, 6-bisphosphate (hexose) molecule dephoshorylated to a molecules of fructose-6- phosphate in the presence of fructose phosphatase enzyme.



Regeneration Phase: To continue Calvin cycle RuBP should be regenerated. The sequence of reactions in regeneration phase is as follows:

7.. One molecule of fructose -6 - phosphate react with one molecule of G3P to form one molecules of xylulose-5 - phosphate (Pentose) and one molecule of erythrose-4 - phosphate (Tetrose). Transketolase catalyses the reaction.



8.. One molecule of erythrose-4 - phosphate react with one molecule of DHAP to form one molecule of sedoheptulose bisphosphate (7C.compound) in the presence of tarnsaldolase.

9.. One molecule of sedoheptulose bisphosphate undergo dephosphorylation to form one molecule of sedoheptulose - 7 - phosphate in the presence of phosphatase enzyme.

10. One molecules of seduoheptulose phophate reacts with one molecules of G3P and forms one molecules of ribose -5 - phosphate (pentose) and one molecule of xylulose-5 - phosphate (pentose). The enzyme transketolase catalyses the reaction.

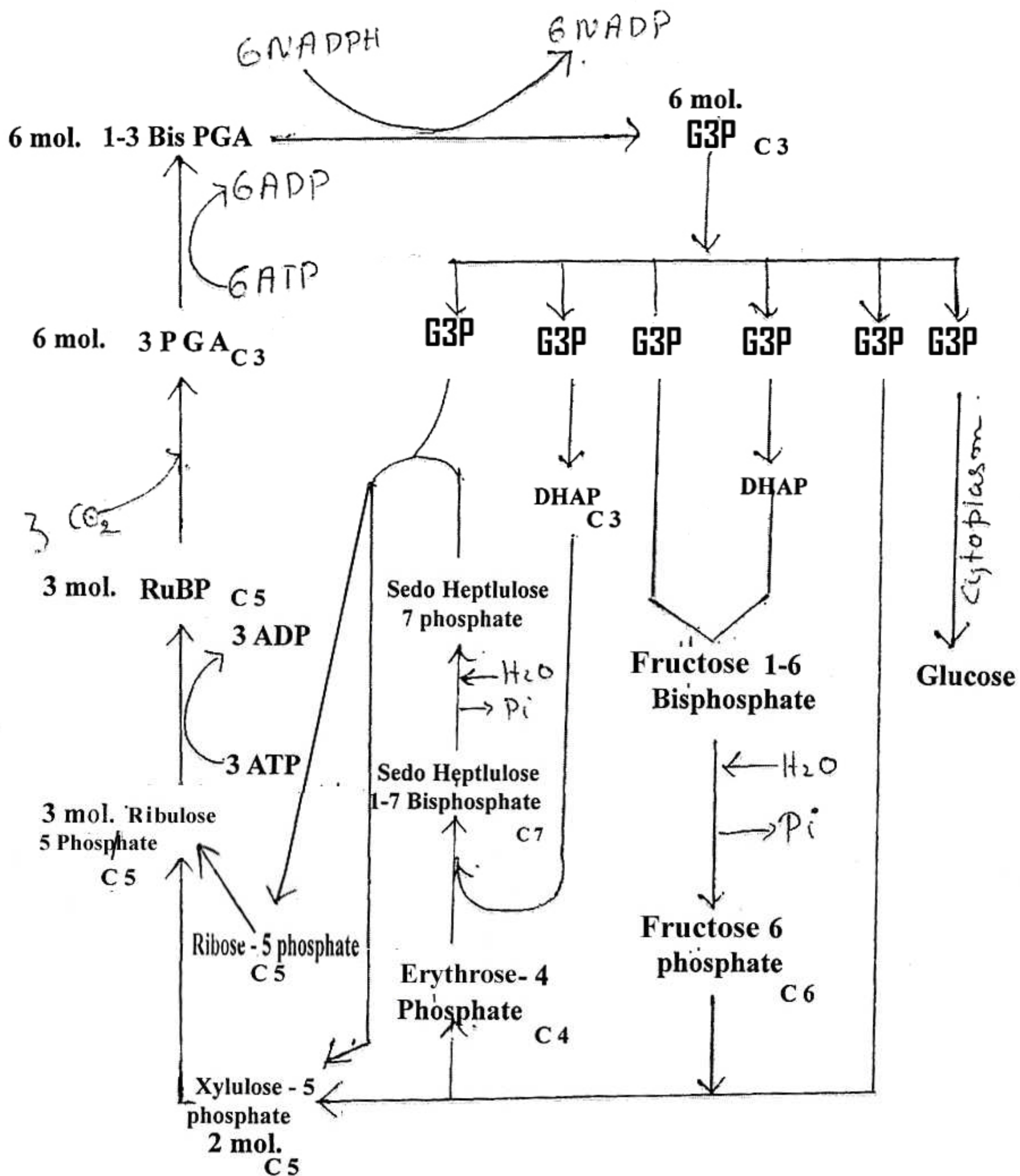
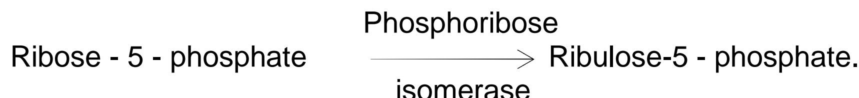


Fig. 3.0 Calvin Cycle

11. Two molecules of xylulose 5 - phosphate (from reactions 1 and 4) are converted into two molecules of Ribulose 5 Phosphate in the presence of epimerase.

2 Xylulose - 5 - phosphate $\xrightarrow{\text{epimerase}}$ 2 Ribulose-5 - phosphate.

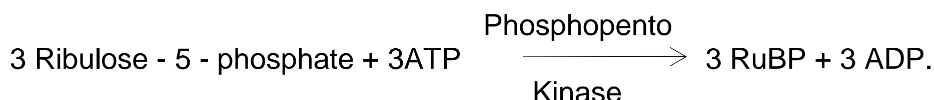
12. One molecule of ribose-5-phosphate is isomerised into one molecule of ribulose -5 - phosphate in the presence of phosphoribose isomerase.



Ribose - 5 - phosphate $\xrightarrow[\text{isomerase}]{\text{Phosphoribose}}$ Ribulose-5 - phosphate.

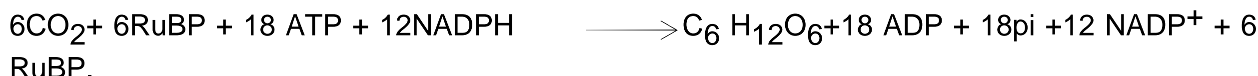
Thus 3 molecules of Riblose 5 phosphate are formed through reactions 11 and 12.

13. The 3 molecules of ribulose-5-phophate are converted to 3 molecules of ribulose bisphosphate (RuBP) by phosphopentokinase. 3 ATP are utilised in this reaction.



3 Ribulose - 5 - phosphate + 3ATP $\xrightarrow[\text{Kinase}]{\text{Phosphopento}}$ 3 RuBP + 3 ADP.

Thus 3 molecules of RuBP are regenerated. Calvin cycle can be summarized in the following equation.



$6CO_2$+ 6RuBP + 18 ATP + 12NADPH $\longrightarrow$ $C_6H_{12}O_6$+18 ADP + 18pi +12 $NADP^+$ + 6 RuBP.

The above equation indicates that six assimilatory units (18 ATP + 12 NADPH) and six CO_2 are necessary for the formation of one molecule of glucose.

4.13 CARBON FIXATION IN C_4 PLANTS

H.P.Kortschack and C.E.Hartt (1965) reported the synthesis of β - carboxylic acid (4C compound) as first stable product of photosynthesis in sugar cane. Australian biochemists *M.D. Hatch and C.R.Slack* (1970) worked out an alternate pathway of carbon fixation in maize, sugar cane and other tropical grasses. This is popularly called *Hatch-Slack cycle.* This is also called β *- carboxylation pathway.* The first stable product is oxaloacetic acid which is a 4 carbon dicarboxylic acid. So it is also called *C_4 cycle* or *Dicarboxylic acid cycle.* It is also called *co-operative photosynthesis* because there is cooperation between mesophyll chloroplasts and bundle sheath chloroplasts for the synthesis of carbohydrates.

Prof. V.S.Ramdas and his co-workers of *Sri Venkateswara University, Tirupathi* reported C_4 pathway in plants belonging to Zygophyllaceae (*Tribulus terrestris).* Euphorbiaceae (*Euphorbia hirta).* Amaranthaceae (Amaranthus viridis) etc., by using radioactive isotope of carbon C^{14}.

C_4 plants have*'Kranz'* type of leaf anatomy. They have dimorphic chloroplasts. The chloroplasts of mesophyll cells contain grana and stroma. The stroma does not contain

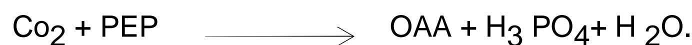
enzymes of Clavin cycle (dark reaction). It consists of enzymes of C_4 cycle. The chloroplasts of bundle sheath cells are large and centripetally arranged. They do not contain grana. The stroma contains enzymes of Calvin cycle.

C_4 PATHWAY

Kranz anatomy: Most of the plants exhibiting a C_4 pathway have a special leaf anatomy called 'Kranz anatomy' ('Wreath' in German). It is characterized by presence of big parenchymatous cells around the vascular bundles called the bundle sheath. In addition to this the C_4 plants are known to posses dimorphic chloroplast. The chloroplast in the bundle sheath cells lack grana while those of mesophyll cells posses them. Dimorphic condition need not occur in all C_4 plants.

C_4 Path way :- This pathway operates in two photosynthetic cells Mesophyll and Bundle sheath cells.

Reactions in Mesophyll Cells : 1. The atmospheric Co_2 is initially accepted by Phosphoenol, Pyruivic acid (PEP) a C_3 compound to form oxaloacetic acid (OAA) which is a four carbon compound in presence of PEP carboxylase.



$$Co_2 + PEP \longrightarrow OAA + H_3PO_4 + H_2O.$$

The C_4 species can be divided into three sub-groups depending upon the enzyme. responsible for decarboxylation of C_4 acid.

1) Malate formers : In these members the oxaloacetic is reduced to malic acid by malic dehydrogenase and $NADPH+H^+$ is oxidised to $NADP^+$.

$$OAA + NADPH \longrightarrow \text{Malicacid} + NADP^+$$

The malicacid thus formed is transferred to the chloroplast of Bundle sheath cells & there it undergoes oxdative decarboxylation.

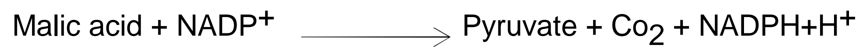
2) Asparate formers: In some members oxoloacetic is converted into asparitic acid by asparatic amino transferase.

$$OAA + NH_3 \longrightarrow \text{Asparaitic acid.}$$

This aspartate is transported to the bundle sheath cells. Where it is transaminated to oxaloacetic acid. Later this OAA is reduced to malic acid and it is further decarboxylated.

3. P C K Type : The OAA is converted into aspartate and it is translocated to the bundle sheath cells. There it is converted back into OAA. Later OAA is decarboxylated to form Phosphoenol pyruvic acid which yields pyruvate. This type is called phophoenol pyruvate carboxy kinase (PCK).

Reactions in Bundle sheath Cells : In the bundle sheath cells the Co_2 is released by the oxidative decarboxylation of malic acid.


Malic acid + $NADP^+$ ⟶ Pyruvate + Co_2 + $NADPH+H^+$

The released Co_2 is now fixed in the calvin cycle pathway of C_3 plants to syntheisise sugars. The NADPH and pyruvate travel back into the mesophyll cells.

Reactions in Mesophyll Cells :

The pyruvate that enters into the mesophyll cells is converted into phoshoenol pyruvic acid with the utlization of 2 ATP molecules in presence of Pyruvicphosphate dikinase.

Pyruvic + 2 ATP + Pi ⟶ PEP + 2 AMP +2 PPi

To syntheseis one molecule of Glucose, C_4 plants utilize 30 ATP and 12 NADPH.

Significance of C_4 pathway :

C_4 plants show higher rate of photosynthesis because of the following the reasons:-

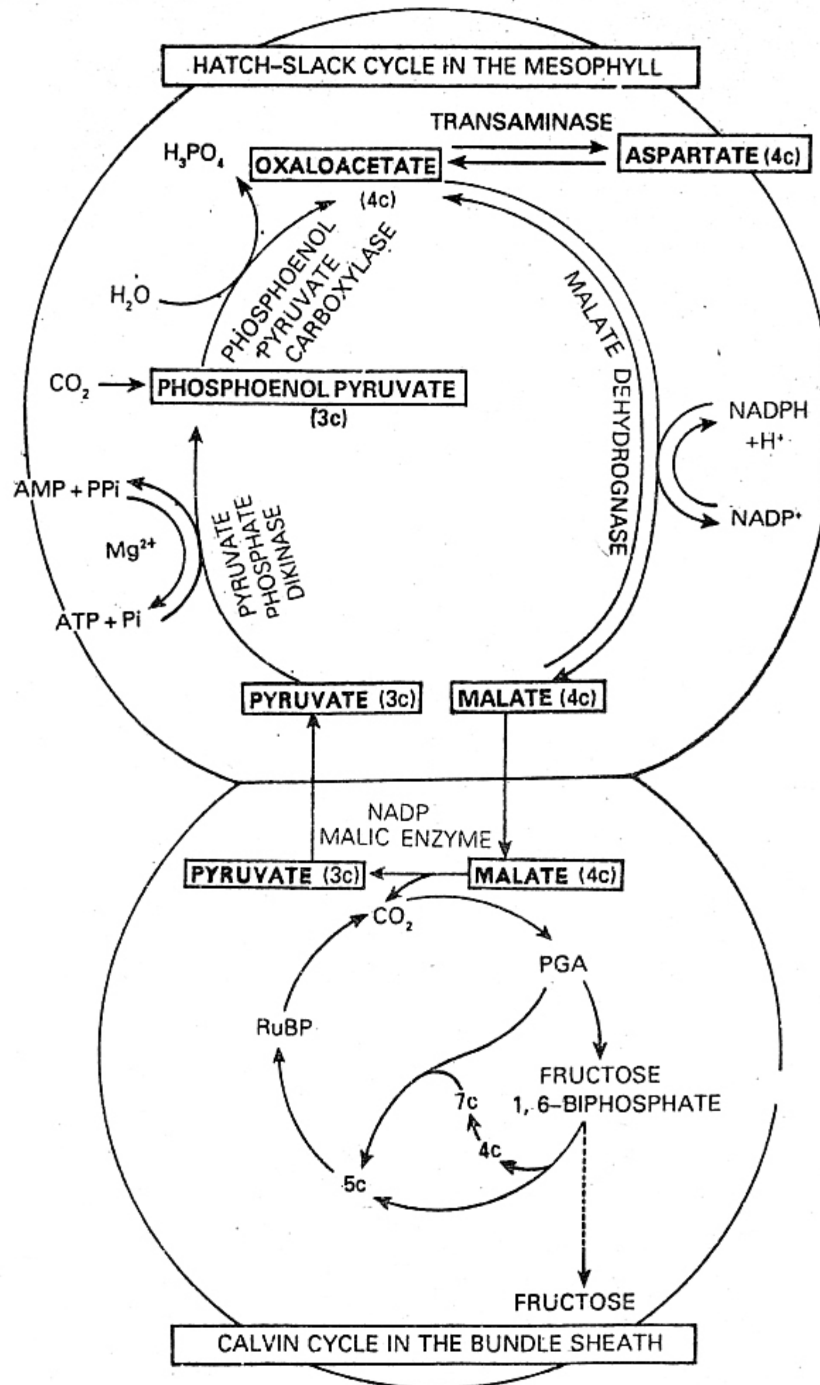
i) In C_4 plants two carboxylations occur one in the Mosophyll and other in Bundle sheath cells.

ii) The high affinity of PEP carboxylase to Co_2.

iii) C_4 plants can carry on photosynthesis even under very low Co_2 concentration.

iv) C_4 plants show higher rate of translocation of photosynthetic products.

v) O_2 is not an inhibitory factor.



Hatch-Slack cycle and Calvin cycle as they occur within the mesophyll and bundle sheath of the tropical grasses.

Fig: 3.1 C_4.Cycle

4.14 Differences between C_3 & C_4 plants :

No.	Character	C_3 Plants	C_4 Plants
1.	CO_2 acceptor	RuBP	PEP
2.	First stable product	PGA	Oxaloacetate
3.	Type of Chloroplast	One type	Dimorphic, bundle sheath chloroplasts lack grana. Mesophyll cells have normal Chloroplasts.
4.	Leaf anatomy	Normal	Kranz anatomy
5.	Enzyme of C_3 pathway	Found in mesophyll cells.	Found in bundle sheath cells.
6.	CO_2 compensation point	50 - 150 ppm	0 - 10 ppm
7.	Photorespiration	Present	Absent
8.	Net rate of photo synthesis in full sunlight	15-35mg of CO_2 per dm^2 of leaf	40-80mg of CO_2 per dm^2 of leaf.
9.	Bundle sheath Cells.	Not prominent	Very prominent; Kranz like
10.	CO_2 fixation	C_3 pathway	Both C_3 and C_4
11.	High rate of O_2	Inhibits photosynthesis	has no effect on photosynthesis
12.	Temperature (optimum)	10-25^{0}C	30-45^{0}C
13.	ATP molecules required to synthesize one molecule of glucose.	18	30

4.15 Crassulacean Acid Metabolism:

It is a metabolism occurring in certain succulent plants of the family Crassulaceae. In xerophytic plants to check the rate of transpiration the stomata are closed during day time and open at night. (Scotoactivity).

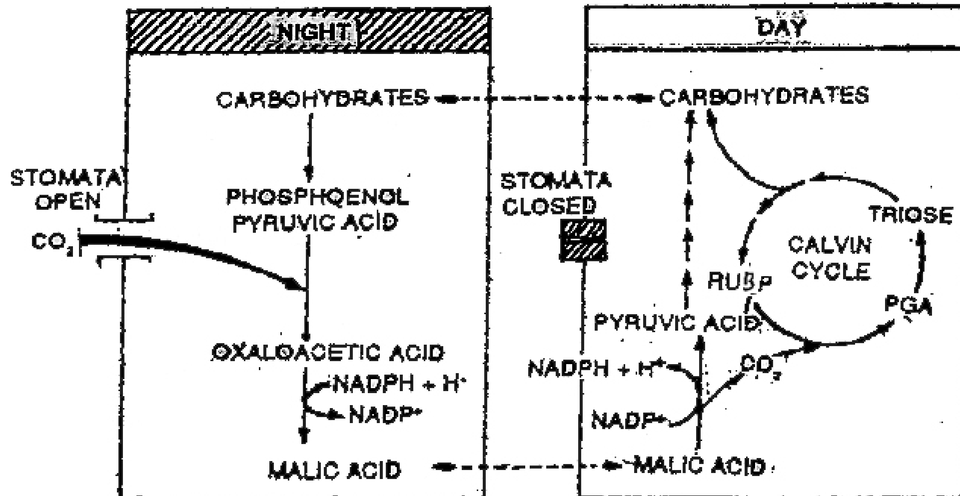


Fig: 3.2 C A M

During Night :

- When stomata are open, Co_2 is fixed by phosphoenol pyruvic acid to form oxaloacetic acid (OAA) Catalysed by PEP carboxylase.
- Later the OAA gets reduced to malic acid with the help of malic dehydrogenase.

During day time : In the presence of light Malic acid is decarboxylated to form pyruvate and Co_2. This Co_2 enters the C_3 cycle and is fixed by RuBP to form sugars. The metabolic fixation of Co_2 is essentially same in both C_4 plants and CAM plants. In CAM plants both C_3 and C_4 pathways occur in mesophyll cells, while in C_4 plants they are separated between mesophyll and bundle sheath cells.

4.16 PHOTORESPIRATION

The process of photorespiration is demonstrated in tobacco plant by Decker(1955). Since then it has been reported in large number of plants particularly those which perform C_3 cycle. Decker_indicated that the respiration is more in light than in dark. It is estimated to be 3 to 5 times more than the rate of respiration in dark.

In photorespiration the ribulose bis phosphate instead of Co_2 takes O_2 and synthesizes glycolic acid and in subsequent reaction Co_2 is released. This entire process takes place in Chloroplast, Peroxisome and Mitochondria.

Reaction in Chloroplast : 1. When the Co_2 concentration in the atmosphere is below 1% the ribulose bisphosphate combines with O_2 and forms one molecule of phospho glyceric acid and Phosphoglycolic acid in the presence of enzyme RuBP oxygenase.

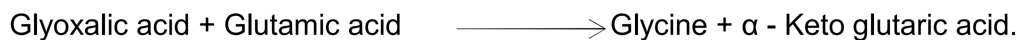
2. The phospoglycolic acid is dephosphorylated to form glycolic acid in the presence of Enzyme phosphatase.

glycolic acid enters into Peroxisome.

Reactions in Peroxisome :-

3. The glycolic acid reacts with O_2 and oxdises to glyoxylic acid and H_2O_2 with the help of glycolic acid oxidise.

4. The glyoxalic acid is converted into glycine by transamination in the presence of glyoxylate transaminse.



This glycine is transported to mitochondria.

Reactions in Mitochondria :- In Mitochondria 2 molecules of glycine combine to form one molecule of serine. In the process Co_2 and NH_3 are released and NAD is reduced to NADH

Serine returns to peroxisome.

Reactions in Peroxisomes:- Serine is converted successively into hydroxypyruvic acid and then into glyceric acid.

NH_3 NADH

The glyceric aicd is transported to chloroplast.

Reactions in Chloroplast :- In chloroplast the glyceric acid undergoes phosphorylation by ATP to form 3 PGA. They join the pool in the Co_2 reduction cycle.

Significance of Photorespiration:- Photorespiration is a necessary evil. It may help photosynthesis by increasing the volume of intercellular Co_2 It provides the protective mechanism against light destruction of chloroplast in C_3 plants. It may utilise excess of ATP and NADPH and prevents solarization.

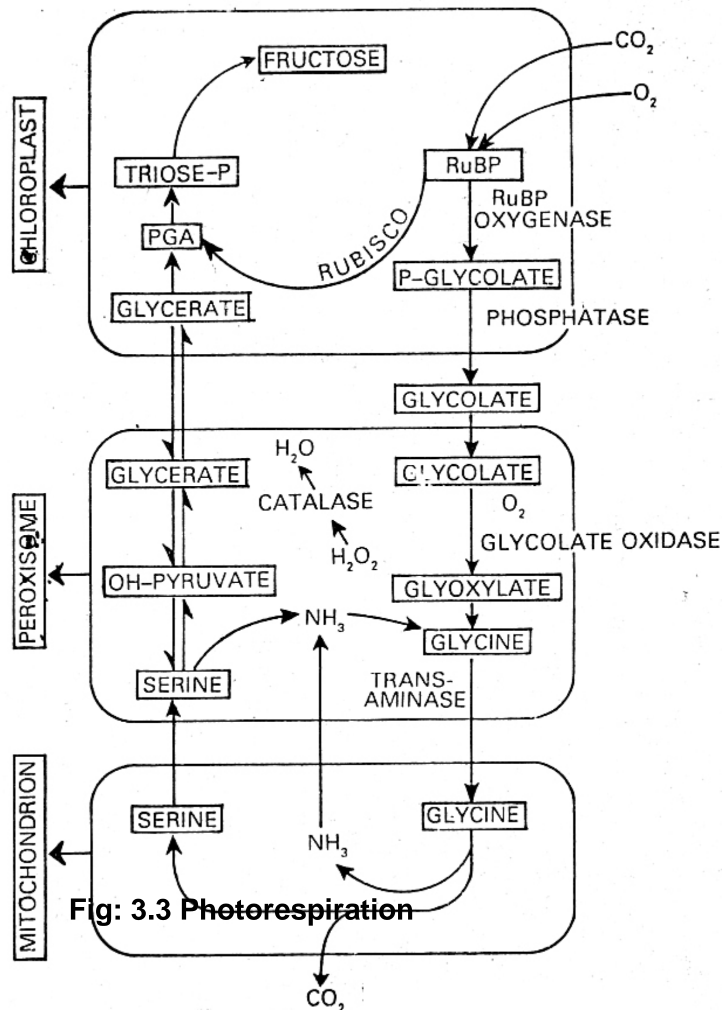


Fig ; 3.3 Photorespiration

4.17 TRANSLOCATION OF ORGANIC SOLUTES

Introduction : Food material is mostly manufactured by the green cells of the leaves of a plant. Hence leaves are considered as the supply end of the food material. The food has to be translocated rapidly from the supply ends to different parts of plant for consumption and utilization. In most cases, the organic food from the leaves is translocated to the lower parts of the plant for consumption or storage. Sucrose is the chief carbohydrate transported in plants.

Path of translocation :- There is controversy regarding the elements which are concerned with translocation of food material. However it is confirmed that the down ward translocation occurs through the phloem.

Mechanism of Phloem conduction :- A number of theories have given to explain mechanism, but none of them seems to be entirely satisfactory. The following are certain major theories for phloem conductions.

1. Diffusion hypothesis.
2. Protoplasmic streaming.
3. Electro osmotic theory.
4. Pressure flow hypothesis.

1. Diffusion Hypothesis :- Many workers are of the opinion that nutrient translocation takes place by simple diffusion. Since nutrient flow is from the region of higher concentration to the region of. lower concentration i.e., source (leaf) to lower sink.(root).

Objections: - The rate of flow of solutes in the sive tubes is at least 40,000 times more than what is possible by the process of diffusion.
- Respiratory poisons affect the rate of flow, indicating its depends on metabolism.

2. Protoplasmic Streaming : It is proposed by Hugo devries. The theory suggests that protoplasmic streaming movements in the sieve elements is responsible for the translocation of organic solutes.

Objections :- 1. The rate of streaming of protoplasm is much less than the rate of movement of solutes in the phloem.

2. There is no protoplasmic streaming but still they are able to perform translocation.

Electro Osmotic Theory: Fenson and spanner proposed that an electric potential across the sieve plates causes the movement of water along with the solutes. K^+ ions are pumped into the sieve tubes at the expense. of ATP. Sugar molecules adhered. to K^+ ions are carried through the negatively charged. sieve pores, thus sugar molecules migrate across the plate with K^+. The hypothesis however does not provide answers to several problems of solute translocation.

3. Pressure Flow Or Mass Flow hypothesis :- This theory was proposed by *Munch* (1930) and elaborated by *Crafts* (1938). According to this theory organic solutes are translocated *" en masse"* through the sieve tubes from the supplying end or *source* (leaves) to the consumption end or *sink* (roots, fruits, tubers).

Important features of this theory are :

a. Mesophyll cell synthesize sugars during photosynthesis. They dissolve in cell sap thereby increasing the osmotic concentration and (ψ_W decreases).

b. Water enters the mesophyll cells from the xylem. Turgor pressure or pressure potential (ψ_p) of the mesophyll cells increases.

c. Sugars dissolved in water move from mesophyll cells into the *symplast* system of sieve tubes (protoplasts of all the sieve tubes form a part of the symplast system).

d. Solutes are carried *"en masse"* through the symplast and finally reach the consumption centers.

e. In the consumption end, food materials (solutes) are either *used up* (as in roots) or *Stored* in an insoluble form (as in fruits, tubers). Therefore the osmotic concentration and consequently the turgor pressure in these cells will be low.

f. Thus continuous pressure gradient is established across the symplast between the cells of the *source* (leaf) and those of the *Sink* (root).

g. Water returns to the source (leaf) through the *apoplast system.*

(The chain of xylem elements that connect the supply end and consumption end form a part of the apoplast system). Here water acts as a *carrier of solutes.* It picks up the solutes at the supply end (leaf) and carries them to the consumption end (root).

PHYSICAL MODEL FOR PRESSURE FLOW :

Much explained his theory with the help of a physical model as shown in fig 3.5. It consists of two semi permeable *osmometer* bulbs A and B. The bulbs A and B are connected by a tube C. Bulb A is filled with higher concentrated coloured sugar solution and B is filled with dilute sugar solution.

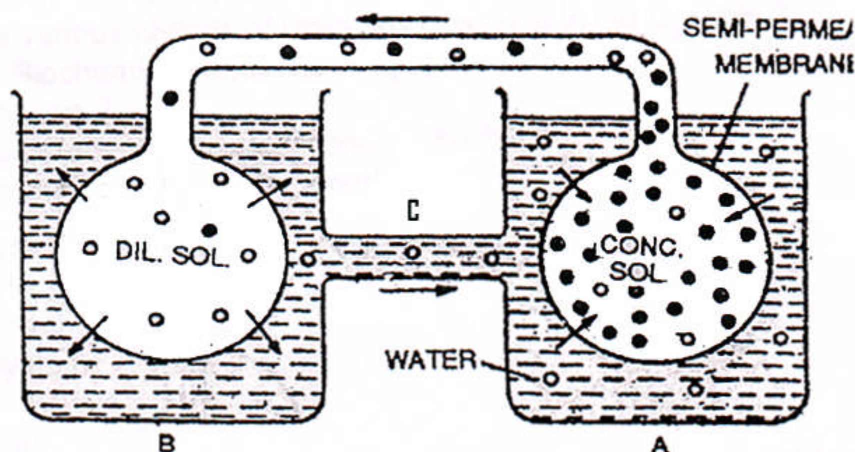


Fig: 3.5 PHYSICAL MODEL FOR PRESSURE FLOW

Both the osmometers A and B are immersed in vessels D which is filled with water. Water enters more rapidly into bulb A from D by osmosis than into B. As a result pressure (=TP) develops within the bulb A. At the same time pressure in bulb B is less. Thus a pressure gradient develops between the two bulbs. Solution from bulb A flows "en masse" with some pressure into bulb B along with water. This flow of solution from A to B continues until the concentration of the solutions in both the bulbs becomes equal.

This physical system can be kept working continuously by maintaining higher concentration in bulb A and lower concentration in bulb B.

Bulb A is comparable to the production centre (leaf). bulb B - consumption centre (root), tube C - *symplast,* vessel D - *apoplast* of the plant body.

In plants, higher concentration of the solutes at the supply and (leaf) is always maintained due to photosynthesis. Lower concentration of solutes at the consumption end (root) is always maintained due to respiratory breakdown of sugars (roots, apices) or osmotically inactive reserve forms (fruits, tubers).

OBJECTIONS :

1. Bidirectional transport of solutes in the same sieve tube is not explained.
2. Slime content and other fibrils of the sieve tube minimize the speed of flow of solutes even under high pressure.
3. Mass flow is not a physical process as described by *Munch*. Phloem cells utilize 0.3 - 5.0 per cent of sucrose translocated through them. This is an evidence to show that phloem translocation is an active process and requires metabolic energy.

4.18 SYNOPSIS

Photosynthesis is the unique property of plants involving an energy transduction reaction by which light energy is converted into chemical energy necessary for the vital functions of living organisms. By enzymatic reactions, light energy is conserved as phosphate bond energy of ATP and as reducing power in the form of NADPH, which are then utilized by green plant cell for the reduction of CO_2 to form carbohydrates.

Technical Terms : Photon, Light harvesting complex, Antenna molecules, Absorption Spectrum, Action Spectrum, Photolysis, Phosphorylation, Red Drop, Porphyrin, Phytal, Rubisco,

4.19 Self Assess Questions:

Essay Questions:

1. Write an essay on Non Cyclic Photophosphorylation ?
2. Explain the various phases of reactions occurring in the C_3 cycle ?
3 Explain the Biochemical reactions in C_4 plants and tabulate the differences between C_3 and C_4 pathways ?
4. Describe photorespriratiion ?
5. Give an account on phloem transport ?

Short Answers :

1. Cyclic photophosphorylation
2. Emerson effect
3. Pigment system.
4. CAM.
5. Hill reaction.

- K.V.S. Durga Prasad

Lesson 5

RESPIRATION - AEROBIC AND ANAEROBIC RESPIRATION, GLYCOLYSIS, KREB'S CYCLE, ELECTRON TRANSPORT MECHANISM (CHEMIOSMOTIC THEORY), OXIDATIVE PHOSPHORYLATION

5.0 OBJECTIVES

(1) Students will compare aerobic and anaerobic respiration.

(2) Students understand the importance of respiration in plants life.

(3) Students will learn different aspects of aerobic respiration.

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5.0 INTRODUCTION

5.1 DIFFERENCE BETWEEN AEROBIC AND ANAEROBIC RESPIRATION

5.2 GLYCOLYSIS

5.3 KREB'S CYCLE

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5.5 OXIDATIVE PHOSPHORYLATION

5.6 FERMENTATION

5.7 RQ

5.8 SUMMARY

5.9 TECHNICAL TERMS

5.10 SELF ASSESSMENT QUESTIONS

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5.0 INTRODUCTION

Respiration is a catabolic process, by which food material is oxidised and energy is liberated in the form of ATP and heat. ATP is useful form of energy to the plants. Sugars are most generally the starting point. Water and Carbondioxide are commonly the end products. It can be expressed by the following equation.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 673K.\ cal.$$

Respiration is a continuous and life long process on which the entire life of an organism depends. As it releases energy, it is also called exergonic reaction. Respiration is like burning

of fuel in an engine, since both release energy by oxidation of an organic substance. Actually, burning fuel and respiration are very different in the conditions surrounding them. Burning fuel is a physical process occurring outside the cell at very high temperatures whereas respiration is a biological process that occurs inside the cell at more moderate temperatures. This is because they proceed in very small steps, controlled by enzymes.

During respiration, apart from the release of energy, the sugars are modified to form the carbon skeletons, that make up the basic building blocks of cell structure and also used in growth and maintenance of a cell.

History

(1) Malphigi (1679) identified that seeds require air during their germination.

(2) Scheele (1777) proved that all living plants give or liberate CO_2.

(3) Ingen-Housz (1779) showed that all living plants release CO_2 in dark and non-green parts of the plant give out CO_2 in light as well.

(4) De-sassure (1779) told that the volume of O_2 absorbed by the plants is equal to the volume of CO_2 released.

(5) Sachs (1885) First used the terms aerobic and anaerobic respirations for the process occurring with or without the consumption of O_2.

(6) Huxley (1888) studied animal respiration and said that animals absorb O_2 and release CO_2 during respiration.

(7) Meldrum (1938) emphasized that the respiration is a chemical reaction by which organic compounds are burnt by oxidative reactions inside the living cells.

(8) Szent-Gyogryi suggested that many organic acids are oxidised within the cell by removal of hydrogen atoms, these hydrogen atoms are passed through a system of cytochromes and finally react with O_2 to form water, this is known as cellular respiration.

Respiration differs from photosynthesis in the following aspects

	Respiration		Photosynthesis
(1)	It occurs in all living organisms	(1)	It occurs only in the green plants.
(2)	It is a continuous process, occurring throughout life.	(2)	It occurs only during day time, when light is available.
(3)	It is a catabolic process i.e., complex material is broken down into simple substances.	(3)	It is an anabolic process, where simple substances are converted into complex substances.
(4)	It is an exergonic process, energy is released.	(4)	It is an endergonic process energy is consumed.
(5)	Here potential energy (carbohydrates) is changed to kinetic energy.	(5)	Here radiant energy (sunlight) is converted into potential energy.
(6)	It occurs in cytoplasm and	(6)	It occurs in chloroplasts.

(7)	mitochondria.	(7)	CO_2 is used and O_2 is liberated.
(8)	O_2 is taken for oxidising food material and CO_2 is given off.	(8)	Since food material is produced it increases dry weight of the plant.
(9)	Since food material is used, there will be a decrease in dry weight due to respiration. $C_6 H_{12} O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 673$ K. cals.	(9)	$6CO_2 + 6H_2O + \text{energy} \rightarrow C_6 H_{12} O_6 + 6O_2$.

5.1. DIFFERENCE BETWEEN TYPES OF RESPIRATION (AEROBIC AND ANAEROBIC RESPIRATION)

Depending upon the availability of O_2 respiration is classified into 2 types. They are (1) aerobic respiration (2) anaerobic respiration.

	Aerobic respiration		**Anaerobic respiration**
(1)	Respiration occurs in the presence of oxygen.	(1)	It occurs in the absence of O_2.
(2)	It can take place continuously in all plants.	(2)	It cannot be continuous in all plants. Higher plants do not show it, only some parts such as seeds, deeper tissues may perform it. But microorganisms such as bacteria, yeasts can respire anaerobically in a continuous manner.
(3)	It is a complete oxidation process	(3)	It is a partial oxidation process
(4)	End products are CO_2 and H_2O.	(4)	End products are alcohol and CO_2.
(5)	Much energy is liberated.	(5)	Less energy is liberated.
(6)	$C_6 H_{12} O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O +$ 36ATP mols.	(6)	$C_6 H_{12} O_6 \rightarrow 2C_2 H_5OH + 2CO_2 +$ 2ATP mols.
(7)	It shows glycolysis, krebs cycle, electron transport system.	(7)	It shows glycolysis and fermentation.
(8)	Glycolysis occurs in the cytoplasm and krebs cycle, electron transport system occur in mitrochondria.	(8)	Both events occur in the cytoplasm.
(9)	The process is not toxic to cells	(9)	It is toxic to cells.

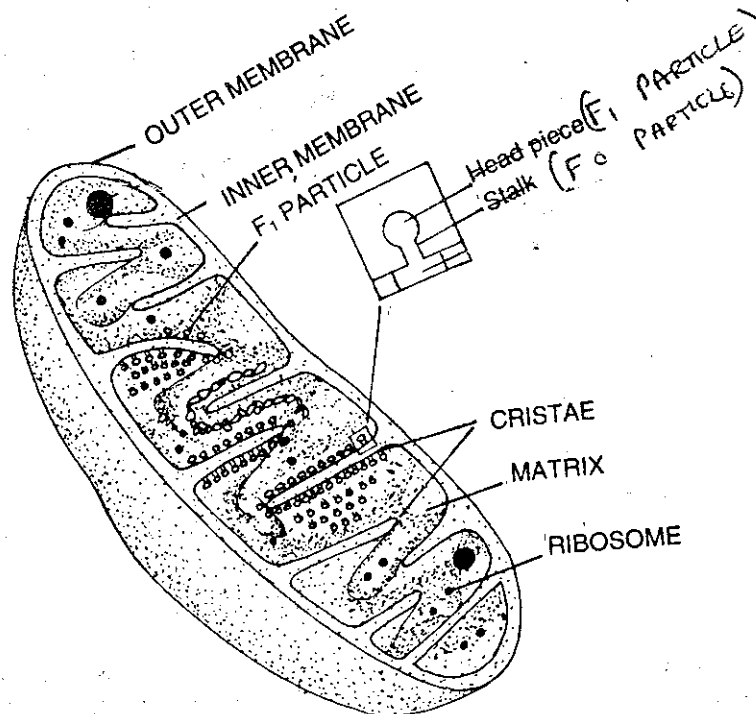
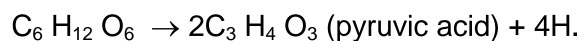


Fig. 5.1 Ultrastructure of mitochondria and enlarged section of mitochondrial crest

5.2 GLYCOLYSIS

6 carbon glucose is broken down into 3 carbon compound. It is called “Glycolysis”. Glycos = sweet, Lysis = splitting.

It is the first phase of both aerobic and anaerobic respiration. It occurs in the cytoplasm. As EMBDEN, MEYERHOF and PARNAS did lot of investigation on this process, it is also named as EMP pathway, in honour of them. Glucose is the starting point and the pyruvic acid is the end product. The overall equation of glycolysis can be represented as:



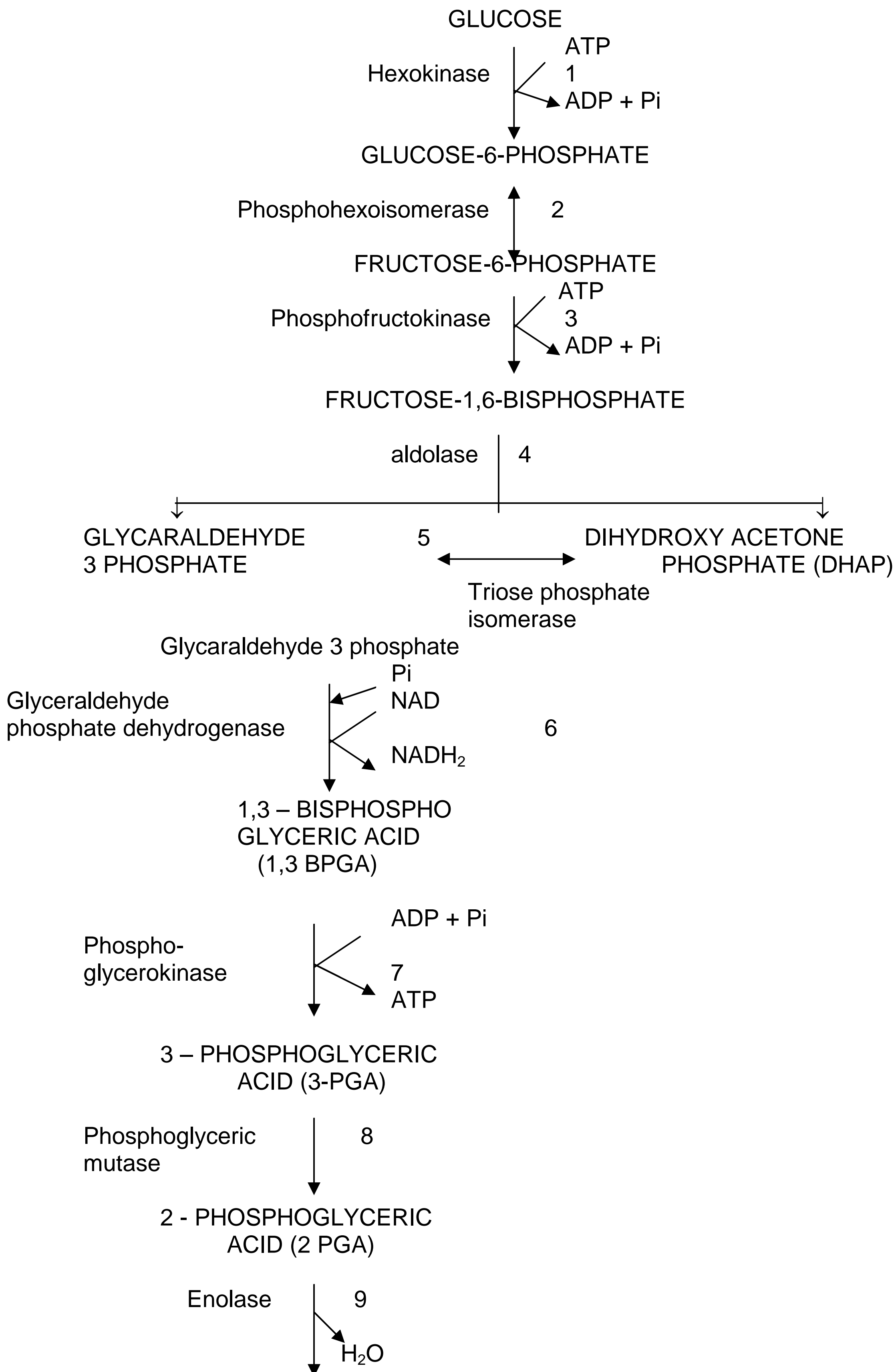
$$C_6H_{12}O_6 \rightarrow 2C_3H_4O_3 \text{ (pyruvic acid)} + 4H.$$

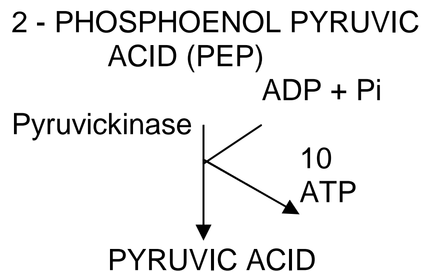
The process of glycolysis is sub divided into two main events.

(a) Conversion of glucose to fructose 1,6 diphosphate.

(b) Splitting of Fructose, 1,6 bisphosphate into two molecules of pyruvic acid, a 3-carbon compound.

Reactions of Glycolysis



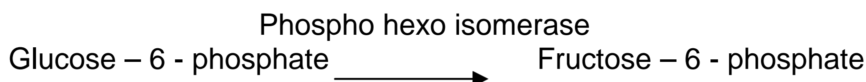


(1) Phosphorylation

This is the first step of Glycolysis. Glucose combines with ATP and is converted into glucose-6-phosphate in the presence of enzyme Hexokinase.

(2) Isomerisation

Glucose – 6 - phosphate is converted into its isomer Fructose – 6- phosphate in the presence of “phospho hexose isomerase”.



(3) Phosphorylation

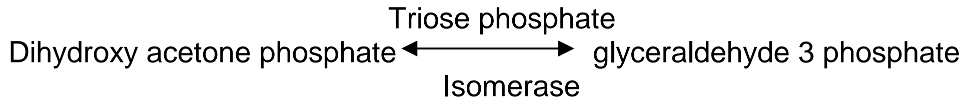
Fructose – 6 - phosphate undergoes phosphorylation and gives fructose – 1,6 – bisphosphate. In this step ATP is the phosphate donor. The reaction is mediated by the enzyme “phospho fructo kinase”.

(4) Cleavage

Fructose –1,6 - bisphosphate is split up into two triose phosphate molecules, namely glyceraldehyde 3 phosphate and dihydroxy acetone phosphate. This reaction is catalysed by aldolase and the products are inter convertible. An equilibrium is maintained between the two trise phosphates by the catalytic action of triose phosphate isomerase.

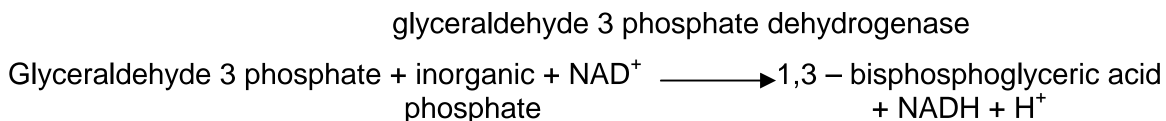
(5) Isomerisation

Among the two trioses, resulted in the above reaction, phosphoglyceraldehyde directly participates in the further reactions. On the other hand dihydroxyacetone phosphate, which is not suitable for direct biological oxidation is converted into glyceraldehyde 3 phosphate in the presence of ‘triose phosphate isomerase’ and participates in the further reactions.



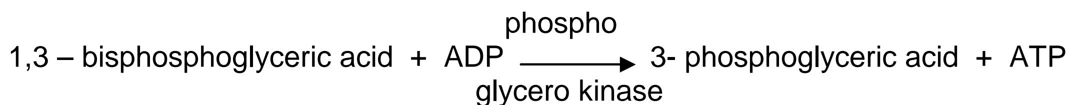
(6) Oxidation

It is the only oxidation step in the entire glycolysis process. Glyceraldehyde 3 phosphate undergoes oxidation as well as phosphorylation and forms 1,3- biphosphoglyceric acid and NADH in the presence of ‘glyceraldehyde 3 phosphate dehydrogenase’. For the phosphorylation, instead of using ATP as in 1 and 3 reactions, inorganic phosphate present in the cytoplasm is used. The 2 hydrogens, released during oxidation are transferred to the NAD^+ and reduce it into $NADH^+$.



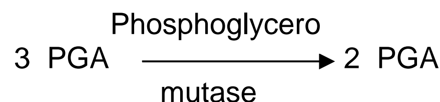
(7) Dephosphorylation

1,3- biphosphoglyceric acid undergoes dephosphorylation and forms 3- phosphoglyceric acid in the presence of ‘phosphoglycero kinase’. ADP molecule accepts the phosphate and gets converted into high energy compound ATP. This process of ATP formation is known as substrate level phosphorylation.



(8) Intra Molecular Shift

The 3- phosphoglyceric acid is later converted into 2-phosphoglyceric acid in the presence of ‘phosphoglycero mutase’.



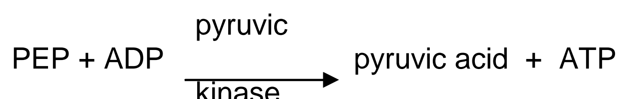
(9) Dehydration

The enzyme ‘enolase’ catalyses the removal of one water molecule from 2-phosphoglyceric acid resulting in the formation of phosphoenal pyruvic acid.

$$2\ PGA \xrightarrow{enolase} PEP + H_2O$$

(10) Dephosphorylation

It is the last step of glycolysis, where phosphoenol pyruric acid undergoes dephosphorylation in the presence of pyruvic kinase and forms pyruvic acid. ADP is converted into ATP. This is second step of "substrate level phosphorylation".



$$PEP + ADP \xrightarrow[kinase]{pyruvic} pyruvic\ acid + ATP$$

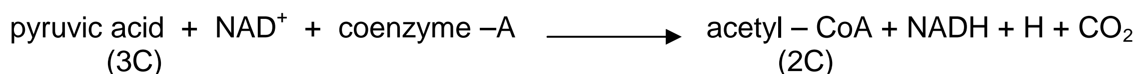
In glycolysis, 2 molecules of ATP are utilized in the (1) and (3) reactions. During 6th reaction $NADH_2$ is released and in (7) and (10) reactions 2 ATP are released. 6th to 10th reactions will occur again, so that 2 pyruvic acids are released and the total ATP are:

at 6th reaction – $2NADH_2$ = $1NADH_2$ = 2ATP so that 4ATP are released*
(*$NADH_2$, released in cytoplasm is equal to 2 ATP)
at 7th and 10th reactions = 4ATP

Total ATP are 8, among them 2 are consumed at (1) and (3) reactions so that total net gain of ATP during glycolysis is 6 ATP.

Oxidative decarboxylation of pyruvic acid

It is the second stage of aerobic respiration and it occurs in mitochondrial matrix. Two molecules of pyruvic acid, released at the end of glycolysis are transported through the mitochondrial membrane into matrix by a special protein called 'pyruvate translocator'. Once the pyruvic acid is inside the matrix. It is first decarboxylated and then oxidised. Finally it condenses with co-enzyme 'A', to form acetyl co-A. It is the connecting link between glycolysis and kreb's cycle.



pyruvic acid + NAD^+ + coenzyme –A → acetyl – CoA + NADH + H + CO_2
(3C) (2C)

The above reaction is under control of pyruvic dehydrogenase enzyme complex. This is cluster of 3 enzymes (a) Pyruvate decarboxylase

(b) Dihydrolipoyl transacetylase
(c) Dihydrolipoyl dehydrogenase

For the formation of acetyl CoA, 6 co-factors are required. They are mg^{2+}, FAD, NAD, TPP (Thiamine pyro phosphate), lipoic acid and co enzyme–A. At the end of oxidative decarboxylation 2 moles of NADH + H^+ are released for 2 mols. of pyruvic acid.

5.3 KREBS CYCLE

Pyruvic acid (3c) enters into the krebs cycle in the form of acetyl –CoA (2C) which is formed during oxidative decarboxylation. The further oxidation of acetyl – CoA can be showed by different 10 reactions called kreb's cycle. It was named as krebs cycle after it's discoverer Sir Hans Krebs (1937). As the first product of this cycle is citric acid, it is also called as citric acid cycle. As the first four intermediate compounds of this cycle are tricarboxylic acids, it is also called as "Tri carboxylic acid" cycle. It is an aerobic process that occurs in the mitochondria.

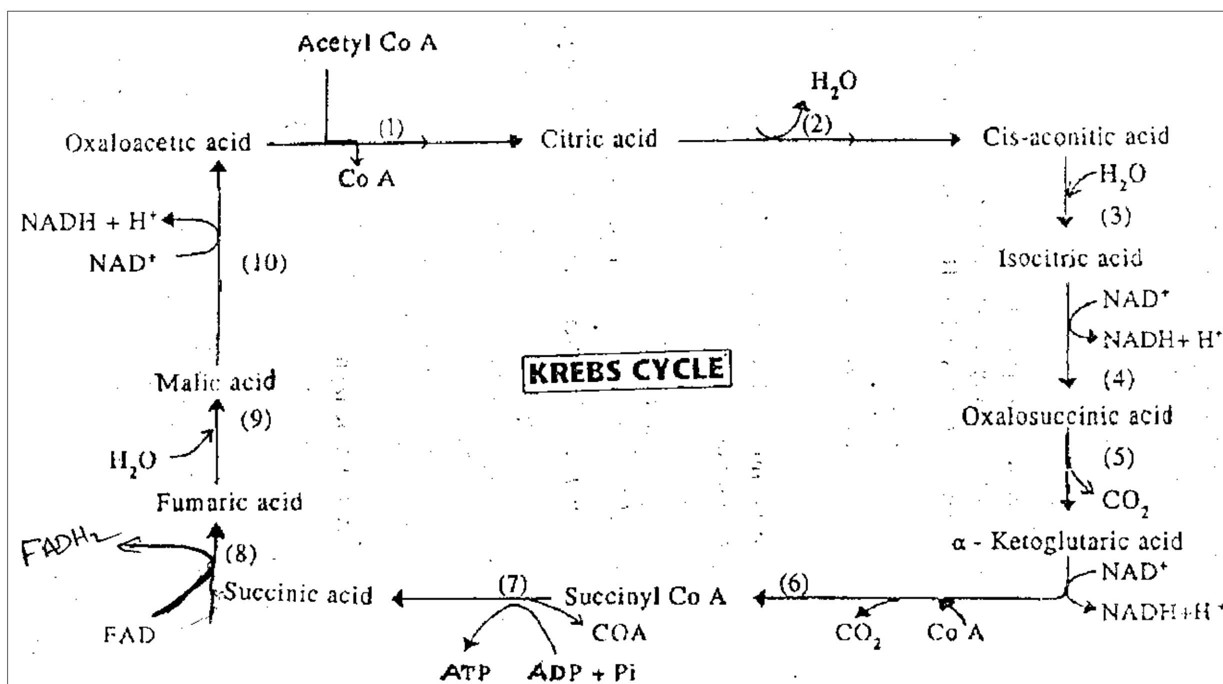
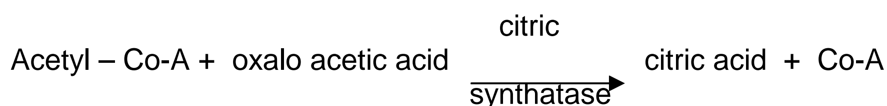


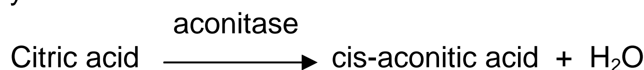
Fig. 5.2 Krebs cycle

(1) The acetyl Co-A(2C) condenses with oxalo acetic acid (4C) in the presence of citric synthatase and forms citric acid and Co-A is released.

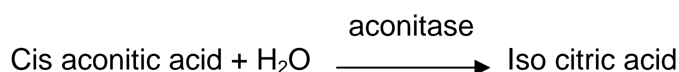


Acetyl – Co-A + oxalo acetic acid $\xrightarrow[\text{synthatase}]{\text{citric}}$ citric acid + Co-A

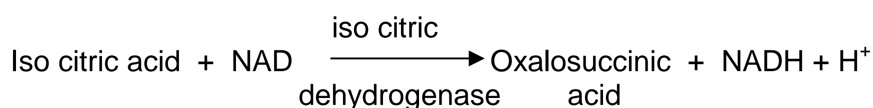
(2) Citric acid is converted into cis-aconitic acid by the dehydration in the presence of an enzyme aconitase.



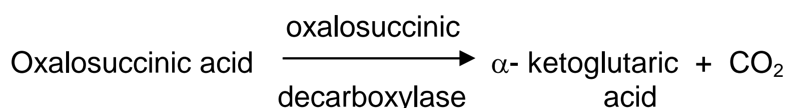
(3) In the next step 'aconitase' enzyme mediates the addition of one molecule of water to cis-aconitic acid. The product formed from this hydration step is '**isocitric acid'**.



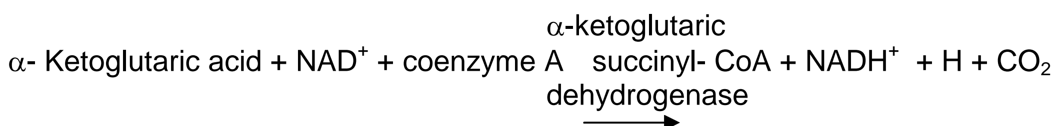
(4) Iso citric acid forms oxalosuccinic acid due to the oxidation in the presence of isocitric dehydrogenase. In the reaction NAD^+ is reduced to NADH + H^+.



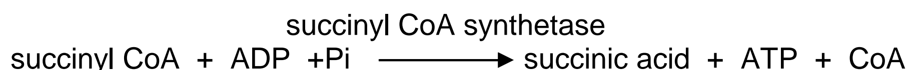
(5) The 6- carbon oxalo succinic acid releases one molecule of CO_2 in the presence of 'oxalosuccinic decarboxylase' and forms a 5-carbon 'α-ketoglutaric acid'.



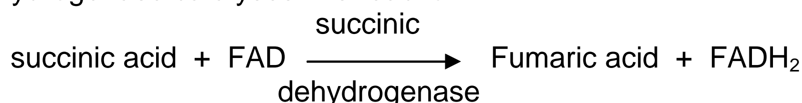
(6) α- keto glutaric acid, a 5 carbon compound undergoes oxidative decarboxylation and condenses with – CoA and forms succinyl Co-A (4C) in the presence of α- keto glutaric dehydrogenase. In this reaction NAD acts as hydrogen acceptor and NADH is formed. CO_2 is also removed.



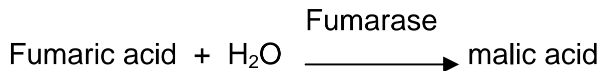
(7) Succinyl Co-A splits into succinic acid and co enzyme-A by the catalytic activity of 'succinyl CoA synthetase'. One ATP is released by the addition of inorganic phosphate to ADP. Because ATP formation is linked directly to conversion of substrate, this reaction is an example of substrate level phosphorylation.



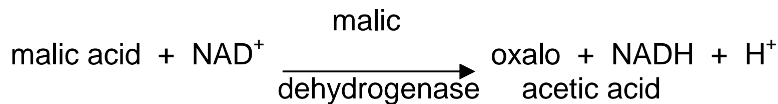
(8) Succinic acid undergoes oxidation and forms fumaric acid. Here instead of NAD, FAD serves as hydrogen acceptor. Therefore FAD is reduced to $FADH_2$. Succinic dehydrogenase catalyses this reaction.



(9) Fumaric acid is hydrated to form malic acid in the presence of fumarase enzyme.



(10) It is the final oxidation step of krebs cycle, malic acid, in the presence of 'malic dehydrogenase' releases 2 hydrogen atoms and gives oxalo acetic acid. In this step NAD^+ acts as hydrogen acceptor and is converted into NADH + H^+.



Net gain

At the end of krebs cycle, for every two molecules of acetyl Co-A undergoing oxidation, it results 2 molecules of ATP at 7th reaction, 6 molecules of $NADH_2$ at 4th, 6th and 10th reactions and two $FADH_2$ at 8th reaction. Therefore the net gain is

7th reaction - 2 Molecules of ATP
4th, 6th and 10th reaction - 6 Molecules of $NADH_2$ = 6 x 3 = 18 ATP
8th reaction - 2 Molecules of $FADH_2$ = 2 x 2 = 4 ATP

During oxidative decarboxylation of pyruvic acid – 2 $NADH_2$ are released, that is equal to 6 ATP. So that net gain is 30 ATP.

Significance of Krebs cycle

Krebs cycle is also called as 'Amphibolic pathway', because it involves in both anabolism and catabolism. In the anabolic role, the keto acids of this pathway serve as the substrate for the synthesis of amino acids. In the catabolic role, it serves as a pathway for oxidation of carbohydrates.

Electron transport system

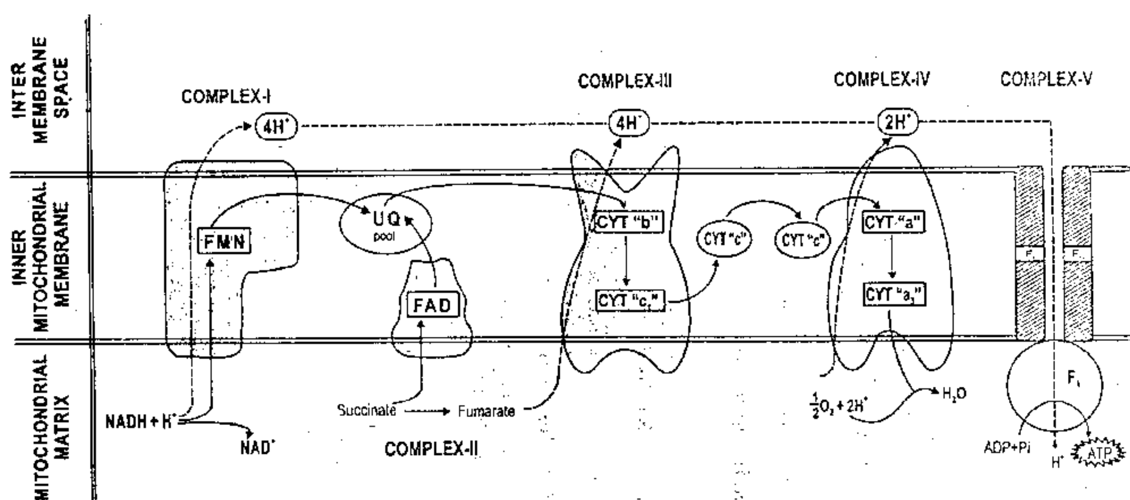


Fig. 5.3 Schematic diagram of electron transport system and ATP synthesis

This is the final stage of aerobic respiration. From the earlier steps such as glycolysis, oxidative decarboxylation, krebs cycle a total of 12 high energy electron pairs are generated as 10 $NADH_2$ and 2 $FADH_2$ for each molecule of glucose.

For keeping respiration in continuous action, these $NADH_2$ and $FADH_2$ must be oxidised into NAD and FAD respectively. During oxidation, the removed electrons can be transported through different intermediate electron carrier complexes and finally reach to oxygen. All the protein complexes involved in electron transport together are called electron transport system. The energy released in this oxidation process will help in the synthesis of ATP molecules.

Electron carriers: They are closely associated with inner mitochondrial membrane. Among different electron carriers Flavoproteins, cytochromes, Iron-sulphar proteins and ubiquinone are very important. Cytochromes are of different types such as a_1, a_3, b and c. Cytochrome a_1 and a_3 are called terminal oxidases or cytochrome oxidases. They play an important role in terminal oxidation.

Different complexes involve in Electron transport

Complex-I: It is also called 'NADH-dehydrogenase'. It is a complex enzyme, containing Fe-S proteins and FMN. It transfers electrons from NADH to Ubiquinone.

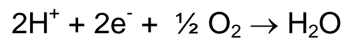
Complex-II: It is called 'succinic dehydrogenase'. It contains Fe-S proteins and FAD. It transfers electrons from succinate to ubiquinone. Ubiquinone is a mobile carrier between Complex I and III and also between II and III.

Complex-III: It is called cytochrome 'C'-reductase. It contains three cytochromes such as b_{560}, b_{565}, C and also contains one Fe-S protein. It transfers electrons from ubiquinol to cytochrome C_1. Cytochrome C is a mobile carrier between Complex III and IV.

Complex-IV: It is called cytochrome 'C'-oxidase. It contains 2 cytochromes such as a_1, a_3 and 2 copper containing proteins. It transfers electrons from cytochrome C to molecular oxygen.

Complex-V: It is called 'ATP synthase'. It contains F_o base piece and F_1 head place. It is involved in ATP synthesis.

The process of Electron transport: When NADH is oxidised to NAD^+, 2 e^- are transported to FMN (prosthetic group of complex I) and $4H^+$ ions are released into intermembrane space. For every two electrons passing through complex I, results in the pumping of $4H^+$ from matrix to the intermembrane space. Ubiquinone transfers these electrons to Complex III. Similarly complex II takes electrons from $FADH_2$ and transfers to ubiquinone but H^+ are not released in this step. $FADH_2$ is oxidised into FAD^+. During the above changes, ubiquinone gets reduced to ubiquinol. Later electrons are transferred to complex III. At this stage, 4 H^+ are released to intermembrane space. A quinone cycle (Q-cycle) operates at complex III. For every $2e^-$ passing through Q-cycle and complex III, $4H^+$ are translocated from the matrix into the intermembrane space. The electrons from complex-III are transferred to the mobile carrier called cytochrome C, thus reducing it. The reduced cytochrome C moves to complex IV and transfers electron to it. The electrons from complex-IV are transferred to molecular oxygen. At this step $2H^+$ ions are transported to intermembrane space and 2 H^+ ions are given to molecular O_2.



$2H^+ + 2e^- + \frac{1}{2} O_2 \rightarrow H_2O$

Thus, during electron transport, H^+ ions are released into intermembrane space at three stages. As electrons pass down in the cytochrome chain proton gradient is established in the mitochondria. It further helps in the formation of ATP molecules. It can be clearly explained by chemiosmotic hypothesis.

5.4 OXIDATIVE PHOSPHORYLATION (CHEMI OSMOTIC HYPOTHESIS)

As previously stated formation of ATP is closely associated with the transfer of electrons from NADH or $FADH_2$ to O_2. The mechanism of mitochondrial ATP synthesis is based on Peter Mitchell's **chemiosmotic hypothesis**. According to this hypothesis the transfer of electrons from NADH or $FADH_2$ to O_2 through electron transport system results in proton (H^+) transfer from the matrix region to intermembrane space through inner membrane. Proton translocation occurs at 3 sites.

(1) from NADH to FMN
(2) Cyt. b to Cyt. c_1
(3) From Cyt a_3 to O_2

It results high proton concentration on outside the inner membrane than the matrix region. The difference in the proton concentration across the inner membrane is called proton gradient. Because of higher proton concentration outside the inner membrane, protons move down the gradient into the matrix across the inner membrane through coupling factor or ATP synthase. The ATP synthase has a stalk and head piece. It is embedded in the membrane with the help of stalk (Fo particle) which opens in the intermembrane space and the head piece (F_1 particles) faces and extends into the matrix. The Fo part is the proton channel through which protons return to the matrix and the F_1 part is the actual site of ATP synthesis. When protons (H^+) move down the gradient, energy is released. This energy helps in combining ADP and Pi, leading to the formation of ATP. The energy from $3H^+$, moving down the potential gradient is enough to form one ATP molecule. Thus one NADH molecule, which adds $10H^+$ to the concentration gradient, accounts for the synthesis of 3 ATP molecules. However the cytosolic NADH of glycolysis and $FADH_2$ of krebs cycle adds only $6H^+$ ions to the concentration gradient and accounts for the synthesis of 2 ATP molecules each.

The chemiosmotic hypothesis is supported by experimental evidences, given by Jogendroff, B.T. Storey *et. al.*

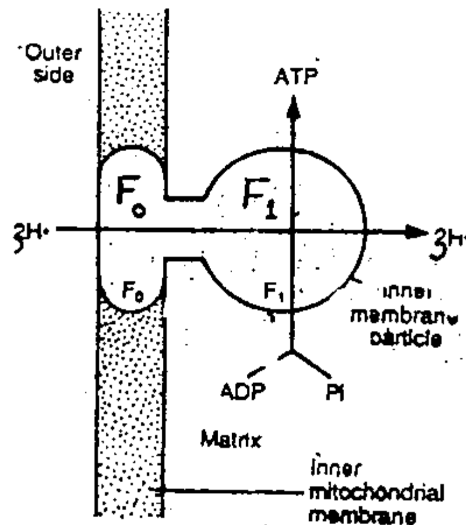


Fig. 5.4 Chemiosmotic generation of ATP

NET GAIN OF ATP DURING AEROBIC RESPIRATION

During Glycolysis = 2 $NADH_2$ molecules are released = 4 ATP (Here one NADH = 2 ATP)
4 ATP molecules are released, among these 2 ATP molecules are consumed
in the 1st and 3rd reactions. So net gain of ATP molecules during Glycolysis
are 6 ATP.

During oxidative decarboxylation = 2 $NADH_2$ ($NADH_2$ = 3 ATP) = 6 ATP

During krebs cycle = $6NADH_2$ + $2FADH_2$ + 2ATP = 24 ATP

Total = 36 ATP

Energy available in Glucose = 673K. cals.

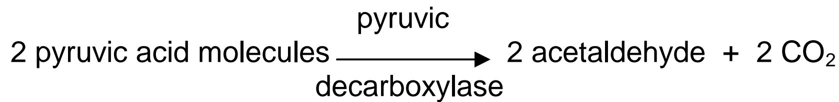
Energy stored in ATP molecules during complete oxidation of glucose 36x12K. cal.

Therefore 66% of the energy is trapped in the form of ATP and the rest of the energy is escaped in the form of heat.

5.6 FERMENTATION

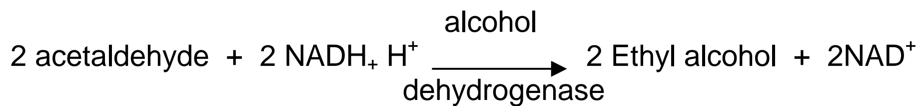
Respiration that occurs in the absence of oxygen, where partial oxidation of food substances occur is called anaerobic respiration. Generally micro organisms and some parts of the higher plants perform it. The mechanism of anaerobic respiration occurs in 2 stages (a) Glycolysis (b) Fermentation. As we know that Glycolysis is a common step occurs in both aerobic and anaerobic respirations, fermentation is only discussed here. Glycolysis yields 6 ATP and 2 pyruvic acid molecules. These pyruvic acids are the substrates for fermentation. It

is a cytosolic process involving the production of ethyl alcohol ($C_2 H_5 OH$) and CO_2 from hexose sugars. It was first reported by 'GAYLUSSAC' and the process, named as fermentation by 'LOUIS PASTEUR'. First and foremost 2 pyruvic acids undergo decarboxylation and form 2 molecules of acetaldehyde in the presence "pyruvic decarboxylase enzyme, accompanied by the release of CO_2.



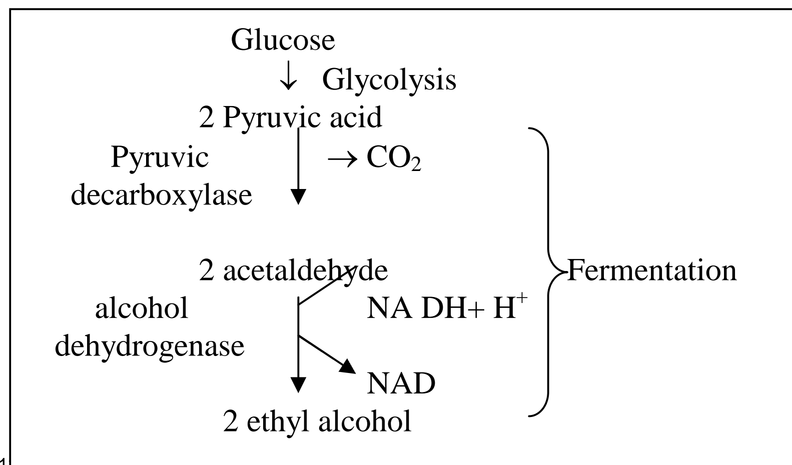
$$\text{2 pyruvic acid molecules} \xrightarrow[\text{decarboxylase}]{\text{pyruvic}} \text{2 acetaldehyde} + 2\,CO_2$$

These molecules of acetaldehyde undergo reduction and form 2 molecules of $C_2 H_5 OH$ (ethyl alcohol) in the presence of alcohol dehydrogenase enzyme. 2 molecules of $NADH_+ H^+$ formed during glycolysis supply the hydrogens essential for reduction.



$$\text{2 acetaldehyde} + 2\,NADH_+ H^+ \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} \text{2 Ethyl alcohol} + 2NAD^+$$

Mechanism of anaerobic respiration



The overall reaction is : $C_6 H_1$

5.7 RQ (RESPIRATORY QUOTIENT)

To measure the rate of respiration, it is essential to estimate the amount of oxygen consumed and the amount of CO_2 released. The ratio between CO_2 liberated and O_2 consumed is known as '**Respiratory Quotient'** (R.Q).

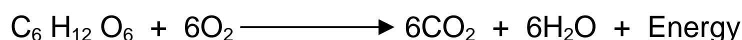
$$R.Q. = \frac{CO_2}{O_2}$$

The value of R.Q is variable from substrate to substrate. So basing upon the value of R.Q., we can know the type of respiratory substrate. However R.Q. is not of major importance in physiological studies of respiration because the respiratory substrate in a plant changes from time to time. Sometimes different kinds of substrates may be oxidised simultaneously.

Different respiratory substrates and R.Q. values

(1)Carbohydrates

When carbohydrates are the substrates of respiration, then the consumed O_2 and released CO_2 would be in same proportions. Thus the R.Q. value becomes one.



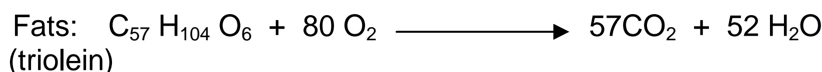
$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

$$\text{R.Q. value} = \frac{\text{Vol. of } CO_2 \text{ released}}{\text{Vol. of } O_2 \text{ consumed}} - \frac{6}{6} = 1$$

(2) Fats and proteins

If fats and proteins are the substrates, consumed O_2 value is more than the released CO_2. So the R.Q. value is less than one.

Eg:



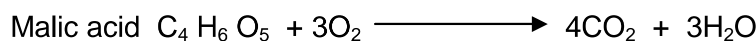
Fats: $C_{57}H_{104}O_6 + 80\,O_2 \longrightarrow 57CO_2 + 52\,H_2O$
(triolein)

$$\frac{57C_2}{80O_2} = 0.71$$

(3) Organic acid

When organic acids are used as substrates then released CO_2 is always higher than the O_2. So the R.Q. values is more than one.

Eg:

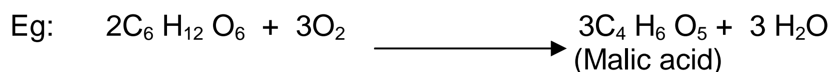


Malic acid $C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$

$$\frac{4CO_2}{3O_2} = 1.33$$

(4) Succulents

During respiration in succulent plants, O_2 is absorbed without the evolution of CO_2. So R.Q. value is always 'O'.



Eg: $2C_6H_{12}O_6 + 3O_2 \longrightarrow 3C_4H_6O_5 + 3\,H_2O$
(Malic acid)

$$\text{R.Q.} = \frac{O}{3} = 0 \text{ (zero)}$$

5.8 SUMMARY

Respiration is a catabolic process, by which food material is oxidised and energy is released in the form of ATP molecules. It is classified into (a) aerobic respiration occurring only in the presence of O_2. (b) anaerobic respiration that occurs in the absence of O_2. Both types start with a common beginning i.e. Glycolysis. It occurs in cytoplasm, where hexose sugars are oxidised into 2 pyruvic acid molecules. From here both aerobic and anaerobic processes deviate. In aerobic respiration, glycolysis is followed by krebs cycle and electron transport system where as in anaerobic, glycolysis is followed by fermentation. Fermentation is a very useful industrial process involved in the preparation of bread, wine and other beverages. Formation of ATP molecules, in the presence of O_2 is called 'oxidative phosphorylation'. It is generally seen in aerobic respiration. It is clearly explained by chemiosmotic theory. The ratio between released CO_2 and consumed O_2 is called R.Q.

5.9 TECHNICAL TERMS

Catabolism, exergonic reaction, R.Q, substrate level phosphorylation, ATP synthase, chemiosmotic hypothesis, NAD, FAD, FMN, oxidative phosphorylation, fermentation, proton gradient.

5.10 Self Assessment Questions

Essay type:

(1) Explain the process with schematic diagrams which is common in both aerobic and anaerobic respirations.
(2) Describe aerobic respiration and write the differences between aerobic and anaerobic respirations.
(3) Write a detailed essay on Krebs cycle.
(4) Explain the importance of electron transport system.
(5) Describe Glycolysis.

Short notes:

(1) Fermentation
(2) Anaerobic respiration
(3) R.Q.
(4) Chemiosmotic Hypothesis
(5) Difference between respiration and photosynthesis

5.11 REFERENCE BOOKS

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Lesson 6

NITROGEN METABOLISM: BIOLOGICAL NITROGEN FIXATION, ASSIMILATION OF NITRATE, BIOSYNTHESIS OF AMINO ACIDS.

6.0 OBJECTIVES

(1) Students will understand the process of biological nitrogen fixation.
(2) Students will understand the process of assimilation of Nitrate in higher plants.
(3) Peoples will learn the bio synthesis of amino acids.

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6.1 INTRODUCTION

6.2 FORMS OF NITROGEN

6.3 NITROGEN FIXATION

6.4 BIOLOGICAL NITROGEN FIXATION

6.5 PROCESS OF NITROGEN FIXATION

6.6 CONVERSTION OF ORGANIC NITROGEN INTO MOLECULAR NITROGEN

6.7 BIO SYNTHESIS OF AMINO ACIDS

6.8 SUMMARY

6.9 TECHNICAL TERMS

6.10 SELF ASSESSMENT QUESTION

6.11 REFERENCE BOOKS

6.1 INTRODUCTION

It is revealed by plant analysis, that 30-40 elements are present in a plant body. Scientists proved that around 70 elements exist in one plant or the other of the plant kingdom. Of these 70 elements 16 elements are treated as essential elements, because they are very important for plant growth and development. Based on the quantitative requirements by the plants, essential elements can be classified into macro elements and micro elements. The elements which are required in large quantities are called macro elements. They are carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Potassium (K), and Sulphur

(S). The elements which are required in small quantities are called micro elements. They are Iron (Fe), Manganese (Mn), Boron (B), Zinc (Zn), Copper (Cu), Molybdenum (Mo) and Chlorine (Cl).

Nitrogen is a very important macro element, after C, H and O. It is also called as 'critical element'. It is an important component of nucleic acids, amino acids, proteins, enzymes, Co-enzymes, vitamins, alkaloids, chlorophylls and cytochromes. About 78% of nitrogen is present in the atmosphere in the form of gas. Though N_2 is present in such higher concentration still it is not available for plant growth and development, because of stable banding between two nitrogen atoms ($N \equiv N$). Plants do not have the enzyme that will break this bond. So N_2 must be converted into simple forms to be consumed by plants.

6.1 FORMS OF NITROGEN

Nitrogen is available to plants in 4 different forms, they are

(1) Molecular Nitrogen

(2) Nitrate Nitrogen

(3) Ammonium Nitrogen

(4) Organic Nitrogen

(1) Molecular Nitrogen

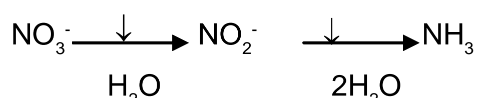
It is the most abundant form of Nitrogen. It cannot be used by higher plants, but some lower organisms can use it. For e.g., *Azatobacter, Clostridium, Chlorobium, Rhodospirillum*, Cyano bacteria. They reduce mol. N_2 into Ammonia with the help of Nitrogenase enzyme. They utilise this N_2 form either directly or indirectly with the help of plants.

(2) Nitrate Nitrogen

This is the assimilative form of N_2 for the plants, present in the soil. Plants absorb these NO_3 ions through roots, but NO_3 ions are not directly used by plants. It must be reduced to ammonia before it is incorporated into nitrogenous compounds of the plants. The above conversion i.e. from nitrate to ammonia needs energy in the form of energy, which can be derived by the oxidation of carbohydrates (Respiration). The reduction of nitrate occurs mainly in leaves and stem during day time. The reducing power for the reduction of nitrate is available from photosynthesis.

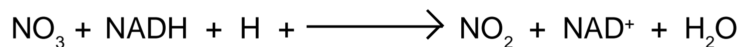
Assimilation of Nitrate

The reduction of Nitrates to ammonia is carried in two steps. In the first step nitrate (NO_3^-) is reduced to (NO_2^-). One atom of oxygen is removed as water. In the second step nitrite (NO_2^-) is reduced to ammonia (NH_3) two atoms of oxygen are removed as water.



The first reaction ($NO_3^- \rightarrow NO_2^-$) needs an enzyme, named '**nitrate reductase'**, present in the cytoplasm and two electrons. The electrons can be supplied by either NADH or NADPH. Nitrate reductase is a complex enzyme, having FAD (Flavin adenine dinucleotide) as a prosthetic group and molybdenum as a co-factor. Both will serve as electron carriers. Molybdenum has an

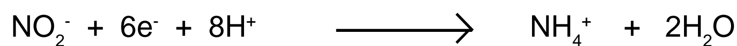
important role in Nitrate assimilation process, that is why in the deficiency of Mo, plants show symptoms of nitrogen deficiency first and later the symptoms of Mo. The enzyme was isolated from soyabean leaves and *Neurospora* by Evans (1953) and Nason (1954).



$$NO_3 + NADH + H + \xrightarrow{\text{Nitrate reductase}} NO_2 + NAD^+ + H_2O$$

In the second reaction nitrite is reduced to ammonia (NH_3) in the presence of another enzyme, named '**nitrite reductase'**. This reaction is carried in chloroplastids or pro-plastids.

This reaction needs 6 electrons and 8 protons. Ferredoxin serves as an electron acceptor. The reduced Ferredoxin donates $6e^-$ to nitrite and reduces it to ammonia. Ferredoxin is a non-heme protein with low molecular weight.



$$NO_2^- + 6e^- + 8H^+ \xrightarrow{\text{Nitrite reductase}} NH_4^+ + 2H_2O$$

In contrast to leaves, ferredoxin does not serve as electron carrier in non-green plant parts. Instead of it some unidentified substance serves as electron carrier in non green parts such as roots. The reducing power for this reaction will be supplied by respiration in lieu of photo synthesis. In green parts, the source of hydrogen is $NADPH_2$ where as in non-green parts $NADH_2$ is the source of hydrogen.

(3) Ammonium Nitrogen

It is the most useful form of nitrogen for plants growth and development. Wet soils are the richest sources of ammonium, because ammonium ions are not oxidised into NO_3 in these soils. The ammonium of nitrogen can be directly assimilated by plants. In low concentration NH_4 is a good source of nitrogen whereas in high concentration, it is toxic.

(4) Organic Nitrogen

Dead and decaying plants or animals are the sources for this type of N_2, mainly the organic nitrogen is available in the form of proteins. These proteins molecules are degraded into amino acids, nitrates and NH_3 by micro organisms. So that they can be easily assimilated by plants. In plants foliae spray of urea is effective method of overcoming the short age of nitrogen.

6.2 NITROGEN FIXATION

Conversion of complex molecular or atmospheric nitrogen into simple and useful forms such as nitrates and ammonia is called N_2- Fixation. It is of 3 types.

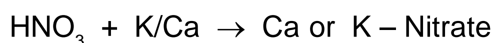
(1) Physical N_2 Fixation

During rains, electric discharges from lighting combine the nitrogen and oxygen and form oxides of nitrogen. It is brought down to soil by rain water in the form of nitric acid. The alkali radicals present in the soil react with nitric acid and from nitrites and nitrates. Noggle and Fritz (1983) contributed research work on physical N_2 fixation.

$$N_2 + O_2 \rightarrow 2NO$$

$$2NO + O_2 \rightarrow 2NO_2$$

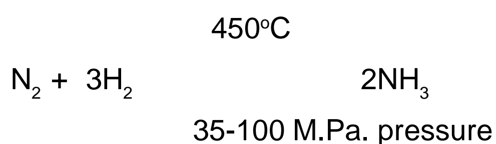
$2NO_2$ + Rain water $\rightarrow HNO_2 + HNO_3$



HNO_3 + K/Ca $\rightarrow$ Ca or K – Nitrate

(2) Chemical N_2 Fixation

The abiological N_2 fixation through an industrial process is called chemical N_2 fixation or *Haber – Bosch* process'.



$$N_2 + 3H_2 \xrightarrow[\text{35-100 M.Pa. pressure}]{450^oC} 2NH_3$$

6.3 BIOLOGICAL NITROGEN FIXATION

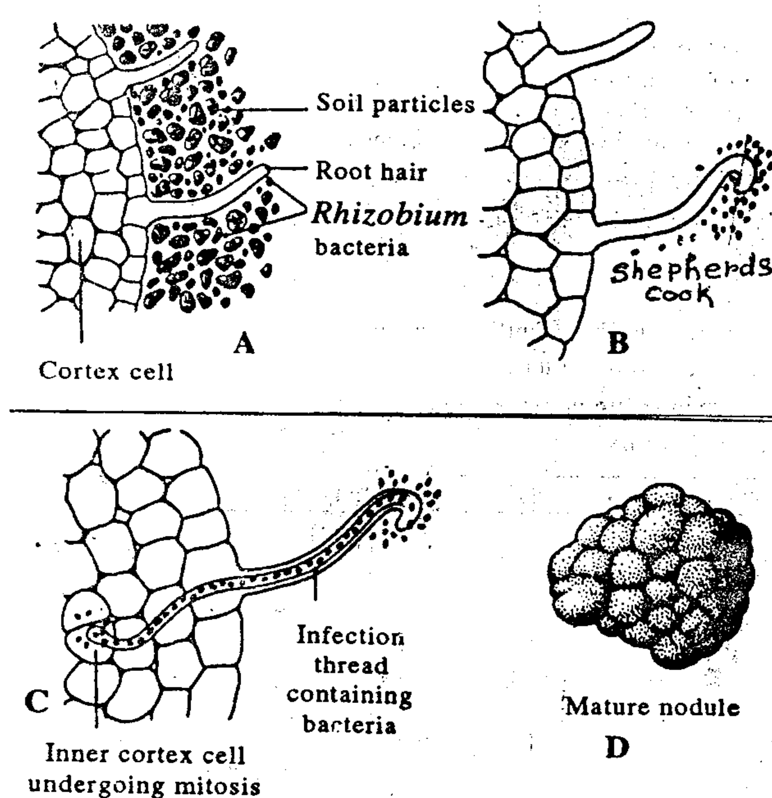


Fig. 6.1 Developed of root nodules in soyabean

A-B: Infection of root hair by Rhizobium bacteria

C: Formation of infection thread; **D**: Mature nodule

Nitrogen fixation, through microorganisms is called as biological nitrogen fixation. Biological nitrogen fixation adds 200 million tonnes of nitrogen to the soil annually. It is of 2 types (a) symbiotic N_2 fixation, where the organisms indirectly fix nitrogen by being symbiotically associated with higher plants. (b) Non-symbiotic N_2 fixation. Microorganisms independently or directly fix nitrogen, e.g. certain bacteria, cyanobacteria.

Bacteria: (i) Aerobic bacteria: e.g. Azotobacter

(ii) Anaerobic bacteria: e.g. Clostridium, Azospirillum

(iii) Photosynthetic bacteria: e.g. Rhodospirillum, Chlorobium

Cyanobacteria: (i) Unicellular: e.g. Gleotheca

(ii) Filamentous: Nostoc, Annabaena, Scytonema, Oscillatoria

In the above microorganisms, nitrogenase is the key enzyme in nitrogen fixation. Nitrogen fixation by Azotobacter inoculated soils is about 10-15 kg/hectare. Azotobactrin is the trade name for the biofertilizer and is used for increasing the crop yield in East European countries. The amount of nitrogen fixed by Cyanobacteria is 35-195 kg/ha/year. Azospirillum in association with rhizosphere of grasses fixes 40-80 kg/hectare.

Symbiotic Nitrogen Fixation

It was first discovered by Hellreigal and Wilfarth. There are certain microorganisms, which are always closely associated with higher plants. Higher plants provide carbon source and shelter to the microorganism where as microorganism fix atmospheric nitrogen and provide simple form of nitrogen to the plant.

Some of the examples of symbiotic N_{2}- fixation

Symbiotic Nitrogen fixing systems occur in several plants. Nearly 10,000 species of higher plants are estimated to be served as hosts for micro organisms. Some lower plants such as fungi and lichens may also serve as hosts.

Host plant	Micro organism associated
Leguminaceae plants	*Rhizobium sps.*
Casuarina & Alnus	*Actinomycetes fungi*
Cycas and Azolla	*Anabaena (BGA)*
Salvinia	*Nostoc*
Anthoceros	*Nostoc*
Grasses	*Azospirillum*

Of all these, Rhizobium species present in the nodules of leguminaceae plants is of great importance. It was estimated that symbiotic rhizobium strains can fix 100-200 times more N_2 than that of the free living forms. Different species of Rhizobium infect the roots of legumes.

Sl. No.	Group	Rhizobium species	Host plant
(1)	Alpha – Alpha	*R. meliloti*	melilotus, medicago, Trigonella
(2)	Lupin group	*R. lupini*	lupinous
(3)	Soyabean group	*R. japaniens*	soyabeans
(4)	Bean group	*R. phaseolus*	Beans
(5)	Pea group	*R. leguminacearum*	Peas
(6)	Clover group	*R. trifoli*	Red, White cloves
(7)	Cowpea group	*Rhizobium* species	Vigna, Cajanus, Cicer, Crotalaria, Arachis

Thus different species of Rhizobium infect the roots of different legume plants, but not the same plant. It is well known fact from the times immemorial cultivation of leguminous crops increases soil fertility. The reason for this was first discovered by Beijerinck, who identified bacteria in the root nodules of leguminaceae plants. Neither the legume nor the rhizobium can fix atmospheric nitrogen independently. When they are associated then only they can fix nitrogen. Rhizobium is an acrobic, gram-ve, saprophytic, rod shaped bacteria and soil habitant.

Firstly, roots of legume plant secretes sugars, amino acids and flavinoids into the soil. Chemically attracted bacteria (rhizobium) move to the root. The bacteria multiply, increase in number and infect the root hair causing it to curl by producing cytokinin and polymixin–B (curling factors). This is known as '**shepherds hook**'. The host recognizes compatible bacteria by specific plant proteins called '**Lectins**'. The rhizobium bacteria secrete cell wall digesting enzymes such as '**cellulase**', pectinase, polygalactorunase and break the cell wall. After breaking the cell wall they reach plasma membrane. The growth of the root hair ceases and the membrane invaginates, due to this a tubular intrusion is developed inside the cell, called infection thread. It contains the invading bacteria.

The infection thread makes the bacteria to reach the cortical cells. Mean while the cortical cells secret growth harmones, and undergo cell division, leading to the formation of young nodule called nodulation or 'Rhizocoenosis'. By the effect of chromosomal number changes host cells become tetraploid. Bacteria loose their shapes and are called bacteriods. Bacteriods are surrounded by a peribacterial membrane. When all these changes are over both the host and the bacteria start nitrogen metabolism.

As a result of symbiotic association between the two organisms a special pink colour pigment is developed in the root nodule, called Leghaemoglobin. It is present in the peribacterial membrane, N_2 fixation is only expected in the pink nodules. Leg Hb contains iron just like blood Hb, but deviating from the blood Hb in having amino acids like globulin and isoleucine. It was proposed that globin part of this pigment is produced by the bacteria and the heme part is by the host. Both parts probably get united in the root cells. The main role of leg Hb is that protecting nitrogenase, an enzyme prerequisite for carrying N_2 fixation. Actually the enzyme is oxygen sensitive but the bacteria are aerobic. For providing O_2 to the bacteria and obstructing the passage of O_2 upto the enzyme, leg Hb behaves like a curtain, hence the pigment is called biological curtain or O_2 scavenger. Nitrogenase is responsible for N_2 fixation. It is regulated by Nif genes. Nitrogenase in the bacteriod then becomes functional. Thus a sort of metabolic compartmentation is seen in the bacteria.

Nitrogenase contains 2 parts one is Mo-Fe protein part and the another one is Fe- protein part. Mo-Fe protein part is made up of two large α polypeptide chains and two short β chains, bounded by <u>Mo</u> and enclosed by Fe^+, <u>S</u> ions. Fe- protein part is made up of two more or less identical polypeptide chains, held together by <u>4Fe⁺</u> and <u>4S</u> ions. Both sub units are held together by Na^+ ions. By the above information we can clearly understand the role of 3 mineral ions in N_2 metabolism. (1) Iron is an important component of leg haemoglobin (2) Cobalt is an essential part of Vitamin B_{12} which is involved in the formation of leg Hb. (3) Molybdenum is an electron acceptor or donor in the reduction of N_2 into NH_3.

6.4 PROCESS OF N_2 FIXATION

The reduction of N_2 to ammonia requires a reduced substrate, capable of donating hydrogen i.e. $NADH_2$. An electron carrier that can transfer hydrogen from the reduced substrata to N_2, i.e. Ferredoxin and ATP's, which can be given by respiration process. The source of electrons and the

nature of electron carrier to transfer electrons to N_2 and the energy source (ATP) may vary in different nitrogen fixing systems but the basic mechanism of N_2 fixation is same in all of them.

(1) reduced Ferredoxin + Nitrogenase → Oxidised Ferredoxin + reduced Fe^- protein

(2) reduced Fe^- protein + ATP → Oxidised Fe^- protein + reduced Fe^- mo protein + ADP + Pi

(3) reduced Fe^- mo protein + N_2 → $2NH_3$ + oxidised Fe^- mo protein

* The overall reaction in the conversion of N_2 → $2NH_3$ is as follows.

$$N_2 + 8e^- + 8H^+ + 16\,ATP \rightarrow 2NH_3 + H_2 + 16\,ADP + 16\,Pi$$

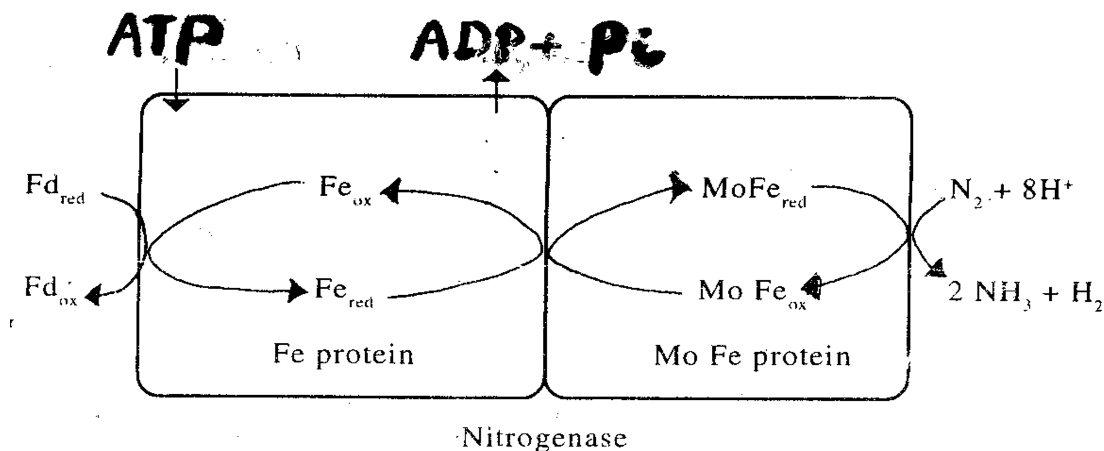


Fig. 8.1 Schematic diagram showing N_2 fixation in bacteria. Electron donor is Ferredoxin.

The reduced ammonia is incorporated into the amino acids and further protein molecules of the plant body this is called '*assimilation*'. Thus molecular N_2 is assimilated and incorporated in the form of organic N_2 by plants with the help of micro organisms.

6.5 CONVERSION OF ORGANIC N_2 INTO MOLECULAR N_2

The continuous use of molecular N_2 by the plants may cause deficiency of N_2 in the environment but it does not happen because after death of these plants and animals the molecular N_2 level in the atmosphere will be balanced by another micro organisms. This large step is sub divided into three small steps.

They are (1) ammonification
(2) nitrification
(3) denitrification

(1) Ammonification: The conversion of organic N_2 present in the body proteins into ammonia is called as ammonification soon after the death of plants and animals, this is carried by ammonifying bacteria, some fungi and actinomycetes. This is the first step of degration of organic matter.

(2) Nitrification: Conversion of above released ammonia into nitrates is called as nitrification. This is carried by nitrifying bacteria such as *Nitrosomonas* and *Nitrosobacter*. Firstly ammonia is converted into Nitrites by 'Nitrosomonas' and later it is changed to Nitrates by Nitrosobacter. It occurs in aerobic conditions.

(3) Denitrification: Conversion of Nitrates (NO_3**)** into N_2 is called denitrification or DNR (Dissimalatory nitrate reduction), sometimes nitrates may be again reduced to ammonia to be used by living plants called ANR (Assimalatory Nitrate reduction). It is carried by denitrifying bacteria called '*pseudomonas denitrificans*' and *Thiobacillus denitrificans*'. It occurs in anaerobic conditions.

All the 4 steps such as N_2 fixation, ammonification, nitrification and denitrification are treated as

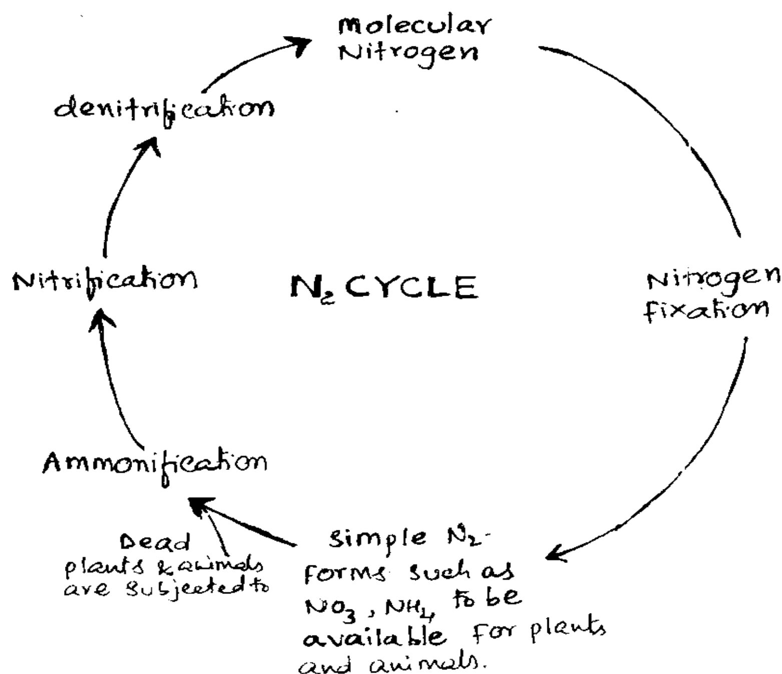


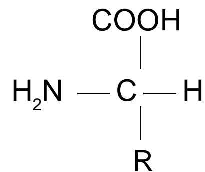
Fig. 6.2 Diagram of Nitrogen cycle

cycle, called N_2 cycle. The cyclic conversion of N_2 between atmosphere, soil and plants is called N_2 cycle.

6.6 BIOSYNTHESIS OF AMINO ACIDS

Amino acids are organic acids, containing both amino group (positive charge) and carboxyl group (Negative charge). Several amino acids link together by peptide bonds and form long polypeptide chain. So amino acids are basic units of protein molecules and organisms.

The primary structure of amino acid is as follows.



The primary amino acids differ in the R group. Amino acids contain carbon (C), Hydrogen (H), Oxygen (O) and Nitrogen (N). Some amino acids also contain sulphar (S). Most of the amino acids contain single amino group and single carboxyl group, they are called neutral amino acids. Some amino acids contain 2 amino groups, called basic amino acids e.g.: Omithine. Some are with two carbaryl groups, called 'acidic type' e.g., aspartic acid. Structural proteins can be made by 20 amino acids, called structural amino acids. The other amino acids

are not structural components, called non-structural amino acids, their function is not known e.g. homoserine.

Properties of amino acids

(1) They are colourless, crystalised compounds.
(2) They are dipolar ions in dilute solvents, they can act as proton donars and acceptors.
(3) On heating they give colouration, called *Nin hydrin reaction*'

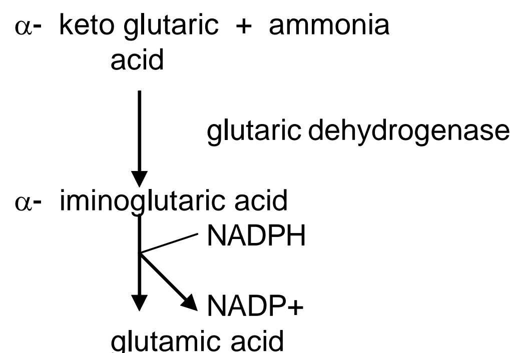
Plants synthesise amino acids by 2 methods.

I. Reductive amination.
II. Trans amination.

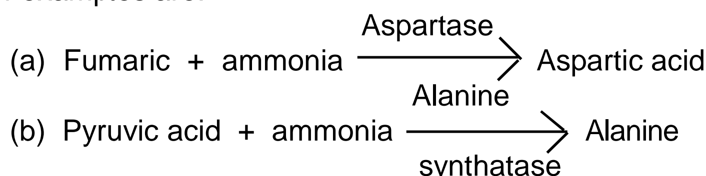
I. Reductive amination

It is a reaction, where the intermediatory organic acids of krebs cycle react with ammonia and form amino acids, e.g., reductive amination of glutamic acid.

Glutamic acid is an amino acid, produced by the reaction between α- ketoglutaric acid and ammonia. The production of glutamic acid occurs in 2 steps. In the first step α- imino glutaric acid is produced, this reaction is catalysed by "glutamic dehydrogenase". In the second step this α-imino glutaric acid is reduced to "glutamic acid". The electron donar for this step is NADPH.

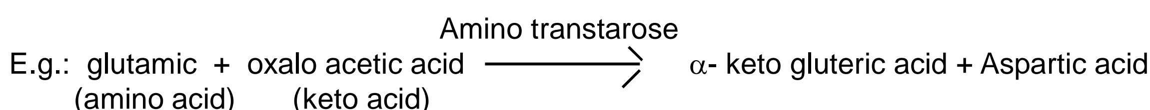


another examples are:



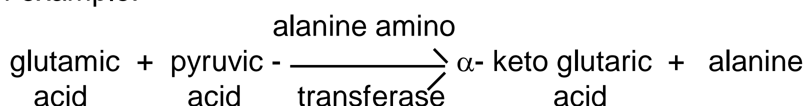
II. Trans amination

Transfer of amino group of an amino acid to keto acid gives a new amino acid. It is catalysed by amino transfarase and vitamin B_6 (Pyridoxol phosphate).



Here pyridoxol phosphate (Co-enzyme) is firmly bound to enzyme amino transfarase, by getting amino group from an amino acid it becomes pyradox amino phosphate. Later it transfers amino group to receptor keto acid and changes it to another amino acid. It is estimated nearly 17 amino acids are synthesised from glutamic acid by trans amination process.

another example:



6.7 SUMMARY

Nitrogen is an important element after **C, H** and **O**. Among different N_2 forms ammonia is the most useful form for plant growth and development. Conversion of complex nitrogen into simple forms such as NO_3, NH_3 is called N_2 fixation. Nitrogen can be fixed physically, chemically and biologically. The assimilated N_2 or organic nitrogen is re converted into molecular N_2 through ammonification, nitrification and denitrification. The cyclic conversion of N_2 among atmosphere, soil and plant is called '**Nitrogen cycle'**. Amino acids are building blocks of proteins, which are structural units of an organism. They are synthesised by reductive amination and transamination.

6.8 TECHNICAL TERMS

Rhizocoenosis, ammonification, nitrification, denitrification, Leg Haemoglobin, Nitrogenase,

Haber–Bosch process, curling factors, Leetins.

6.9 SELF ASSESSMENT QUESTIONS

Essay type

(1) Write a detailed essay on Biological Nitrogen Fixation.

(2) What are amino acids, write about the bio-synthesis of amino acids.

Short notes

(1) Nodulation

(2) Process of N_2 Fixation

(3) Nitrogenase

(4) Physical N_2 Fixation

(5) N_2 Cycle

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2. Subhash Chandra Datla, **Plant Physiology**, Wiley Eastern Limited, New Delhi, 1994.

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Lesson 7

PLANT GROWTH REGULATORS: DISCOVERY, BIO SYNTHESIS, PHYSIOLOGICAL EFFECTS OF AUXINS, GIBBERELLINS, CYTOKININS, ABSCISSIC ACID AND ETHYLENE

7.0 OBJECTIVES

(1) Students will learn about different types of phytohormones.

(2) They will understand the co-ordination between different hormones in plant growth and development.

(3) Students discriminate plant hormones and animal hormones.

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7.2.2 Types of auxins

7.2.3 Natural occurrence of auxins

7.2.4 Bio-synthesis of auxins

7.2.5 Auxins transport

7.2.6 Physiological effects of auxins

7.3 GIBBERELLINS

7.3.1 Bio-synthesis of GA

7.3.2 Physiological effects of GA

7.4 CYTOKININS

7.4.1 Bio-synthesis of Cytokinins

7.4.2 Physiological effects of Cytokinins

7.5 ETHYLENE

7.5.1 Bio-synthesis of Ethylene

7.5.2 Physiological effects of Ethylene

7.6 ABSCISSIC ACID

7.6.1 Bio-synthesis of ABA

7.6.2 Physiological functions of ABA

7.7 Summary

7.8 Technical Terms

7.9 Model Questions

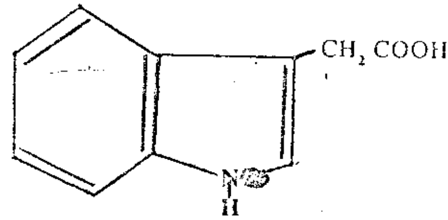
7.10 Reference Books

7.1 INTRODUCTION

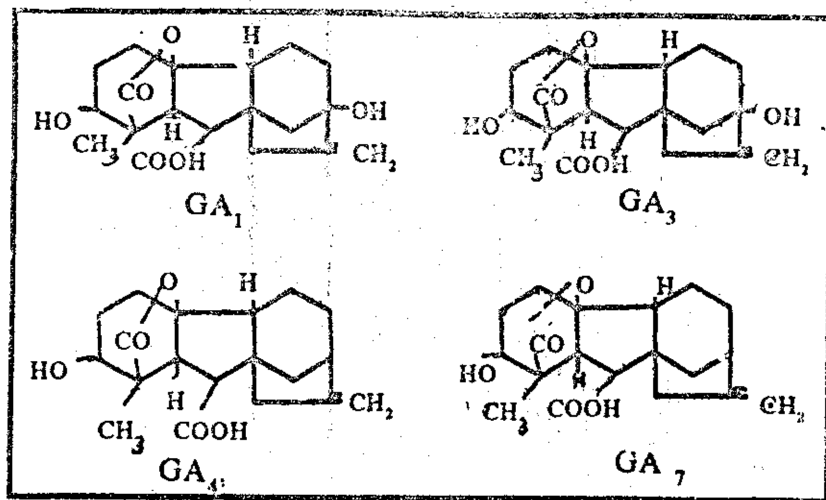
The growth and development of plant body is controlled by two internal factors. They are (1) nutrients, (2) Hormones. Nutrient factors provide crude substances such as mineral ions and primary metabolites – carbohydrates, lipids, proteins. These crude energy giving substances can be systemically utilised by plants with the help of some chemical messengers, other wise called "Hormones". A hormone is defined as a substance which is produced in one part of a living organism and is transferred to another part, to influence some physiological activity.

Our knowledge of hormones in animals and man preceded that of plant hormones. Infact, the study of animal hormones called endocrinology has become almost a science in itself. Extensive enquiries of the plant hormones dates from about 1930, although their existence was suspected much earlier. Like animal hormones plant hormones cannot be produced by special organs (glands) hence, plant hormones are named 'phytohormones', in order to separate them from animal hormones. The term phytohormones was coined by J.S. Huxley. Now-a-days the term plant growth regulators is being used widely.

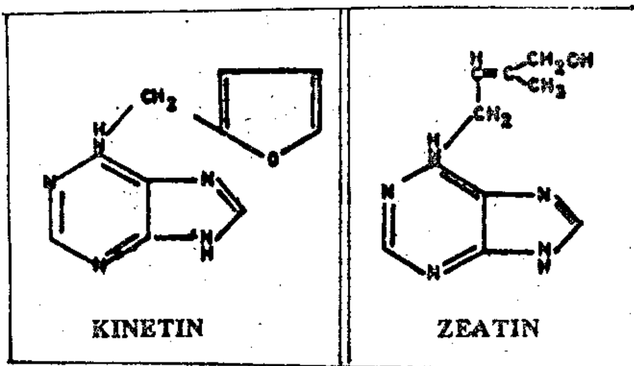
Infact, the classical work of Charles Darwin and Francis Darwin on plant curvatures may be regarded as the starting point. The publication of Darwin's last book, "The power of movements in plant" in 1880 opened the gates that led to the flood of experimentation and information on growth substances. The plant growth regulators are classified into 5 types. They are: 1) Auxins 2) Gibberellins 3) Cytokinins 4) Ethylene and 5) Abscisic acid.



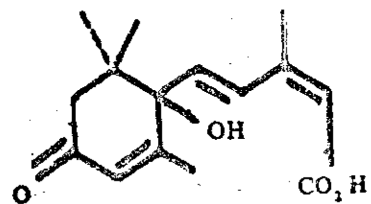
Structure of Indole 3- acetic acid



structure of Gibberellins



Structure of cytokinins



structure of Abscisic acid

Fig. 7.1 Structures of different phytoharmones

7.2 AUXINS

History of Auxins

1. **Julivs Von Sachs** first proposed that leaves produce an organ forming substance, which translocates downwards.

2. **Charles Darwin (1880),** who conducted experiments on *Phalaris canariensis* (canary grass) identified curvature in coleoptile tip. The tip bend towards light. But when the tip is covered, it will not receive the light sensation.

3. **Boyson-Jensen (1911)** found that a substance present in the tip takes the responsibility of bending the tip towards light coming direction, but he could not isolate it.

4. **F.W. Went (1926)** conducted experiments on 'Avena plant' for bioassaying the substance, it is known as "Avena curvature test" and identified the active substance as Indole Acetic Acid (IAA).

5. **Kogl and Haagen Smith (1931)** isolated similar substance in the form of crystals from human urine and named it as auxin-A (Auxentriolic acid). The activity of this substance was determined by "**Avena curvature test**".

6. **Kogl, Erxleben and Heagen Smith (1934)** isolated similar substance from corn germ oil and named it as auxin-B. In the same year they isolated heteroauxin from human urine, it is now known as Indole-3 acetic acid (IAA).

7. **K.V. Thimann *et al.,* 1934** discovered the presence of IAA in several plants. It was first isolated from Rhizopus species.

7.2.1 Avena Curvature Test: (Bio assay of Auxins)

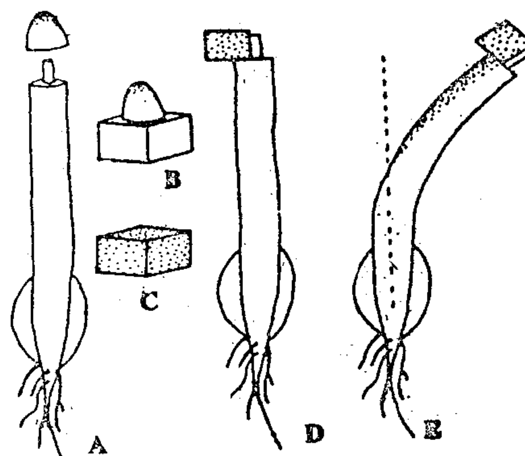


Fig. 7.2 Avena curvature test

Bio-assay means the testing of substance for its activity in causing a growth response of some sort in a living part or plant part. Frequently, the test is quantitative, i.e. a measure of concentration required to produce the effect. The avena curvature test was performed by Went under controlled conditions on oat plant in (1926).

Firstly F.W. Went cut off the extreme tip of the coleoptile and the chemical substance present in it was transferred into an agar block. Later agar block was cut into pieces and placed over the detipped coleoptiles. One block was kept evenly and another one was kept towards a side. After sometime growth was observed in two coleoptiles. First one showed straight growth. This is because the equal distribution of the chemical substance. But in the second case instead of straight growth curved growth was seen this is because unequal distribution of the chemical substance. Here agar block was kept unevenly, so the chemical substance entered into the coleoptile only on that side. That side only showed growth and bending. Basing upon the curvature we can estimate the concentration of chemical substance. There is a linear relationship between concentrations and the degree of curvatures. This method is a bio assay for auxin is called "**Avena curvature test**".

7.2.2 Types of Auxins

The discovery of auxin and the identity of its chemical structure led rapidly to the synthesis of other auxin related substances. Some synthetic compounds are more active than the natural auxins and induce many varied responses. So basing upon the source of availability auxins are of two types.

(1) Naturally occurring auxins, called natural auxins.
(2) Synthetically prepared ones, called synthetic auxins.

Synthetic auxins are again of 2 types (1) compounds similar to IAA and with similar physiological activity. Eg: Indole pyruvic acid
Indole propionic acid
Indole butyric acid

2,4-D and 2,4,5 – T are also synthetic auxins.

(2) Compounds not chemically similar to IAA but similar in activity are α-napthalene acetic acid, β-napthalene acetic acid, phenylacetic acid, napthoxyacetic acid and phenoxy acetic acid. The basic molecular requirements for auxin activity include an aromatic ring, an acidic side chain and some spatial relationship between the two (Koepfli *et al.*, 1938).

7.2.3 Natural Occurrence of Auxins

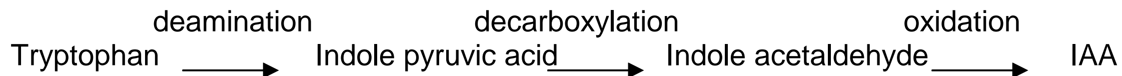
Auxins are present in the highest concentrations in buds, growing tips of the stems, roots and leaves. But however the concentration of auxin in root tip is always lower than the stem tip. The concentration decreases from top to the base. The auxin level varies with time and age of the plant body. Young parts are rich sources of auxins. Dormant plant parts show low auxin levels.

In plants, auxins occur in 2 forms, (1) Free auxins – which can be easily extracted and (2) bound auxins – which are difficult to extract. The initiation and regulation of growth is controlled by the proportions of these two forms.

7.2.4 Biosynthesis of auxins

Tryptophan, an amino acid is considered as the precursor of auxin synthesis. IAA can be formed from tryptophane by two methods.

(1) Tryptophan is first deaminated into Indole pyruvic acid. This indole pyruvic acid is decarboxylated to indole acetaldehyde. Finally it is oxidised to IAA.



(2) In second pathway, tryptophan is decarboxylated into tryptamine. Later it is deaminated indole acetaldehyde, On oxidation it is changed to IAA.

7.2.5 Auxin transport

Auxin moves from top to bottom, the movements are always in single direction. But in roots it moves from bottom to top. Auxin moves very slowly i.e., 1cm per one hour. The movement requires energy. Anaerobic conditions inhibit the transport. The mechanism of Auxin transport is not well understood. Phenomenon such as diffusion, surface tension, electrophoresis and protoplasmic streaming may have some role in the auxin transport.

Effective auxin concentration

Auxins promote growth only in low concentrations, $10^{-}M^{3}$ concentration of auxin inhibits the growth (higher concentration). Generally stem growth needs $10^{-5}M$ concentration and root growth requires $10^{-9}M$ concentration.

7.2.6 PHYSIOLOGICAL EFFECTS OF AUXINS

(1) Cell division and Cell enlargement

Primary growth of stem is accompanied by cell enlargement, it is the most important function of auxin. This particular activity is done by following changes. (a) Increase in plasticity of cell wall by breaking bonds between cellulose and semi cellulose contents. (b) Increase in osmotic content of the cell and cell permeability to water (c) reduction of wall pressure. By the above changes the cell enlarges to its maximum extent and gets ready to division. The divisional activity of auxin is seen in cambium during secondary growth of dicot stem. So that secondary tissues such as secondary and secondary phloem are developed. Jacobs, 1984 observed divisionable and differentiable activity of callus when it was supplied with auxins.

(2) Root formation

In any case if the stem is separated from root system and placed in moist soil. It develops roots with in no time. This is because of transmission of IAA from stem apex to bottom and initiates root formation. This idea is really a boon to horticulturists for propagating plants through stem cuttings by spraying synthetic auxins.

(3) Apical dominance

The domination of apical buds on lateral buds is called 'Apical dominance' only in the absence of apical buds, lateral buds will grow. Besides the apical bud, the young developing leaves are also a source of auxin which inhibits the growth of lateral buds. Physiologists are unable to understand why the apical bud grows vigorously in the presence of high concentration of auxin and why not the lateral buds even in the presence of lower

concentrations. According to Gregory and Veale, the influence of auxin is related to the nutritional status of nitrogen. In some plants the adequate supply of nitrogen could control the domination of apical buds over lateral buds. In the deficient conditions of nitrogen the apical dominance nature is restored by apical buds. This property is very helpful to horticulturists to store potato tubers for nearly 5 years. Similarly bushy appearance can be achieved in ornamentals.

(4) Tropisms

Auxins are involved in both phototropism and geotropism. When the coleoptile is exposed to light on one side, the dark side receives more auxin. This auxin causes cell elongation on the shaded side than on the light exposed side. So, the young stem bends towards light coming direction. But in root, the high concentration of auxin on the dark side inhibits growth and so it bends away from light. This is called phototropism.

Similarly when a plant is placed horizontally, the stem bends upwards and the root downwards. This is due to higher concentration of auxin on the lower side of both stem and root. In a stem, it promotes growth on the lower side but in a root it inhibits growth on the lower side. The explanation of tropical movements in plants, mediated by Auxins is known as "**cholodny-went theory**".

(5) Flowering

Auxins generally inhibit flowering, but artificial auxins like NAA, 2,4-D promote flowering in pine apple. But in cabbage, by delaying flowering auxins indirectly help in increasing the bud size.

(6) Sex determination

Auxins also play an important role in sex determination For eg: Application of NAA on cucurbitaceae plants, increases the production of female flowers.

(7) Parthenocarpy

Parthenocarpy means setting and grwoth of fruit without a developing embryo. Soon after pollination and fertilization, ovules are modified into seeds and ovary is modified into fruit. The stimulus for ovary transformation into fruit can be given by pollination. Especially pollen grains contain tryptophan, which is changed to IAA in embryosac, with the help of IAA seeds and fruits are developed. Gustafson (1939) discovered that auxins could induce parthexocarpy. The seedless varieties of grapes show higher auxin content than seeded variety. When auxins are sprayed increase in fruit set is obtained in grapes, tomato, citrus and pear. NAA and IBA are used to induce parthenocarpy in some plants.

(8) Prevention of Abscission layer

Before falling a plant part, an abscission layer is formed at the base of particular organ. It is a great curse to the horticulturists, even before the maturation the young parts including fruits fall down and cause greater losses. In general auxins present in the leaves will come to the abscission layer and inhibit abscission. So that the plant part remains attached to the plant. Keeping this idea in the view immature fruit falling in the apple trees can be controlled by spraying NAA.

(9) Weed killers

Weed control is a serious problem to agriculturists because they compete with crop plants for nutrients and they also serve as collateral hosts for many harmful pathogens. To get maximum benefit out of the crop, weeds should be controlled. It can be controlled by spraying artificial auxins like 2,4-D (2,4, dichloro phenoxy acetic acid and 2,4,5-T (2,4,5 Trichloro phenoxy acetic acid).

(10) Callus formation

It IAA is applied to the tip of petiole after removing the leaf lamina, a callus tissue is developed as a yellow swelling. This is developed by repeated divisions of parenclyma cells. In tissue cultures, IAA is added for developing callus tissue and roots initiation. During grafting, it was noticed that application of IAA results in the quick formation of callus at the grafted portion.

Along with this activities auxins also mediate activation of enzymes, formation of root nodules in leguminaceae plants and elongation of veins.

7.3 GIBBERELLINS

History: It is popularly known as flowering hormone.

(1) They were first discovered by Kurosawa, a Japan scientist while doing experiments on bakanae disease or foolish seedling disease of rice. The infected plants grew taller. The disease is caused by Gibberella fujikoroi.

(2) Yabuta and Hayashi (1930) isolated the active compound and named it as gibberellin. But this work was neglected due to second world war.

(3) West and Phinney (1956) reported that Gibberellins also exist in higher plants. Gibberellin compounds with similar activity have been found in gymnosperms, ferns, angiosperms as well as algae.

So far 110 different Gibberellins have been isolated. Majority of them are from flowering plants. All of them have the same structure called gibbane ring structure. Gibberellins are the esters of gibberellic acids. Different kinds of GA are referred by their subscript numbers like GA_1, GA_2, GA_3 etc. All types are not found in the same plants. For eg: GA_1 is mostly found in Angiosperms and GA_2 is found in Fungi. GA_3 was one of the first to be isolated and characterised. Gibberellins are chemically diterperoids and contain 20 carbons. They are synthesised from “Acetyl-CoA”.

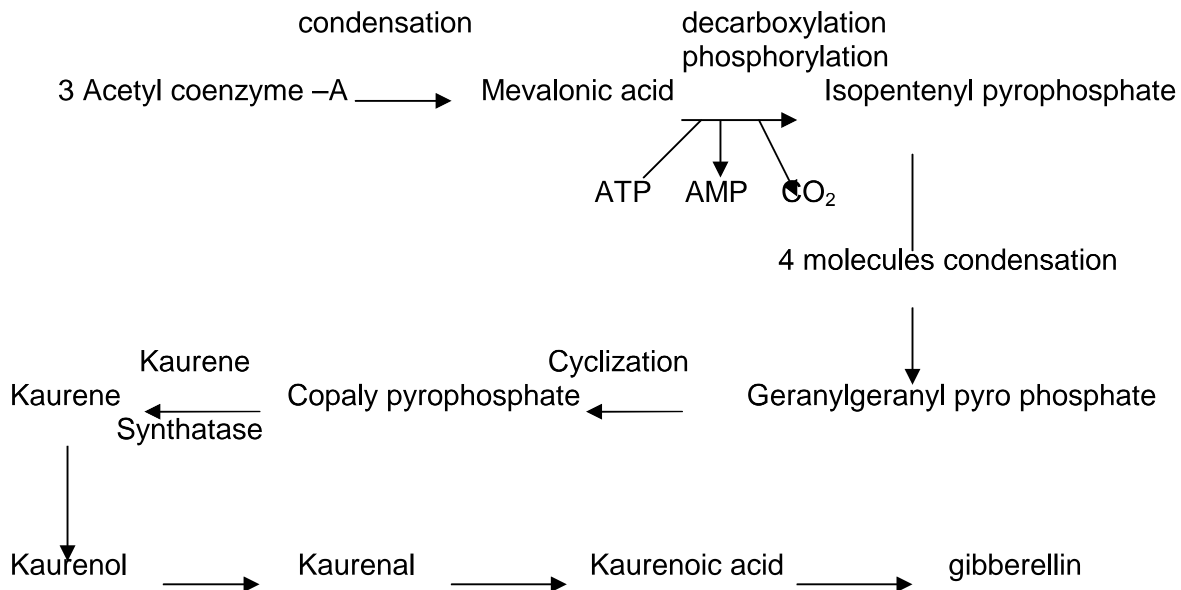
Natural Occurrence of Gibberellins

Gibberellins are found in all the organs of higher plants but they are abundantly present in apical meristems, germinating seeds, growing fruits and young leaves.

7.3.1 Bio-Synthesis

Basically gibberellins are synthesised from terperas through isoprenoid pathway. Gibberellins are synthesised via the terpenoid pathway. Three molecules of Acetyl CoA join together to form mevalonic acid. Isopentenyl Pyrophosphate (IPP) is formed from mevalonic acid. Condensation of 4 units of IPP lead to the formation of Geranyl Geranyl Pyrophosphate

(GGPP), a C_{20} diterpene. This is converted to Kaurene a 4 ringed compound. Kawrene gives rise to Gibberellins.



Gibberellins transport: Gibberellins are translocated in both ways. They can be carried up through xylem and phloem by diffusion.

7.3.2 Physiological effects of Gibberellins

(1) Removal of genetic dwarfism

Stem elongation in higher plants is considered as one of the important effect of gibberellins. In dwarf varieties of pea, maize, and other plants, dwarfism is due to gene mutation. If gibberellins are not synthesised in required concentrations stem remains dwarf due to shorter inter nodes. When, gibberellins are applied to such plants, cell elongation is restored and internodal length increases. The plants attain their original sizes.

(2) Breaking of dormancy

The seeds of lettuce require darkness to germinate similarly the seeds of apple and peach do not germinate until they are exposed to low temperature. Gibberellins can break both types of dormancy.

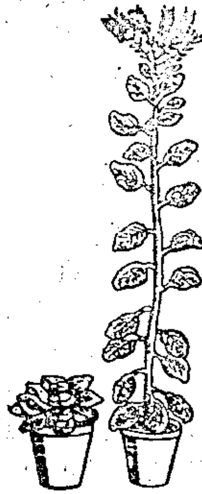


Fig. 7.3 Effect of gibberellin on the cabbage plant

(3) Germination

L.G. Paleg (1950) first recognised the ability of GA's in seed germination by mobilising reserve food materials. In germinating seeds, gibberellins are synthesised in embryo and move to aleurone layer, the protein rich layer surrounding endosperm, where an enzyme named α-amylase synthesis is induced by gibberellins. This is a hydrolysing enzyme. It hydrolyses the starch into sugars in the endosperm. From the sugars, energy is liberated in respiration and this energy is used for seed germination.

(4) Flowering

Some rosette plants need long photo periods or cold treatment for flowering. These treatments increase the gibberellin content of the plants. Gibberellins can induce flowering in such plants which require either long photoperiod or vernalization. But they do not induce flowering in short day plants under long day conditions. GA_7 is the most effective form in the initiation of flowering. Generally just before the floral induction, shar prise in gibberellin synthesis occurs, leading to a sudden increase in shoot length. This type of rapid and dramatic increase in shoot length is referred as *bolting,* and it is followed by flowering. Bolting is observed in biennials (e.g. Cabbage).

(5) Parthenocarpy

Gibberellins are effective in producing seed less fruits in the plants like apple, peach, pears etc. Where GA is effective, auxins may be ineffective. But in case of tomato both are effective.

(6) Sex determination

Gibberellins induce the formation of male flowers in place of the female flowers in cucumber. But in Bryophyllum and Chrysanthemum they directly initiate flowering.

(7) Gibberellic acid and meristems

Gibberellins may promote cell division and cell elongation. But their activity is confined only to subapical and intercalary meristems but not in apical meristems.

Along with the above activities gibberellins also involve in the increase of fruit size, sugar content, peduncle elongation in grapes. Flower formation in roses, rhododendrons, poinsettias and in several other plants. Sugar cane yield and sucrose content in the cane and seed production in conifers can be enhanced by gibberellin treatment.

7.4 CYTOKININS

cyto : cell, kinin : division. The hormone, which mainly involves in cell division is cytokinin.

History

(1) G. Haberlandt (1913) discovered an unknown compound present in the phloem tissue of various plants stimulated cell division in cut potato tubers.

(2) **J. Von over beek (1940's)** used coconut milk, the milky endosperm of coconut to induce cell division in tissue culture studies.

(3) **Skoog and Miller (1955)** isolated a compound from yeast DNA which was capable of stimulating cell division in Tobacco pith cultures. Subsequently, they discovered and characterized a very active compound formed by partial breakdown of herring (a marine fish) sperm DNA. They named this compound as Kinetin (6-furfuryl amino purine) because of its activity in inducing Cytokinesis. However, kinetin cannot be regarded as natural substance as it is only a degradative substance from DNA molecule.

(4) **Miller and Letham (1964)** independently isolated a naturally occuring cytokinin called a zeatin from immature maize seeds. Benzyladenine (BA), Benzylaminopurine (BAP) are synthetic cytokinins.

Natural occurrence of Cytokinins

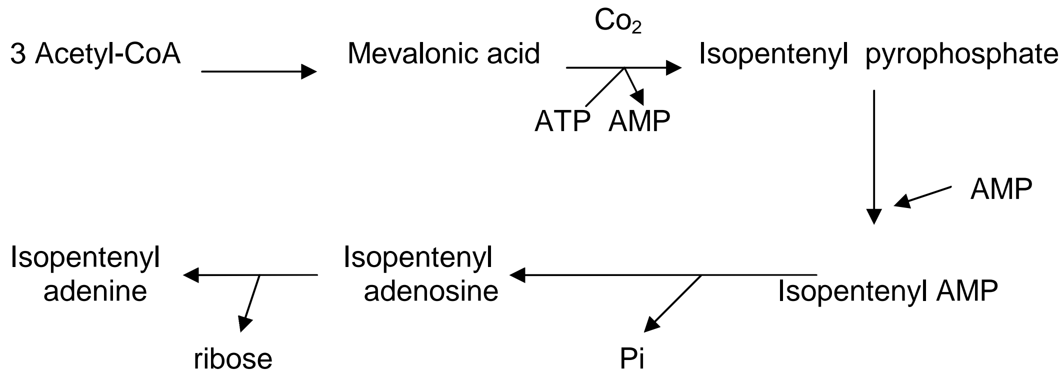
Most cytokinins known are synthetic ones. But several have also been isolated from plants. Generally, natural cytokinins are more active than synthetic ones. Isopentynyl adenine (IPA), Zeatin riboside, dihydrozeatin occur in plants. Zeatin is the most abundant natural cytokinin. Some plant pathogenic bacteria secrete cytokinins that induce cell division in host tissue. e.g. *Agrobacterium tumifaciens. Corynbacterium fascians* causes witches broom- a disease in which there is loss of apical dominance and subsequent development of side shoots. Cytokinins are present in many lower and higher plants. They have been recognised in several bacteria, yeasts and fungi. In vascular plants, they are found in actively growing parts like embryo, endosperm, seedlings, special meristems and developing fruits. Some tRNAs contain cytokinin.

7.4.1 Bio-Synthesis of Cytokinins

Chemically cytokinins are Isopentenyl adenine. They are synthesised in root tips and developing seeds. The information about bio-synthesis of cytokinin is very little.

(1) Conjugation of three acetyl-CoA molecules form mevolonic acid.
(2) This mevolonic acid is phosphorylated into Isopentenyl pyrophosphate by consuming ATP molecule.
(3) On conjugation with AMP this isopentenyl pyrophosphate is changed to Isopentenyl – AMP phosphate.
(4) This will be changed to Iso pentenyl adenosine by losing phosphate group.

(5) Finally it gives Isopentenyl adenine by losing one ribose sugar molecule.



Translocation of Cytokinin

Cytokinin translocates very slowly through xylem from lower parts to upper parts.

7.4.2 Physiological effects of Cytokinin

(1) Cell division: Induction of cell division is considered as major function of cytokinins in plants. Cytokinins promote cell division in tissue culture practices.

(2) Growth of vegetative parts: Cytokinins restrict verticle growth of roots and stem. But initiates horizontal growth (secondary growth). Leaf growth requires cytokinin activity Cytokinins convert small leaves into broader especially in the plants, grow in dark. During seed germination, the growth of cotyledons is exclusively promoted by cytokinins.

(3) Morphogenesis: Cytokinins in association with auxins, induce differentiation of organs. Higher cytokinin to auxin ratio is maintained in culture medium, favours differentiation, stems and leaves from callus. On the other hand low ratio of cytokinin to auxin favours root formation only.

(4) Delay in senescence: The ageing process in plants is known as 'Senescenee'. Senescenee process is manifested by yellowing of plant parts. This is because of break down of chlorophyll molecules and decreased synthesis of RNA and proteins. Senescence is followed by death of the whole plant or plant organs. Cytokinin delays this process. The ability of cytokinins to delay the process of senescence is called as '**RICHMAND LANG EFFECT'**. If plant parts contain enough amounts of cytokinins, they remain green and healthy.

(6) Dormancy: Temporary suspension of growth and metabolic activities is called dormancy. Cytokinins remove the affect of dormancy in Tobacco seeds, which are phototrophic seeds. After treatment with cytokinins they can germinate even in the absence of light. Similarly striga (a parasitic plant) on application of cytokinin can germinate even in the absence of suitable host.

Cytokinins also play an important role in the opening of stomata. They counter act the usual dominance of the apicalbud.

7.5 ETHYLENE

History

Ethylene is an important harmone having a role in plant growth, development and ripening of fruits.

(1) Denny (1924) found that ethylene stimulated ripening of fruits.
(2) Gane (1935) identified it abundantly in ripen bananas.
(3) Burg and Thimann (1959) recognised physiological significance of ethylene as a plant hormone. At normal physiological temperatures it is a gas. It is similar to other hormones by being effective at lower concentrations, having simple chemical structure. ($H_2C = CH_2$)

Occurrence: It is mainly found in ripened parts of the plant body.

7.5.1 Bio-synthesis

Several concepts regarding the ethylene synthesis mechanisms have been emerged. It is synthesised from the amino acid methionine.

7.5.2 Physiological functions

(1) Ripening of fruits: It was found that application of ethylene hastens ripening of fruits. But it is not well understood, how it brings about ripening. Frenkel (1968) suggested that some enzymes, related to ripening process may be activated by ethylene. Giving weightage to this process scientists found the presence of ethylene in many ripening fruits.

(2) Triple response growth: Generally seed germinates and grows vertically, if at all it gets any barrier in the way of its growth, ethylene causes horizontal growth habit in seedlings. This peculiar pattern of growth induced by ethylene is known as "**triple response growth**". Ethylene inhibits stem elongation, increases in thickness of the stem and promotes horizontal growth.

(3) Apical dominance: Ethylene inhibits the growth of lateral buds and causes apical dominance along with auxins.

(4) Geotropism: The geotropic movements of root was thought of higher concentrations of auxins which suppress the growth on that side and cause geotropic movements. But according to Burg and Burg this is due to higher concentration of ethylene on that side.

Along with these activities ethylene also causes leaf abscission, stem swelling, inhibition of root and stem growth.

7.6 ABSCISIC ACID

It is a growth inhibitory hormone, also called as 'stress hormone'.

History

(1) **D.J. Osborne (1955)** found that senescent leaves contain a substance different from other hormones which can accelerate abscission of plant parts.

(2) **Adicott *et al.,* (1963)** discovered two substances in cotton fruits responsible for the abscission of fruits. They named them as abscisin–I and abscisin–II.

(3) Wareing *et al*., isolated a dormancy inducing substance from the leaves of **Acer** and named it as "**dormin**". Later it came to be known that abscisin-II and dormin were structurally similar and a common name abscisic acid (ABA) was given in 1967 by '**Cornforth'**.

Natural occurrence

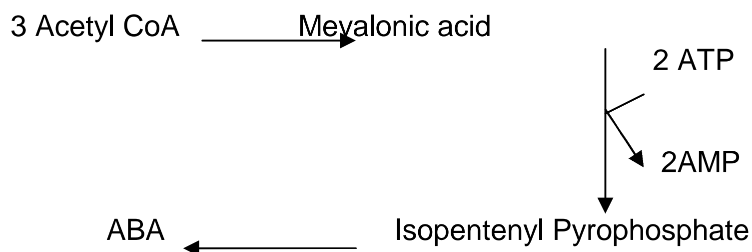
It is present abundantly in mosses, ferns, gymnosperms and angiosperms, but absent in liverworts, bacteria, algae and fungi. In angiosperms it is mainly found in fruits sanascing leaves dormant seeds, buds and their presence is not reported in roots. According to recent investigations ABA is present in green algae and some fungi.

7.6.1 Bio-synthesis

Chemically ABA is a 15 carbon terpeniod compound (sesquiterpene). ABA is synthesised anabolically through mevalonate pathway or catabolically from carotenoids.

(1) Through mevalonate pathway:

(1) 3 Acetyl CoA molecules conjugate and form mevalonic acid. On consuming 2 ATPmols. It is changed to mevalonic pyrophosphate.
(2) This mevalonic pyrophosphate is modified into IPP (Isopentenyl pyrophosphate) by losing one Co_2 molecule.
(3) Three IPP moelcules conjugate and form single molecule of ABA.



(2) From Carotenoids:

(1) Violoxanthin is oxidised into xanthoxin, which further changes to ABA.

7.6.2 Physiological Functions

(1) Growth and development of the plant: ABA inhibits the growth of stem, root and leaves.

(2) Apical dominance: It controls dominated nature of apical buds over lateral buds.

(3) Dormancy: The inability of the seeds to germinate or buds to grow even under favourable conditions is called 'Dormancy', ABA promotes dormancy of both seeds and buds.

(4) Closure of Stomata: When plants are encountered to water stress conditions, ABA concentration will be enhanced and induce the closure of stomata. So that water loss will be reduced.

(5) Abscission: It is basically a hormone much related to abscission. By enhancing cell wall degrading enzymes it causes abscission.

7.7 SUMMARY

A substance, produced in one part and having its effect on other part is called hormone. Plant hormones are named phytohormones. They are (1) Auxins (IAA) (2) Gibberellins (GA) (3) Cytokinins (4) Ethylene and (5) Abscissic acid (ABA). Though they have individual activities still plant growth and development is controlled by co-ordinatory action of these hormones. Auxins are responsible for root initiation, apical dominance, tropical movements and parthenocarpy. Gibberellins mainly involved in seed germination, flowering, removing genetic dwarfism and parthenocarpy. Cytokinins major function is in cell division and it also controls apical dominance, senescenee and dormancy. Ethylene is mainly concerned in fruit ripening. ABA is a growth inhibitory hormone.

7.8 TECHNICAL TERMS

Apical dominance, Parthenocarpy, Bolting, Senescence, Abscission, Richmond lang effect, Triple response growth, Bio assay etc.

7.9 SELF ASSESSMENT QUESTIONS

Essay type

(1) Explain the biosynthesis and physiological activities of IAA.
(2) What are phytohormones, explain them in detail?
(3) Write an essay on Gibberellins.

Short notes

(1) Role of different hormones on apical dominance.
(2) Richmond – Lang effect.
(3) Triple response growth.
(4) Avena coleoptile curvature test.
(5) Physiological changes occur during seed germination.
(6) Cholodny – went theory.

7.9 REFERENCE BOOKS

1. Subhash Chandra Datta, **Plant Physiology**, Wiley Eastern Limited, New Delhi, 1994.
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- D. Raja Sekhar

Lesson 8

PHYSIOLOGY OF FLOWERING: PHYTOCHROME, CONCEPT OF PHOTOPERIODISM, FLORIGEN CONCEPT AND VERNALIZATION

8.0 OBJECTIVES

(1) Students will learn the physiology of flowering.

(2) Learners comprehend the role of phytochrome in flowering process.

(3) Students will compare florigen concept and vernaline concept.

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8.1 INTRODUCTION

Growth and development are common to all living organisms. Growth is a quantitative change whereas development is a qualitative change. Growth can be measured but not development. Plants show two types of growths (1) vegetative growth and (2) reproductive growth. Generally vegetative growth is followed by reproductive growth. The growth is controlled by number of external and internal factors. Among different external factors light is

the most important factor which mediates different metabolic activities of plant. In the present chapter light response in flowering has been discussed.

8.2 PHOTOPERIODISM

The phenomenon of photoperiodism focusses the switch over form vegetative to the flowering studies. It is one of the most mysterious events in the life of a plant. The foundations of our present knowledge on photoperiodism were laid by Garner and Allard in 1920, who conducted experiments on Maryland Mammoth, a mutant tobacco variety. Generally seasonal variations are mostly observed in temperate countries rather than tropical and sub tropical countries. In temperate countries the day length period changes from season to season. For eg: In winter season day length is decreased whereas in summer it is increased. According to these changes the plants show development, it is called photoperiodism. Keeping this idea in the view Garner and Allard could induce the experimental plant to flower in summer, by placing it in darkness i.e., by providing a short photoperiod.

Meanwhile, some other researchers have showed that some species which flower in summer can be induced to flower in winter, by providing artificial light along with natural day light. It clearly tells us the light requirement for development is vary from species to species. When required light is given irrespective of seasons a plant shows regular development and flowering too. In the absence of required photoperiods, plants may show only vegetative growth but not reproductive growth i.e., flowering.

8.3 TYPES OF PLANTS

Depending upon the influence of day light length on the flowering three groups of plants were recognised. They are (1) long day plants (LDP), (2) Short day plants (SDP) and (3) day neutral plants (DNP). Whether a plant is classified as SDP or LDP depends on its behaviour relative to critical day length. The photoperiod required to induce flowering is known as critical day length or critical photoperiod.

(1) Long-day plants (LDP): These plants require more or long periods of light for flowering. They flower when light is given more than the critical period. Critical period denotes the minimum limit of phtoperiodic hours. In Hyocyanus (Henbane) the critical day length is 11 hours and it will flower when the day length exceeds of light. The critical period varies in different plants e*.g.,* most of the agricultural plants such as Wheat, Potato, Spinach, Radish and Henbane.

(2) Short-day plants (SDP): These plants require short periods. They flower when light is given below the critical period. If the light hours exceed critical period, they do not flower. Here critical period denotes the maximum limit of photoperiodic hours. Thus the critical dry length for SD plant xanthium is 15.5 hours meaning that it will floer whenever the day length is less than 15.5 hours out of 24 hours. The critical period varies from species to species, e.g., Cereals, Cosmos, Crysanthemum, Xanthium, Billoxi soybean and winter rice (Aman variety).

(3) Day–neutral plants (DNP): The flowering of plants of this group is independent of day length. That means they flower all the year round. They have no critical periods, e*.g.* Cotton, Zinnia, Tomato.

In tomato flowering seems to be controlled by temperature effect. An alternation between night temperature of 15^oC and a day temperature of 25^oC appears to be optimal for flowering in these plants, called thermoperiodism or thermoperiodicity.

Besides above types there are certain plants which have both lower critical period and upper critical period. They flower only when the range of photoperiod is in between lower and upper critical periods, such plants are called intermediate day plants (IDP) e.g. Phaseolus, Polystachys, Mikania, Andropogon sp. Short day plants will flower even when exposed to long day conditions, because they have been previously exposed to a sufficient number of short days. Similarly, long day plants flower in short days if they have been previously exposed to a sufficient period of long days. This is called “Photoperiodic induction”.

8.4 PHOTOINDUCTIVE CYCLES

The required photoperiod duration is called photo inductive cycle. The number of cycles required for inducing flowering varies in different species. For example Xanthium is a short day plant, single short day cycle initiates flowering in this plant. Another short day plant *Impatiens balsamina* requires eleven photo inductive cycles, three for flower primordia initiation and eight for maturity. The number of cycles depend on the length of photoperiod and irradiance. In *Plantago lanceolata*, a long day plant, flowering occurs after 25 long days. The plant can flower even the cycles are given discontinuously. But SDP require continuous photo inductive cycles.

8.5 SITE OF PHOTOPERIODIC RESPONSE

It was showed that the flowering was not initiated in some plants if the photoperiod was given to apical meristem. But when the same light was given to leaves, the flowering was initiated. It clearly conveys us the importance of leaves in receiving the stimulus. This stimulus is transferred to apical meristem and induces flowering activity. The fully expanded youngest leaves are more effective in taking stimulus than that of the young unexpanded leaves e.g., Xanthium. Similarly when a light treated branch of the same plant (SDP) is grafted to light untreated branch, flowering was induced. That means the stimulus of photoperiodic induction is transmitted across graft unions. It is thought that a flower inducing hormone is produced in leaves subjected to photoperiodic induction. The flowering stimulus is transmitted probably through phloem and may be translocated both in upward and down ward directions.

8.6 SIGNIFICANCE OF DARK PERIOD

Hamner and Bonner identified the importance of dark period in the flowering process in Xanthium (SDP) sp. When the plant is given a photo inductive cycle of 16 hours of dark and 8 hours of light, flowering occurred. But when the dark period is interrupted by a brief light period, flowering is not occurred. This shows that the dark period should be continuous. The same plant flowered even light period is disturbed. This experiment asserts the importance of dark period over light period. So that SDP plants can be called as long night plants, LDP plants are called as short night plants. In view of the greater importance of the dark period some consider that the term photoperiodism could be replaced by “Nyctoperiodism”. But in reality both the day and the night are important in the time measurement aspects of photoperiodism.

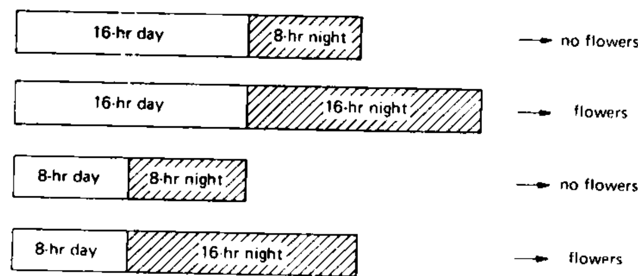


Fig. 1.A Diagram to show the effects of long and short days or nights on flowering of the cocklebur (*Xanthium stromanum*)

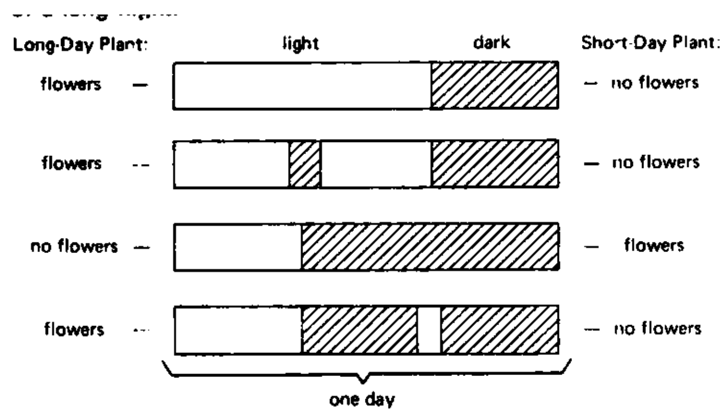


Fig. 1.B Diagram to show the effects on long and short day plants of a dark interruption of a long day and of a light interruption of a long night.

8.7 ROLE OF HORMONES IN PHOTOPERIODISM

The general assumption was that in all plants a specific substance is synthesised in the leaves, from where it is translocated to the growing points and induces flowering. The rate of movement of this flowering stimulus from the leaves of certain plants has also been measured.

Chailakhyan, a Russian plant physiologist named the stimulus of flowering as "**Florigen**". Lincoln, Mayfield and Cunningham isolated a chemical substance from flowering Xanthium plants, which induced flowering in similar plants under non-induced photoperiods. As the isolated substance was acidic in nature it was named as "**Florigenic acid**". This substance could enhance the flowering when applied to the leaves but it had no effect when applied to the buds. So the isolated substance is not florigen, but it is the substance necessary for the synthesis of florigen. In Xanthium it was effective when gibberellic acid was given.

Auxins have no importance in induction of flowering. But gibberellins have important role in flowering, especially for long day plants. They can flower even under short days when they are given with GA. It is found that when Maryland Mammoth (Tobacco variety) a short day plant is grafted with Henbane, a long day plant, either long days or short days cause flowering in both plants. If they are kept in short days continuously, the tobacco plant is induced and after some time henbane also blooms. Similarly when the both plants are subjected to long

days henbane blooms first and later tobacco flowers. Such grafting experiments show that the flowering hormone is same in all species of plants.

Chailkhyan modified his florigen theory to explain the influence of GA on LDP. He thought that florigen consists of two hormones namely 'gibberellin' and a hypothetical hormone called 'anthesin'. He suggested that both hormones are essential for flowering. In the absence of any hormone the flowering does not occur. Generally LDP produces anthesin under any condition and so when GA is supplied flowering occurs. But SDP produces gibberellin under any condition but anthesin only under short days. That is why SDP does not flower under long days. Day neutral plants produce both hormones under any conditions. But this explanation is totally hypothetical.

When plants are stimulated by the proper photoperiods, florigen is induced to develop and causes a morphological change. It results in converting the vegetative primordia into floral primordia. 'Gifford' and 'Jensen' discovered that florigen alters the kind of mRNA synthesised. So the enzymes of meristem are changed their action to induce flowering.

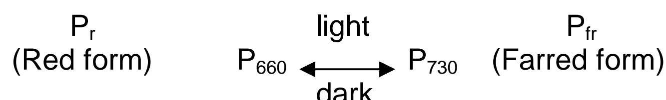
8.8 PHYTOCHROME

Though florigen is not yet isolated. It is supposed that the release of florigen is dependent upon a blue photo-receptor pigment called phytochrome. It is chemically related to phycobilins of algae. The active spectrum of flower formation is strikingly similar to the absorption spectrum of 'allophyocyanin'. Phytocrome is a bili protein or chromoprotein and present in lower concentrations. It plays an important role in almost all light mediated responses called 'photomorphogenesis'.

H.A. Borthwick and S.B. Hendricks first identified the existence of phytochrome while conducting experiments on lettuce seeds. The pigment was extracted from etiolated seedlings of maize and other grain plants. It is present in all green plants and also all parts of the plants such as coleoptile, cotyledons, leaf lamina, hypocotyl, petioles, roots, stems, buds, developing fruits, floral receptacle and inflorescence.

Phytochrome is a chromoprotein with a molecular weight of about 1,20,000. It consists of a protein part and a prosthetic group called chromophores. The protein part is covalently bound to the chromophore. Chromophore is an open chain of tetra pyrrole. Each pyrrole ring shows 4 carbons, one nitrogen and two double bonds.

Phytochrome exists in two forms one absorbing the red is the P_{660} and the other absorbing far-red is the P_{730} form. The two forms are photochemically inter-convertible. During day time red form i.e. P_{660} is converted into farred form i.e. P_{730}.



P_r is biologically inactive. Formation of P_{fr} initiates active developmental processes such as (1) photoperiodic floral induction (2) Nyctinastic leaf movements (3) Seed germination (4) Stem elongation (5) Leaf and catyledon expansion (6) Chloroplast development (7) Chlorophyll & Carotenoid synthesis (7) Anthecyanin synthesis (8) Enzyme activation.

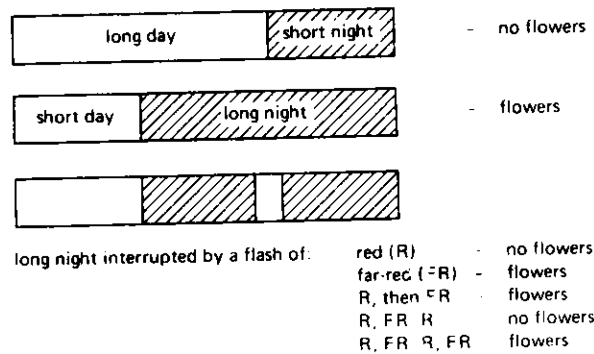


Fig. 2. The effect of a light flash of red (R) or far-red (FR) light or of sequences of R and FR during the inductive night of a short-day plant.

Similarly during night time farred form changes to P_{660} form. In short day plants P_{660} plays a dominant role in promoting flowering where as P_{730} takes a dominant role in promoting flowering in long day plants. So when SDP is supplied with long dark periods immediately P_{730} is converted into P_{660}, because of much accumulation of this form, flowering is initiated in short day plants. When the dark period is interrupted by brief light period, P_{660} accumulation is obstructed so that flowering does not occur. Xanthium, a SDP requires 10-12h of dark period for conversion of P_{735} into P_{660}. If the interrupted light is red, flowering will be inhibited in short day plants, but if it is followed by farred light flowering takes place due to accumulation of P_{660}. In long day plants P_{735} form stimulates flowering. These plants can flower even the dark period is interrupted by a flash of orange-red which can convert P_{660} form into P_{730}.

The above responses can be explained in this manner. In short day plants P_{660} form releases florigen essential for inducing flowering. Whereas in long day plants P_{730} form releases the flowering agent which further stimulates the flowering process.

8.9 VERNALIZATION

Some plants especially which grow in temperate and polar regions require cold treatment before they flower. This treatment is known as 'Vernalization'. Vernalization is an agricultural practice of treatment of seeds with low temperature in initial stages of seedling growth, accelerating its development. This treatment induces a rapid development and results in shortening of the interval between vegetative growth and flowering.

Klippart (1857) first used the concept of vernalization to convert winter wheat variety into spring variety of wheat. Gassner in 1918 and Lysenko in 1928 worked on the same phenomenon and gave more details of the process. The term vernalization was coined by Lysenko (1928). Chonard another scientist defined it as "acquisition of the ability to flower by chilling treatment". Generally vernalization occurs during winter season and flowering occurs in the following spring. It has been used as a technique of promoting flowering in many biennials. It is different from photoperiodism. Photoperiodism initiates and prepares the plant for flowering but vernalization only prepares the plant for flowering. Using vernalization technique may crop plants have been developed, which can grow even in unfavourable conditions. Dormancy of some seeds can also be over come by cold treatment.

8.10 THE SITE OF VERNALIZATION

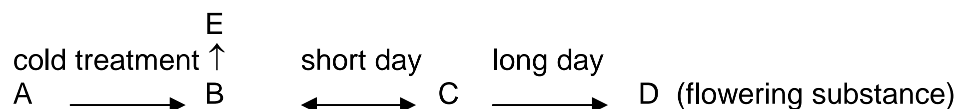
The site of vernalization is the growing tip. G. Melchers, a German botanist proved that the stem apex is the site of vernalization. From this the stimulus is passed to other parts. The stimulus can be passed even to unvernalised part through a graft union. Wellensiet (1961-70) demonstrated the isolated leaves and roots of *Lunaxia biennis* can be vernalised. He said that actively dividing cells are pre-requisite for the perception of cold treatment. No matter where they occur in the plant. Chakravarti (1970), who did experiments on Linum, concluded that the cells of the fully formed epidermis are capable of perceiving the stimulus.

Time duration: Time duration varies from species to species. Generally temperature and time relation is directly proportional. For e.g., 13-17°C temperature is effective if the period of vernalization is 105 days. If the cold treatment is for 40 days, the most effective temperature is between 3-6°C. In Petkus rye, vernalization effect is improved when temperature is increased from –4° to 4°C. The age of plant should also be considered for cold treatment. That is also variable from species to species. In cereals the germinating seeds can be vernalised. Henbane, a biennial receives the stimulus of vernalization only after 10 days of vegetative growth. Oenothera receives the stimulus only after producing 6-8 leaves. It clearly tells us only after some vegetative growth plants are capable to receive the stimulus.

Effect of light on vernalization: Vernalization experiments were done on *Hyoscyamus niger* (henbane) and *Secale cereale* (petkus rye), both are long day plants. Henbane, the annual plant flowers within a growing season. But the biennial variety will flower only when cold treatment is followed by long day condition. It flowers in the next growing season. If the cold treatment is followed by short day, it may grow vegetatively but not flower.

Petkus rye shows two varieties: (1) spring variety, which completes its life cycle in one growing season, annual type. (2) winter variety, it is biennial only flowers in next season after getting natural cold treatment. If the seeds of winter variety are treated with cold treatment it can flower in a growing season like spring variety.

Purvis explained the flowering process in cereals by following scheme.



The process of flowering includes series of reactions. All the reactions must be completed before flowering. In spring rye variety firstly a substance denoted by (A) is converted into (B). It occurs naturally at normal temperature. But in winter rye variety conversion of (A) into (B) requires cold treatment. It may occur naturally but at a very slow rate. The further reactions are under control of photoperiods. Under continuous light, (B) is slowly converted to (C), and initiates the early stage of flowering and finally converts into another substance (D). (D) is the flowering hormone, causes flowering. If proper photo period is not available (B) will be changed to another substance (E) instead of (C) and (D). The substance (E) promotes only vegetative growth. The above process occurs successfully when long light is available. If at all short light period is encountered, the conversion of (C) to (D) is prevented and flowering does not occur.

8.11 PHYSIOLOGY OF VERNALIZATION

Experiments conducted previously assert the importance of meristem in vernalization process. It is considered as site for cold treatment. When a vernalized plant is grafted with an unvernalised plant unvernalised plant also undergoes bolting and blooming. It indicates the presence of a substance in vernalised plant. This substance was named as "**Vernaline**" by Melchers. But It has not been isolated. Vernaline resembles gibberellins in its physiology. But gibberellins first induce bolting, followed by flower initiation, where as vernalization induces flower initiation first and then bolting. Vernalization process needs oxygen, food and water. That means it needs energy. But still much work has to be carried to get the complete information on the physiology of vernalization.

8.12 DEVERNALIZATION

Vernalisation effect is reversible. Completely vernalised rye or wheat varieties, may entirely revert to normal conditions on being dried for several weeks or simply by storing them under anaerobic conditions. The reverse effect of vernalisation is called "**Devernalization**" e.g., the vernalised petkus winter rye seeds can be devernalised in 8 weeks by storing them at dry conditions. Similarly vernalised henbane plants and grains of wheat can be devernalised by exposing to about $35^{o}C$ temperature.

8.13 SUMMARY

Development is a qualitative change, whereas growth is a quantitative change. Reproductive growth in plants refers flowering and fruiting. Two different physiological aspects play an important role in the flowering. One is effect of light duration, called photoperiodism and the second one is effect of cold treatment, called vernalisation. Basing on the photoperiodic effect plants are classified into: (1) LDP, (2) SDP and (3) DNP. Phytochrome is a pigment, plays a paramount role in photoperiodism. A hypothetical chemical substances named Florigen & Vernaline play important roles in photoperiodism & vernalization respectively. However the clear physiological changes in both processes are still not known.

8.14 TECHNICAL TERMS

Photoperiodism, Nictoperiodism, Vernalization, Devernalization, Photoinductive cycles.

8.15 SELF ASSESSMENT QUESTIONS

Essay type:

(1) Write a detailed essay on photoperiodism.
(2) What is vernalization? Explain the physiology of vernalisation in detail.

Short notes:

(1) LDP, SDP and DNP.
(2) Florigen Concept
(3) Vernaline Concept
(4) Phytochrome

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- D. Raja Sekhar

Lesson 9

SEED PHYSIOLOGY – FACTORS AFFECTING SEED GERMINATION AND SEED DORMANCY

9.0 OBJECTIVES

(1) Students learn about different factors involved in seed germination and seed dormancy.

(2) Students will know different causes for dormancy and also know their control.

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9.1 INTRODUCTION

9.2 FACTORS AFFECTING GERMINATION

9.3 DORMANCY

9.4 FACTORS AFFECTING DORMANCY

9.5 METHODS OF BREAKING SEED DORMANCY

9.6 SUMMARY

9.7 TECHNICAL TERMS

9.8 SELF ASSESSMENT QUESTIONS

9.9 REFERENCES

9.1 INTRODUCTION

Germination is a process which activates the embryo to grow. The physiology of germination is under control of a hormone, gibberellin. During seed germination, firstly the dry seed imbibes water present in the soil. So that protoplasm is hydrated and enzymes present in it are activated. These activated hydrolysing enzymes such as amylase, lipase and protease act upon the reserve food materials such as starch, lipids and proteins present in the endosperm and convert them into simple substances. These simple substances become available to the embryo present in the seed. Thus imbibition is the first physiological process occurring in the seed, followed by anaerobic respiration. Because of these changes seeds germinate radicle comes out first and plumule later.

Seed germination is of three types (a) epigeal - cotyledons come above the ground, (b) Hypogeal – cotyledons remain in the soil. Vivipary - seeds germinate within the fruit, e.g. mangrooves. Varner showed that gibberellins formed from the embryo induce formation of hydrolytic enzymes in the aleurone layer of barley seeds. These enzymes hydrolyse complex food material into simple substances and make available to embryo to be grown. Generally amylase is the enzyme which hydrolyses starch into sugars, lipase hydrolyses lipids into fatty acids and glycerol. Similarly proteases break proteins into amino acids.

9.2 FACTORS AFFECTING GERMINATION

Factors, involved in seed germination are broadly classified into: (1) external factors (2) internal factors. No single factor is sufficient for seed germination. The interaction among all these factors is essential for seed to germinate. External factors are mainly water, light, temperature, oxygen, carbondioxide and germination inhibitors. The main internal factors are seed viability and seed longevity.

EXTERNAL FACTORS

a) Water: Dry seeds contain 5-15% of water but this is bound to the colloids and not available for hydrolysis of foods. So germination cannot be commence unless water is available. Seed germination is generally most rapid when water content in the soil is equal or near to field capacity. Seed absorbs water from the soil by imbibition and osmosis. pH of the soil is also an important factor for seed germination. Generally neutral soils are the best for seed germination.

b) Light: Seeds of many species can germinate in the presence or absence of light, but some seeds cannot germinate without light e.g., Lepidium and Tobacco. Some seeds germinate only in the absence of light e.g., Tomato, Onion and some lillies. Phytochrome, a light mediated pigment plays an important role in light mediated germination. The morphogenetic changes in seedlings are the result of phytochrome mediated light responses.

c) Temperature: The temperature range for germination varies from species to species. For e.g., the temperature range for wheat seeds is I-35°C and for corn is 6-45°C. Generally at high temperatures, the enzymes essential for germination are destroyed. If seeds are subjected to cold treatment i.e., vernalisation (0–5°C), gibberellins are induced and germination takes place. Some seeds do not require cold treatment. The temperature requirement of some seeds depends on their age and physical condition. There are certain seeds which can germinate even at high temperatures (45–50°C) e.g., *Cucumis melo*.

d) Oxygen: Seeds do not germinate in the absence of oxygen. Germination does not occur even in low concentrations of oxygen i.e, less than 20% of the atmospheric air. But some hydrophytes germinate under water where oxygen availability is poor. In the beginning of seed germination, seeds respire anaerobically but when the seed coat once imbibes water and becomes permeable to oxygen, they conduct aerobic respiration. But seeds with hard seed coat perform anaerobic respiration for longer duration because of impermeability of O_2 into the seeds. When seed coat is ruptured, then they perform aerobic respiration. Oxygen is very essential for oxidation of stored food material and release of energy.

e) Carbon dioxide: Higher concentration of carbondioxide inhibits germination of many seeds. But this conditions is not present in the soil. Seeds of *Xanthium, Lettuce, Trifolium* can germinate even at high CO_2 concentration. Seeds of Pea, Tomato and Wheat can germinate under 20% CO_2 concentration.

f) Germination inhibitors: There are certain chemicals, present in the soil that inhibit the seed germination. Some examples of such chemicals: Hydrogen cyanide, some alkaloids, essential oils, coumarins, mustard oil, ferulic acid etc. High salt concentration in the soil also inhibits seed germination. Some industrial wastes like acids, alkalis, heavy metals, flourides and phenolics also obstruct seed germination. Similarly seeds of tomato do not germinate unless they are washed thoroughly to remove all the substances, present around the seeds.

Internal Factors: Not only the external factors, some internal factors also play an important role in seed germination. The physiological maturity of embryo, softness of seed coat, seed viability and seed longevity are some of the important internal factors involve in seed germination. Seed viability means the capacity of seed to live and germinate for some specific time period. Generally viable capacity is lost as time passes. Longevity means the length of viable period, that is variable from species to species, for *e.g.,* crop plants have 1 to 2 years, whereas lotus has viable capacity nearly for 200 years. Seed viability also depends on external environment to which the seed is exposed. Seeds can be preserved keeping the viable duration in the view. The viability of seeds can be prolonged by storing them at low temperature.

9.3 DORMANCY

Seeds usually germinate when suitable conditions are provided. Seeds do not germinate in the absence of any one external factor and such a condition is called '**Quiscent'**. But some seeds do not germinate even when all the essential environmental factors are available, because of unsatisfactory internal factors. These seeds are called dormant seeds. On one hand dormancy seems to be a disadvantageous process but on other hand it seems to be useful because seeds overcome unfavourable conditions by being dormant at some stage of their life cycle. In a seed, the growth of embryo is arrested during the period of dormancy. All parts of the seed such as seed coat, endosperm and embryo may be the cause for dormancy. The mechanism of dormancy is quite complex as both external and internal factors are involved in it.

9.4 FACTORS AFFECTING SEED DORMANCY

(1) Thick seed coat: Several plants like legumes and lotus have thick and hard seed coat. It is the main factor of dormancy in these plants. The seed coats may be impregnated with waxes or other substances. These seed coats are impermeable to water and gases and cause seed dormancy. The hard seed coat prevents germination in 3 ways.

(a) Imbibition is the first step of seed germination, leads to the activation of hydrolysing enzymes. When water is not available imbibition does not occur and enzymes can not be activated. So seed germination does not occur. Seeds of *Leguminosae, Malvaceae, Chenopodiaceae, Convolvulaceae* and *Solanaceae* come under this category.

(b) The hard seed coat does not give entry for gases especially to oxygen. When oxygen is not available respiration does not occur properly. So that dormancy is prolonged Eg: grasses and Asteraceae members.

(c) In some plants hard seed coat gives entry to water and O_2, but it doesn't allow the tender embryo to grow e.g., *Amaranthus.*

(2) Immature embryos: In some species, the embryo is not fully mature at the time of seed dispersal e*.g.,* Ginkgo, some orchids, Anemone and Fraxinus. In some cases the embryo attains maturity during dormancy period.

(3) Physiological immaturity of embryo: Although the embryos are morphologically mature, they remain dormant. Such embryos are physiologically immature because enzymes

essential for seed germination are absent or lack of adequate amounts of growth regulators. This phenomenon is observed in apple, pear, cherries, lettuce, pines and Iris.

(4) Dormancy due to chilling requirement: Plants like apple, peach and walnut do not germinate soon after harvest. These plants require cold treatment, that would be 0-5°C for some days to some weeks. When these conditions are given to them, they germinate immediately.

(5) Germination inhibitors: Abscissic acid is a growth inhibiting harmone, which inhibits the seed germination. Similarly 2,4 –D, some organic acids, phenolics, alkaloids, aldehydes, essential oils, unsaturated lactones, ammonia and cyanide releasing substances inhibit germination. Of these some are hormonal growth inhibitors and some other cause unfavourable pH. These inhibitors may be produced by any part of seed. Tomato juice contains caffeic and ferulic acid. These are formed in the fruit and diffuse into the seeds.

(6) Role of light in seed dormancy: Basing upon the light requirement for seed germination, seeds are classified into 3 types: (1) Seeds which germinate equally well in dark as well as light are called non photoblastic seeds *E.g.*: Tomato, Cucumber. (2) Seeds which germinate in the presence of light are called positively photoblastic seeds Eg: *Rumex, Chenopodium, Bidens radiata* etc., (3) Seeds, which germinate in the absence of light are called negatively photoblastic seeds. The effect of light on seed germination is mediated by phytochrome.

9.5 METHODS OF BREAKING DORMANCY

Several methods have been developed to break the dormancy of seeds. The type of treatment depends upon the type of seed dormancy. Some methods of breaking dormancy are discussed here.

(1) Scarification: Dormancy, caused by hard seed coat can be removed by this method. Softening of hard seed coat is called 'Scarification'. The soft seed coat allows water, gases and embryo to grow, again scarification is of 2 types.

a) Mechanical scarification: Softening of seed coat by rupturing the seed against hard surface or scratching the seed coat with knife or shaking the seeds with vigorously with abrasive material (sand). Care has to be taken to avoid injury to the embryo.

b) Chemical scarification: Dipping seeds in strong H_2SO_4 (or) HCl (or) hot water removes dormancy. Treating seeds with organic solvents (ether, alcohol, acetone) also breaks dormancy. Fabaceae family seeds contain strophilor plugs which do not allow water and gases inside the seed. In such conditions shaking the seeds, keeping them inside the sand bottle remove these plugs and dormancy called '*Impaction*', e.g. *Melilotus alba.* Naturally some soil microorganisms, birds and animals carry scarification and remove dormancy.

(2) **Stratification:** Dormancy due to lack of cold treatment can be removed by stratification. The low temperature treatment given to the seeds to break dormancy is called stratification. For giving this treatment, the seeds must be imbibed. In natural conditions, this treatment is available during autumn season. Artificially these conditions can be given by mixing seeds with moistened soil and stored at low temperature. Stratification results in disappearance of inhibitors and promotes build up of hormones like gibberellins and cytokinins.

(3) Alternating temperatures: Providing high and low temperatures alternately can break seed dormancy caused by hard seed coat. But the difference between the temperatures should not be more than 10-20°C. This treatment allows gases through the seed coat. Alternating temperature along with scarification breaks dormancy in the seeds of *Asclepias*. Treatment with gibberellin would replace the temperature requirement but not the requirement of scarification.

(4) Exposure to high concentration of oxygen: Dormancy, caused by growth inhibitors can be removed by this method. It was proved in Xanthium seeds by Peach (1953).

(5) Light: Basing upon the light requirement seeds are classified into (a) Positively photoblastic seeds (b) non photoblastic seeds and (c) negatively photoblastic seeds. Positively photoblastic seeds germinate only in the presence of light. The length of exposure to light may vary from a few seconds to several minutes. Flint and McAlister found that red light was more effective in breaking dormancy. If we provide the required light they germinate, negatively photoblastic seeds do not germinate in the presence of light. So by providing dark conditions we can make them to germinate.

(6) Growth substance: Gibberellins are the growth harmones which promote germination by removing dormancy. So by spraying gibberellins or soaking seeds in gibberellins, seeds can be germinated e.*g.* lettuce.

(7) Chemical substances: Thiourea, Potassium nitrate like substances are used to remove dormancy.

9.6 SUMMARY

The growth and development of an embryo into a seedling is called “seed germination”. Seeds first imbibe and activate hydrolysing enzymes, which convert complex stored food material into simple substances and make available them to the embryo and allow it to germinate. Some external factors like water, light, temperature, oxygen, Co_2 and some internal factors like seed viability, longevity play an important role in seed germination. Generally seeds do not germinate when favourable conditions are not available. When seeds which do not germinate in the absence of any external factors the condition is called ‘**quiscent’**. Seeds which do not germinate because of unsatisfactory internal factors is called ‘dormancy’. Dormancy is because of thick seed coat, immature embryo, physiological immaturity of embryo, germinating inhibitors and dormancy due to chilling requirement. The dormancy of seeds can be removed by scarification, stratification, impaction, treatment with alternating temperatures, exposing to high concentrations of oxygen etc.,

9.7 TECHNICAL TERMS

Imbibition, Germination, Dormancy, Scarification, Stratification, Impaction, Seed Viability, and Longevity, Quiscent.

9.8 SELF ASSESSMENT QUESTIONS

Essay:

(1) What is seed germination, explain the role of different factors involving in seed germination.
(2) What is seed dormancy, explain different factors that cause dormancy.

Short notes:

(1) Scarification

(2) Stratification

(3) Physiology of seed germination

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- **K.RAMA KRISHNA**

LESSON 10

STRUCTURE & FUNCTIONS OF CELL MEMBRANE AND NUCLEUS

10.0 OBJECTIVES

The objectives of this topic are

- To know about the structure of cell membrane
- To learn functions of cell membrane
- To know about structure and functions of nucleus.

STRUCTURE

10.1 INTRODUCTION
10.2 CHEMICAL COMPOSITION
10.3 STRUCTURE
10.3.1 TRILAMELLAR MODEL
10.3.2 BIMOLECULAR LEAF LET MODEL
10.3.3 LATTICE MODEL
10.3.4 FLUID MOSAIC MODEL
10.3.5 MICELLAR MODEL
10.4 ORIGIN
10.5 FUNCTIONS
10.6 INTRODUCTION
10.7 OCCURRENCE
10.8 SHAPE & SIZE
10.9 STRUCTURE OF INTERPHASE NUCLEUS
10.9.1 NUCLEAR MEMBRANE
10.9.2 NUCLEAR SAP
10.9.3 CHROMATIN MATERIAL
10.9.4 NUCLEOLUS
10.10 FUNCTIONS
10.11 SUMMARY
10.12 TECHNICAL TERMS
10.13 SELF ASSESSMENT QUESTIONS
10.14 REFERENCES

CELL MEMBRANE

10.1 INTRODUCTION

Plasma membrane or **cell membrane** is the outermost living boundary of the cell which separates it from the environment and through which the materials entering or leaving the cell must pass (Fig. 10.1). The term plasma membrane was coined by **Nageli** in 1885. The existence of this selectively permeable membrane was inferred even before the advent of electron microscope. This inference was based on the fact that cells could swell and shrink, and when their surface was torn out by a dissecting needle, the contents would ooze out. Studies with electron microscope confirmed the universal existence of plasma membrane. As the plasma membrane is the limiting membrane present in all cells and cell organelles it is also called **bio membrane**.

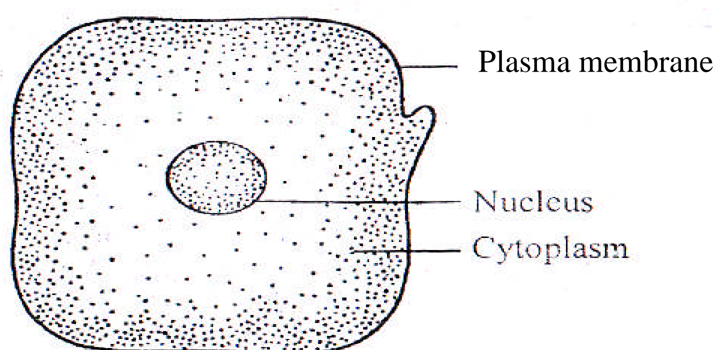

Fig: 10.1 Plasma membrane

10.2 CHEMICAL COMPOSITION

Plasma membrane is about 75 A^0 in thickness and closely appressed to the cell wall. However, it can be seen in a plasmolysed cell when it detaches itself from the wall. The plasma membrane is selectively permeable membrane. Therefore it regulates the entry and exit of substances. Plasma membrane is formed of proteins and lipids. These molecules are arranged in a definite pattern. Some contain polysaccharides, RNA and DNA. It also contains water. The main bulk of plasma membrane is formed of lipids 75%. The main lipid component of the plasma membrane is phospholipids. About 5 important phospholipids are seen. The lipids of the cell membrane are polar lipids. They contain hydrophilic heads and hydrophobic tails.

The protein of plasma membrane has high molecular weight. Three different classes of proteins occur in plasma membrane. They are structural proteins, carrier proteins and enzymes. The structural proteins form the backbone of the cell membrane. The carrier proteins are involved in active transport. The enzymes include ATPase, phosphatase, hexokinase, RNAase and esterase. In some cases the plasma membrane contains carbohydrates. It forms a cell coat around the plasma membrane. Plasma membrane of RBC contains carbohydrates such as hexosamine, fucose and sialic acid. Plasma membrane of Amoeba proteins contains a large amount of polysaccharides. The plasma membrane of nucleus, endoplasmic reticulum contain RNA, and mitochondria, chloroplasts contain DNA.

10.3 STRUCTURE

The plasma membrane is invisible under light microscope. The permeability properties of the membrane gave first indication of the membrane structure. The permeability properties observed for plasma membrane are

(a) Ionic (or) electrically charged compounds penetrate more slowly than non- ionic compounds.

(b) Small molecules penetrate more rapidly than large molecules.

(c) More soluble a compound in a lipid, more rapidly it can enter a cell.

These properties of plasma membrane suggest that

(a) The membrane is carrying a positive electric charge that could impede the passage of positive ions.

(b) The membrane contains pores through which small molecules can pass.

(c) The membrane consists of at least a part of a lipid or fatty film into and out of which the fat soluble compounds can move.

The plasma membrane is mainly formed of proteins and lipids and the term unit membrane can be applied to this. The protein constituent gives to the cell the structural integrity and flexibility. Since the protein molecules can fold and unfold the cell membrane can also expand and contract and through molecular spacing control which molecules can enter the cell from outside environment or pass to the environment from inside of cell. This sort of membrane is said to be selectively permeable. Physiological, analytical and microscopical studies are consistent to the membrane structure that a lipid layer is sand witched between two protein layers.

Many models are proposed to explain the structure of plasma membrane. They are

1. Trilamellar model
2. Bimolecular leaflet model
3. Lattice model
4. Fluid mosaic model
5. Micellar model

10.3.1 TRILAMELLAR MODEL

This was proposed by **Robertson**. In electron microscopic view the plasma membrane shows three layers (trilamellar) (Fig. 10. 2).

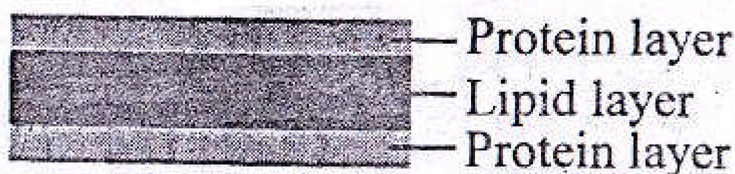

Fig. 10.2 : Trilamellar Model

Outer protein layer, middle lipid layer and inner protein layer. The middle layer is 35 A^0 thick and the inner and outer layers are 20 A^0 each. Such membranes with protein lipid protein arrangement are called unit membrane. According to Robertson all biological membranes are unit membranes and this concept is called **Unit membrane hypothesis** (Fig. 10.3).

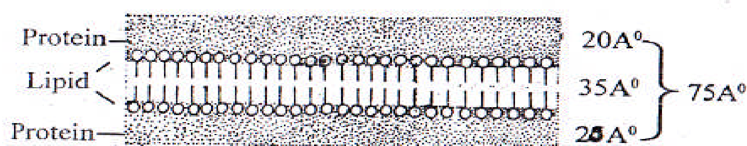

Fig. 10.3: Unit membrane model

Robertson believed that all biological membranes are unit membranes. The plasma membrane of prokaryotes, eukaryotes, membranes of endoplasmic reticulum, golgi bodies, mitochondria, lysosomes, plastids and nucleus are unit membranes.

10.3.2 BIMOLECULAR LEAFLET MODEL

This was proposed by **Daveson** and **Danielli**. According to this model the middle colorless layer shows two rows of phospholipid molecules sandwitched between two dark layers composed of protein molecules. Each lipid molecule has a hydrophobic tail and hydrophilic head. The hydrophilic heads face outwards and the hydrophobic tails of the two layers face each other (Fig. 10.4).

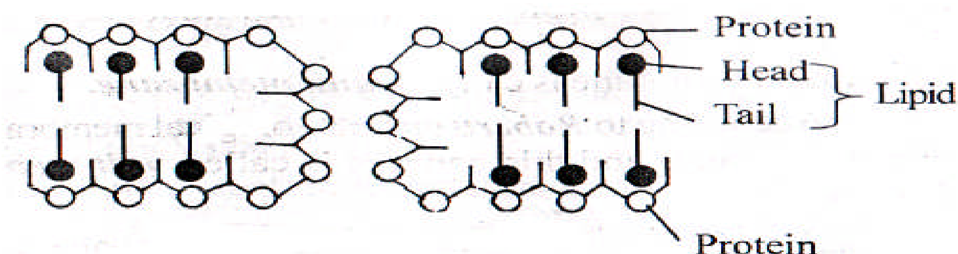

Fig. 10.4 : Bi-molecular leaflet model

Since the protein molecules can fold and unfold the cell membrane can also expand and contract and through molecular spacing control which molecules can enter the cell from outside environment or pass to the environment from inside of cell. This sort of membrane is said to be selectively permeable. Physiological, analytical and microscopical studies are consistent to the membrane structure that a lipid layer is sandwitched between two protein layers.

10.3.3 LATTICE MODEL

This model was proposed by **Wolpers** in 1941. According to this model, in the plasma membrane lipids and proteins are arranged as a lattice or network. Proteins form a kind of mechanical frame work. The lipid component is distributed in the meshes of the protein frame (Fig. 10.5).

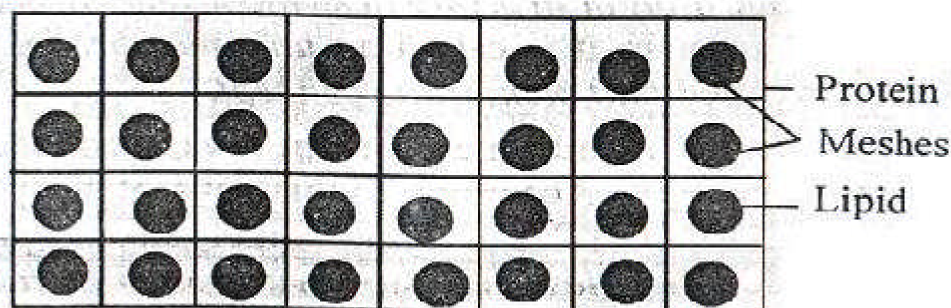

Fig **10.5** *: Lattice model.*

10.3.4 FLUID MOSAIC MODEL

This was proposed by **Singer** and **Nicholson**. According to this model the plasma membrane consists of two layers and the protein molecules are embedded among the lipid molecules. The two layers of lipids are an outer lipid layer and an inner lipid layer. Each lipid molecule has a hydrophobic tail and a hydrophilic head. The hydrophilic heads face outwards and the hydrophobic tails of the two layers face each other. The protein molecules are globular and are of two types peripheral or extrinsic proteins and integral or intrinsic proteins. The peripheral proteins are arranged on the surface and are loosely bound to lipid. The integral proteins are deeply embedded and are tightly bound to the lipid molecules (Fig. 10.6).

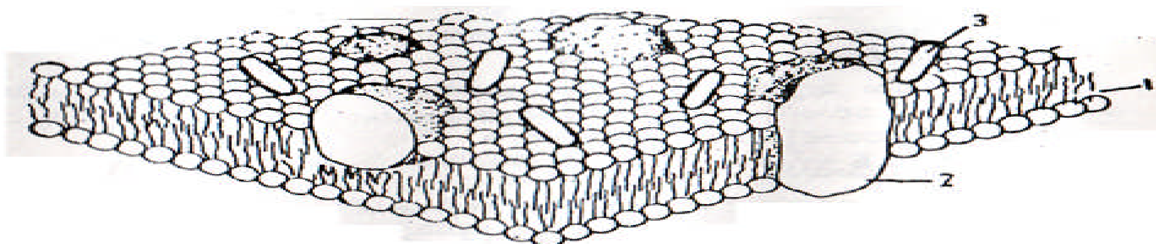

Fig: 10.6 Fluid Mosaic model

1. Lipid staram 2. Proteins 3. Paratheeya Proteins

This model also states that the plasma membrane is a semisolid layer which shows elasticity and differential permeability is facilitated by the arrangement of lipid molecules in a liquid matrix state accommodating protein molecules as floating masses in a mosaic pattern. As the ice bergs float in an ocean, similarly the protein molecules appear to be floating in the ocean of lipid molecules. This arrangement of protein and lipid molecules help the membrane to regulate the entry and exit of substances through it. Hence it plays an important role in the osmo-regulatory process of cells.

10.3.5 MICELLAR MODEL

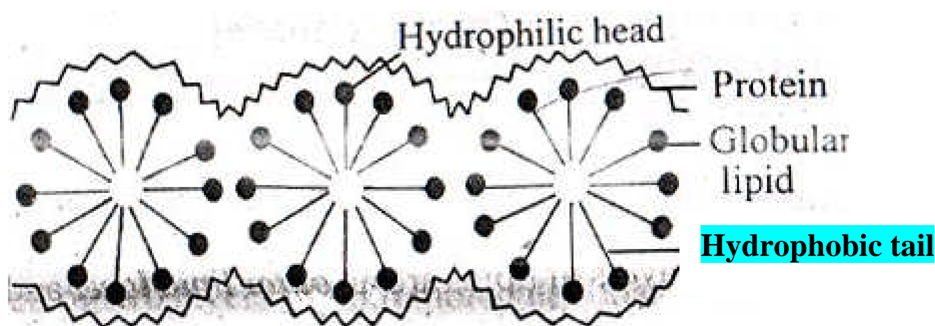

Fig 10.7 Micellar Model

This was proposed by **Hiller** and **Hoffman**. According to this model, the molecules in the plasma membrane are arranged in the form of globular subunits called **micelles**. The lipid micelles are the building blocks of the membrane and the protein globules are arranged on either side. In the lipid micelle, the lipid molecules are arranged in the form of a circle. Each lipid molecule has a hydrophilic head and hydrophobic tail. The tail is directed inwards (Fig. 10.7).

10.4 ORIGIN

It is believed that plasma membrane is formed by the self assembly of proteins and lipids present in the cytoplasm. In the plasma membrane specialized structures are seen here and there. These may be due to outgrowths or ingrowths or contact with adjacent membranes. These structures include microvilli, desmosomes, gap junctions, tight junctions, interjunctions, basal infoldings, plasmodesmata etc.

10.5 FUNCTIONS

(1) Plasma membrane gives the shape to the cell.

(2) It protects cell contents, cell organelles, and keeps the cell contents in place.

(3) Plasma membrane is selectively permeable. It regulates the exchange of materials into and out of the cell selectively. Plasma membrane is a semi permeable membrane. It is used for the transport of molecules and for maintaining equilibrium.

(4) Helps in digestion through picno and phagocytosis.

(5) Being permeable to water, water can pass through it both actively and passively.

(6) lasma membrane controls the ions transport into the cell or outside to the cell. Plasma membrane helps to improve the chemical and constructive relationship between neighbouring cells.

(7) Helps in the formation of cytoplasmic organelles.

(8) Regulates the out flow of excretory material and water from the cell.

(9)Maintains the balance between the osmotic pressure of the intercellular fluid and that of the interstitial fluid.

NUCLEUS

10.6 INTRODUCTION

Nucleus was first discovered by **Robert Brown** 1831. The nucleus is the most essential organelle of the cell which commands cell metabolic events. So it is referred to as **controlling centre of the cell.**

In eukaryotes the nucleus is surrounded by a nuclear membrane (Fig. 10.8).
But in prokaryotes the nucleus is not surrounded by a nuclear membrane. Such a nucleus with a nuclear membrane is called **nucleoid**.

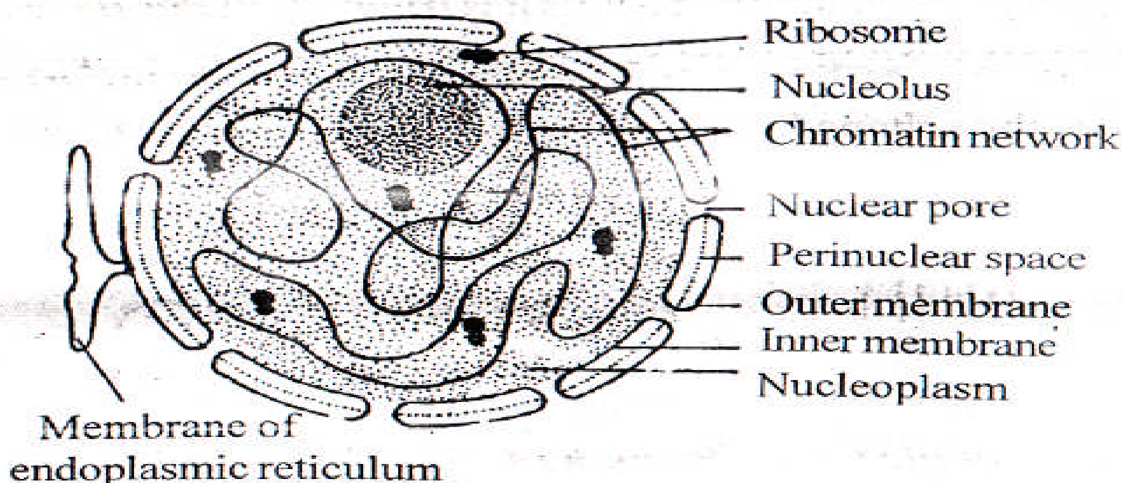

Fig 10.8 A typical nucleus.

10.7 OCCURRENCE

A single well defined nucleus is present in all eukaryotic cells (**monokaryotic**). However, it is absent in RBC and some lens cells of eye (**anucleate**). In ascomycetous and basidiomycetous fungi two nuclei are found in each cell (**dikaryotic**). In algae like *Vaucheria*, fungi like *Mucor* and *Rhizopus* many nuclei are present (**multinucleate**).

The position of the nucleus in a cell is variable. In meristematic cells generally it is situated in the centre of the cell. As the cell undergoes physiological differentiation, there will be a change in the position of the nucleus. In some glandular cells, it lies in the basal region.

10.8 SHAPE AND SIZE

It is usually correlated to the number of chromosomes. The nuclei having triploid or tetraploid chromosomes are comparatively larger than those having diploid chromosomes. The shape of the nucleus varies considerably. It is generally spherical. But it may be oval, fusiform elliptical or flattened. Shape of the nucleus also depends upon the cell size.

The size of the nucleus also varies in different organisms and in different cells of an organism. Generally it is 5-25u in diameter. It is very small in fungi and very large in *Trillium.*

In general a relation exists between the nuclear volume and the volume of the cytoplasm. **Hertig** has identified a numerical formula to this relationship called **nucleoplasmic index (Np).** The lack of maintenance of NP ratio acts as a stimulus to cell division.

NP = vn/(vc-vn) where

Np = Nucleoplasmic index

Vn = Nuclear volume

Vc = Cytoplasmic volume

10.9 STRUCTURE OF INTERPHASE NUCLEUS

A cell has two phases, an interphase or period of nondivision and divisional phase. The interphase cell is metabolically active. The nucleus of the interphase cell is called interphase nucleus . The interphase nucleus shows four main parts. Nuclear membrane, nuclear sap, chromatin material and nucleolus.

10.9.1 Nuclear Membrane (or) Karyotheca

The nucleus is separated from the cytoplasm by a semipermeable membrane, the nuclear membrane or nuclear envelope. It is present in interphase and prophase nuclei. It is double layered, and both membranes are unit membranes similar to plasma membrane. Each layer is about 70-80A^o thick. The outer layer is called **ectokaryotheca** and the inner layer is called **endokaryotheca**, they are separated by a **perilnuclear space** which is about 200-400 A^o . The space is filled with a fluid. The outer surface of the outer membrane is rough due to the presence of ribosomes attached to it, while the inner membrane is smooth. At places, the outer membrane is continuous with the membranes of ER. ER is rapidly attached to the nuclear membrane. Formation of nuclear membrane from ER at the end of cell division, absence of NM in bacteria where ER is absent, demonstrates the close relationship of NM and ER. In the last stage of cell division nucleus is surrounded by nuclear Membrane.

Nuclear Pore

The nuclear membrane contains many pores called nuclear pores. They are spherical or octagonal in shape. At the rims of pores, the two inner and outer nuclear membranes are continuous. The nuclear pores were first demonstrated by **Callan** and **Tomlin** (1950) in amphibian oocytes. The number of pores in unit area of nuclear envelop varies with the cell type and with the physiological state of cell. The pores may be arranged in rows, clusters or hexagonal pattern (or) they may be randomly distributed. Sections through the nuclear pore show the presence of an electrondense ring or cylinder within the pore. This electrondense material has been called as the annulus. The outer end of the annulus extends into the cytoplasm and the inner end extends into the nucleoplasm. The annulus along with the pores form a pore complex (Fig. 10.9).

Franke and his coworkers (1966-70) suggested a model for the pore complexes. According to this model the annulus consists of 8 radially arranged subunits called microtubules (or) microcylinders (100 A^o). These are present around the periphery of the pore. So these are also called peripheral granules, within the pore, a central granule (40-350 A^o) is present. The central granule is connected to the peripheral granules by means of fine fibrils (30 A^o) forming a cart wheel like structure. These fibrils are called **axial filaments**.

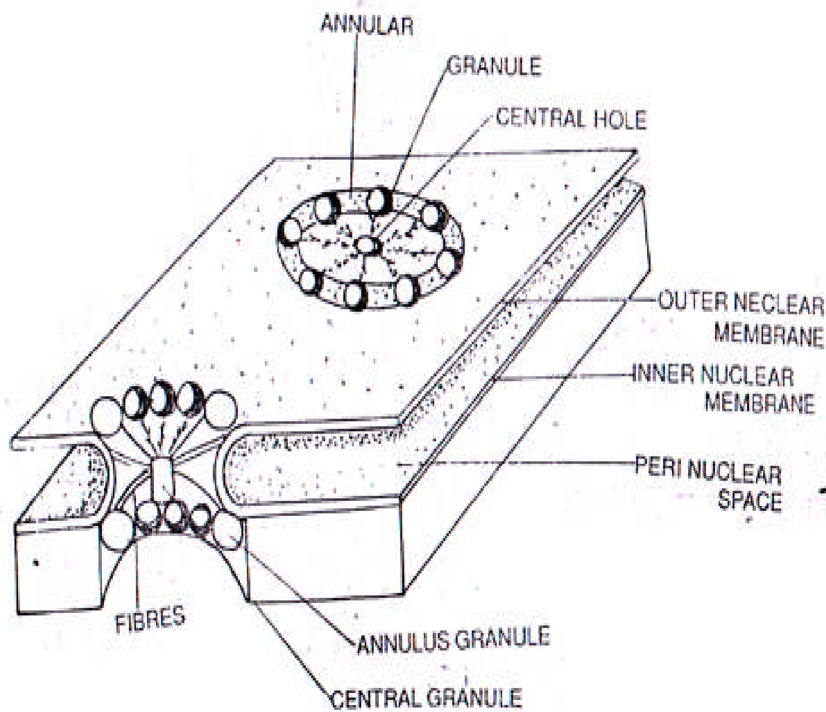

Fig: 10.9 Franks model of Nuclear proe

Recently **Unwin** and **Mulligan** (1982) proposed a computerized image processing technique for the pore complex. According to this, pore complex consists of two rings (annuli) with an inside diameter of 80 mm. Large particles are present forming the central plug (c). Eight "**radical spokes**" (s) are present that extends from the plug to the rings. Sometimes particles are seen attached to the cytoplasmic side of the ring (p) which are arranged octogonally. These particles may be inactive ribosomes attached to the periphery of the pore complex (Fig. 10.10).

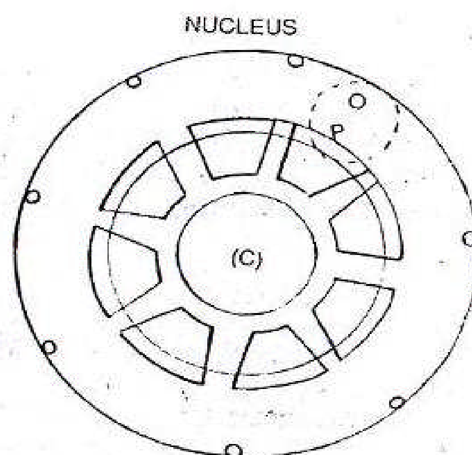

Fig 10.10 : Diagramatic representation of pore comples (R-peripheralring S-central spoke, C-central plug, P-cytoplasmic particle)

The pores can be opened and closed. Exchange of materials occurs through the nuclear pores. The annulus regulates the exchange of macromolecules in relation to their size and chemical nature.

FUNCTIONS OF NUCLEAR ENVELOPE

(1) Nuclear envelope is an interface between nucleoplasm and cytoplasm. It serves to separate the genetic component of the cell (chromosome) from the protein synthesis machinery (ribosomes and ER). It is thus provides protection to DNA against the mutagenic effects of cytoplasmic enzymes.

(2) Transport of macromolecules, small organic materials, water and ions of various substances enter or leave the nucleus through nuclear membrane.

(3) **Dupra** (1965) and **Okada** (1970) found that the chromatin fibers of interphase nucleus are attached to the annuli of the nuclear pore. The attachment sites probably maintain the interphase chromatin fibers in fixed position inside the nucleus. They may also function as initiation points for DNA synthesis.

10.9.2 NUCLEAR SAP

The nucleus is filled with a homogenous, transparent, granular, acidophilic, semifluid known as **nuclear sap** or **nucleoplasm** or **karyolymph** or **nuclear matrix**. It does not take any stain. A variety of substances are dissolved or suspended in it. It is rich in proteins, but a small percentage of DNA, RNA and phospholipids are also present. It is the site of enzyme activity. It has enzymes such as DNA polymerase, RNA polymerase, ribonuclease, alkaline phosphatase and dipeptidase.

NUCLEAR SAP FUNCTIONS

(1) Replication of DNA takes place in the nuclear sap.

(2) Transcription of RNA takes place in the nuclear sap.

(3) Transport of materials occurs in the nuclear sap.

(4) Formation of spindle takes place during cell division.

10.9.3 CHROMATIN MATERIAL

The chromosomes as such are not visible in interphase nucleus. During this stage, the chromosomes exhibit little coiling and appear as fine thread like structures. These fibers are called **chromatin fibers** or **chromonemata**. The chromatin fibers disperse in the form of distended network reticulum. It takes deep stain. This deeply stained network like substance embedded in the nucleoplasm is called **chromatin material** or **chromatin reticulum**. It appears as a network of fine threads. It was first observed by **Hofmeister**. This network readily stains with basic dyes during cell division. This reticulum condenses into thick rod shaped called chromosomes. The chromosome number is constant for every species. Chromatin is regarded as the most important chemical constituent of the nucleus. It contains DNA, RNA and proteins in a compact form. Proteins are of two types, **Histones** and **Nonhistones.**

The Histones are the basic protins and have a fixed ratio with DNA, usually 1:1. Five classes of histones have been recognized (H_2A, H_2B, H_3, H_4 and H_1). The first four classes of

histones have a high content of acidic and basic amino acids. Nonhistone proteins are concerned with gene expression. RNA polymerase is a major enzyme which is a Nonhistone protein.

The interphase chromatin is either distributed loosely or form variously shaped dense clumps (coiled). The uncoiled regions take little stain and are called **euchromatin**. The dense clumps are densely stained and are called **heterochromatin**. Both euchromatin and heterochromatin are formed of DNA. The heterochromatin contains inactive genetic material, whereas the euchromatin contains active genetic material. During synthetic period (S-phase) of interphase the heterochromatin regions replicate after the replicataion of euchromatin regions. Two types of heterochromatin can be recognized, Constitutive and Facultative. **Facultative heterochromatin** represents a temproary state of inactivation of chromatin in which one chromosome of the pair becomes partially or totally heterochromatic. **Constitutive heterochromatin** represents a more permanent future, in which both chromosomes of a pair becomes heterochromatic. It is chiefly associated with nucleolus in both plants and animals.

Electron microscopic and biochemical evidences indicate that the chromatin is like a string of beads, Each bead is a discrete unit and is called **nucleosome**. Each nucleosome is a flat disk shaped particle with about 11nm diameter and 5-7 nm length. Each nucleosone consists of 200 base pairs of DNA and two copies of each of histone proteins H2A, H2B, H3 and H4 called **core histones**. The histone proteins from an octamer, which is coiled by DNA in a helical manner, making two turns. The nucleosomes are connected by the linker DNA comprising about 146 base pairs and H1 histone proteins seals the octomer.

FUNCTIONS OF CHROMATIN MATERIAL

Chromatin is regarded as the most important chemical constituent of the nucleus. It contains chromosomes which are involved in heredity. The majority of the activities of the cell which are under the control of nucleus occur in chromatin material only.

10.9.4 NUCLEOLUS

The number of nucleoli varies from species to species and depends on the number of chromosomes sets. The size of nucleoli is related to the synthetic activities of the cell.

In every nucleus 1, 2 or many spherical, deeply stained bodies called nucleoli are found. These are first reported by **Fontana** (1781). Nucleoli are distinct in the interphase nucleus. They disappear at prophase, remain indistinct during metaphase and anaphase and reappear only during telophase. Nucleoli are often associated with the nucleolar organizing portion of the chromosomes.

The amorphous potion has low electron density. It is the proteinaceous ground substance in which granules and fibrils remain suspended. The fibrillar portion consists of many fibrils called nucleonema, which are formed of ribonucleoproteins.

The chromatin consists of fibrils present around the nucleolus extending into it contains DNA. The granular portion occurs at the periphery of the nucleus. It is composed of RNA and proteins called ribonucleo protein (RNP) granules.

Under the light microscope, the nucleolus appears as a fluid or semifluid or semisolid body of homogenous consistency. Under Electron microscope, it shows amorphous matrix, chromatin granules and fibrils.

Nucleolus contains nucleotides, ribosomal RNA, phospho proteins, acid phosphotase, nucleoside phosphorylase. Nucleoli are useful in synthesis of ribosomes.

FUNCTIONS OF NUCLEOLUS

Nucleous is one of the most active sites of RNA synthesis. It produces about 70-90 percent of cellular RNA . Nucleolus is the main source of RNA.

- Nucleous is related to the biogenesis of ribosomes this process includes

(1) Transcription of the genes that code for RNA .

(2) Processing of periribosomal molecules.

(3) Assembly of ribosomal subunits.

- Nucleolus plays a significant role in mitosis.

10.10 FUNCTIONS OF NUCLEUS

Nucleus is the dynamic centre of the cell. It regulates all physiological activities of the cell. It is therefore referred to as **Cell brain.** Cell division starts with this.

It carries the characters of parents to their offspring in heredity. It has the following functions.

(1) Nucleus controls majority of the activities of cell.

(2) Since the nucleus contains DNA molecules in its chromosomes, it plays an important role in heredity.

(3) The Synthesis of ribosomal RNA takes place inside the nucleus

(4) Nuclear membrane is an interface between nucleoplasm and cytoplasm. Transport of macromolecules, small organic materials, water and ions of various substances enter or leave the nucleus through nuclear membrane only.

(5) During cell division the events like replication of DNA, transcription of RNA, transport of materials takes place in the nuclear sap.

(6) Chromatin is regarded as the most important chemical constituent of the nucleus. It contains chromosomes which are involved in heredity.

10.11 SUMMARY

A plant cell has two main parts, cell wall protoplasm. The protoplasm is the seat of a number biological processes. It is bounded by a thin membrane called plasma lemma or plasma membrane or cell membrane. It is about 7.5 nm, or 75 A^{o} in thickness and closely appressed to the cell wall. It is selective permeable and is made up of proteins and lipids. These molecules are arranged in a definite pattern. Models such as trilamellar model, bimolecular leaf the model, lattice model, fluid mosaic model, micellar have been proposed to explain the structure the plasma membrane. It is believed that plasma membrane is originated by the self assembly proteins and lipids present in the cytoplasm. It is involved in may functions of the cell such a giving shape to the cell, protection of cell content controlling diffusion of ions into and out of the nucleus was first discovered by **Robert Brown** 1831 and is the most essential organelle of the cell. It is referred to us the controlling centre of the cell. A single well

defined nucleus is present in all eukaryotic cells, however variation is observed in lower groups of plants. It shape and size usually are correlated to the number of chromosomes. The cells having more sets of chromosome number are usually larger in size than those having diploid set of chromosomes. The interphase cell is metabolically active and it shows mainly four parts. They are nuclear membrane, nuclear sap, chromatin material and nucleus. Each part is having various functions and in general nucleus is involved in heredity of the cell.

10.12 TECHNICAL TERMS

Cell membrane, plasma membrane, plasma lemma, Trilamellar model, bimolecular leaf let model, lattice model, fluid mosaic model, micellar model, nucleus, controlling centre, interphase, nuclear membrane, nuclear sap, chromatin material, nucleolus, heredity.

10.13 SELF ASSEMSEMNT QUESTIONS

1. Give an account on structure and functions of plasma membrane.
2. Enumerate an account on different models explaining the structure of plasma membrane.
3. Write an account on structure and functions of nucleus.
4. What is the structure of interphase nucleus?

SHORT NOTES

1. Fluid mosaic model
2. Nuclear pore
3. Chromatin
4. Nucleolus
5. Unit Membrane
6. Functions of Plasmamembrane
7. Nucleus

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LESSON 11

STRUCTURE & ORGANIZATION OF CHROMOSOME

11.0 OBJECTIVES

The Objectives of this topic are

- To know about the general morphology of the chromosome such as shape, size, number and structure
- To understand about euchromatin and heterochromatin
- To learn organization of eukaryotic chromosome and functions of chromosomes
- To learn different types of chromosomes

Structure

11.1 INTRODUCTION

11.2 GENERAL MORPHOLOGY OF CHROMOSOME

11.2.1 CHROMOSOME SHAPE

11.2.2 CHROMOSOME SIZE

11.2.3 CHROMOSOME NUMBER

11.2.4 CHROMOSOME STRUCTURE

11.2.4.1 CHROMONEMATA

11.2.4.2 PRIMARY CONSTRICTION

11.2.4.3 SECONDARY CONSTRICTION

11.2.4.4 SATELLITE

11.2.4.5 TELOMERES

11.3 EUCHROMATIN AND HETEROCHROMATIN

11.4 ULTRASTRUCTURE OF CHROMOSOME

11.4.1 MULTISTRANDED MODEL

11.4.2 FOLDED FIBRIL MODEL

11.5 ORGANIZATION OF EUKARYOTIC CHROMOSOME

11.5.1 ARRANGEMENT OR PACKING OF CHROMATIN IN CHROMOSOMES

11.6 FUNCTIONS OF CHROMOSOMES

11.7 TYPES OF CHROMOSOMES

11.7.1 POLYTENE OR SALIVARY GLAND CHROMOSOMES

11.7.2 LAMP BRUSH CHROMOSOMES

11.7.3 SUPERNUMENARY CHROMOSOMES

11.8 SUMMARY

11.9 TECHNICAL TERMS

11.10 SELF ASSESSMENT QUESTIONS

11.11 REFERENCE BOOKS

11.1 INTRODUCTION

Chromosomes are self-reproducing, thread like structures located inside the nucleus. They are called chromosomes (chroma-colour, soma-body) because they are easily stained with dyes. They are the carriers of genes and are popularly known as '**hereditary vehicles**'. **Karl Nageli** (1842) observed deeply stained thread like structures in the nucleus. Later **Hofmeister** (1848) identified such deeply stained dark rod structures in the pollen mother cells of *Tradescantia*. However, they were named as **chromosomes** by **Waldeyer** (1888).

11.2 GENERAL MORPHOLOGY

The chromosome under light microscope consists of two rod like structures called **chromatids** joined with each other by **centromere**. The portions of chromatids on either side of the centromere are called **chromosome arms** and ends of the chromosomes are called **telomeres**. In eukaryotic chromosome there is ample increase in DNA content followed by an increase in the size of the chromosome. They are associated with basic proteins called histones. The shape, size number, and structure of chromosomes are species specific and are remarkably constant under normal environmental conditions.

11.2.1 Chromosome Shape

The shape of the chromosome is largely governed by the position of the centromere. According to the position of the centromere, the chromosomes are classified into four types. They are (Fig. 11.1)

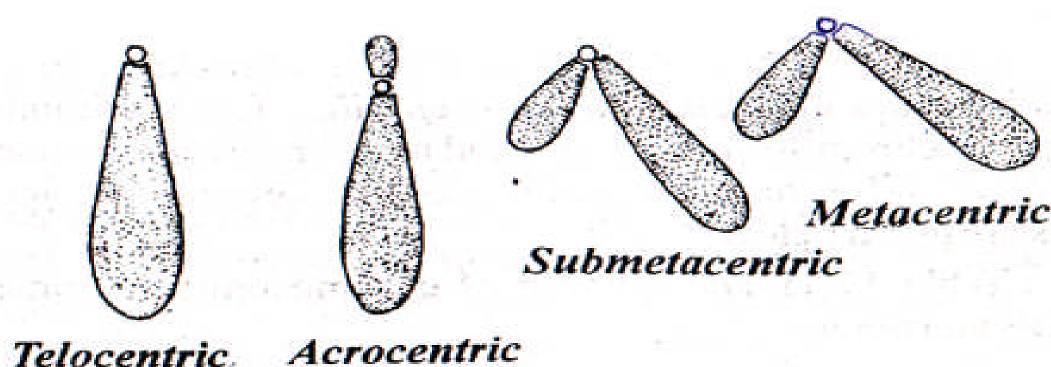

Fig. 11.1 : Different shapes of chromosomes

(a) **Metacentric**: Centromere is present is the middle point. Both arms are equal in length. During anaphase they appear in the shape of 'V'.

(b) **Submetacentric**: Centromere is slightly away from the middle point and arms are unequal. During anaphase they appear in the shape of 'L'.

(c) **Acrocertric**: Centromere is present towards one side. One arm is very long and the other arm is very short. These are rod like chromosomes.

(d) **Telocentric**: Centromere is present at the end of the arm. Only one arm is present. They appear in the shape of `I' during anaphase.

11.2.2 Chromosome Size

Chromosomes are long, cylindrical structures and appear during cell division. The size of a chromosome is generally constant. Mitotic metaphase is convenient for measuring the chromosome size since they are thick, rod shaped and reach maximum condensation during that period. In general, chromosomes of plant cells are larger than those of animal cells. Chromosomes may be as short as 0.25μ (I micron = 0.0001 mm) as in fungi and birds or as long as 32μ as in some plants like *Trillilum*. In angiosperms, monocots generally possess larger chromosomes than dicots.

11.2.3 Number of Chromosomes

The number of chromosomes may vary from species to species but it remains constant for a particular species. The number of chromosomes serves as an aid in the determination of phylogenetic status and taxonomic position of plant and animal species. The number of chromosomes is represented by **diploid** condition which shows 2 sets of chromosomes. Vegetative cells generally show diploid condition (2n). In gametes, only one set of chromosomes is found. This is called **haploid** condition (n). The haploid set of chromosomes which is inherited through a gamete is known as the basic set or **genome**. Two basic sets constitute a diploid set. Sometimes a cell may contain more than two sets of chromosomes. This number is called **polyploidy** (3n, 4n, 5n etc). The diploid complement of an organism which depicts their size, shape, number and structure is called a **karyotype**. The diagramatic representation of karyotype is known as **ideogram**. This chromosome number varies very widely in plant and animal species. In animal kingdom the lowest chromosome number 2n=2 is seen in *Ascaris megalocephala* and in plant kingdom *Haplopappus gracilis* of Asteraceae has the lowest chromosome number 2n=4. In animal kingdom the highest chromosome number of 1600 chromosomes is recorded in *Aulocantha*, a radiolarian (protozoa). In plant kingdom *Ophioglossum* exhibits the highest chromosome number of 2n=1260+10 fragments (Table: 11.1).

Table: 11.1 Number of Chromosomes in some plants.

	Plants		Chromosome number
1.	*Haplopuppus gracilis*	-	4
2.	*Pisum sativum*	-	14
3.	*Allium cepa*	-	16

4. *Brassica oleracea* - 18

5. *Zea mays* - 20

6. *Oryza sativa* - 24

7. *Nicotiana tabacum* - 48

8. *Gossypium hirsutum* - 52

9. *Saccharum officinarum* - 80

10. *Ophioglossum* (pteridophyte) - 1260

11.2.4 Structure of Chromosome

The most ideal stage for studying chromosome structure is metaphase or anaphase at which there is maximum condensation. A typical somatic chromosome has an elongated cylindrical body with two arms called **chromatids.**

In light microscope, the chromosomes appear as elongate bodies with a matrix and two chromatids. The two chromatids are embedded in the achromatic substance known as **matrix.** Matrix is enclosed in a sheath or **pellicle**. However, the electron microscope studies revealed that there is no definite membranous pellicle surrounding the chromosome. A typical metaphase chromosome consists of 5 parts. **Chromonemata, primary constriction, secondary constriction, satellite** and **telomeres** (Fig. 11. 2).

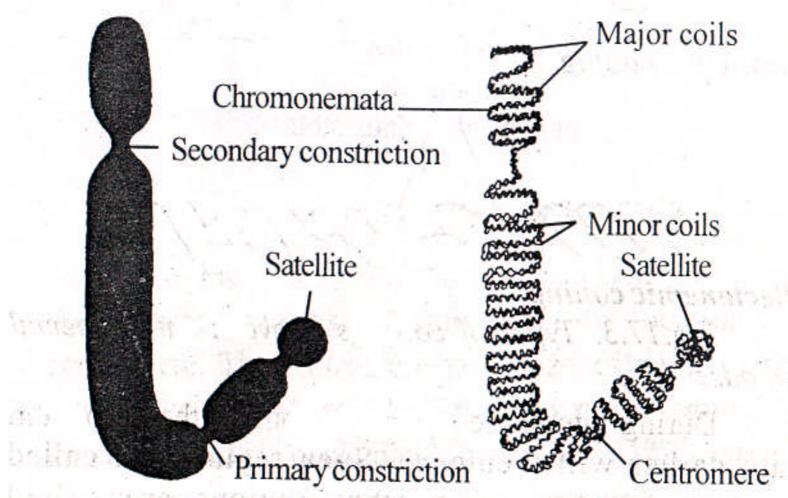

Fig. 11.2: Structure of a typical somatic chromosome

11.2.4.1 Chromonemata

During interphase or early prophase, the chromosomal material becomes visible as very thin, spirally coiled filaments. These filaments are called chromonema(ta). These fibrils of the chromomemata are coiled with each other. The coiling of chromonemata is of two types

namely **paranemic coil** (loose coil, that can be separated easily) and **plectonemic coil** (tight and interwined coil that cannot be separated easily) (Fig. 11.3).

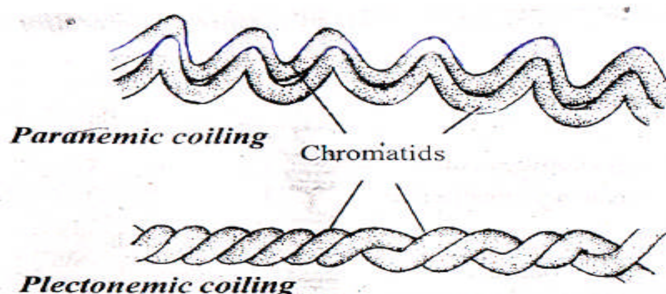

Fig. 11.3 : Types of coiling between chromonemal threads

During early prophase, the chromonemata bear bead like structures called **chromomeres**. The chromomers are the regions of superimposed coils. Sometimes the chromomeres are split up to form to chromatids. The two chromonemata give rise to chromatids at late metaphase. Chromatids are vertical halves of a chromosome attached to each other at centromere.

11.2.4.2 Primary constriction

The chromosome has a constricted, non-stainable part called the primary constriction or centromere. The position of the centromere is constant for a particular chromosome. It divides the chromosome into two arms. The shape of the chromosome is determined by the location of the centromere. Centromere is the region to which spindle fibers are attached during cell division. There are two cup-like discs in the centromere which are called **kinetochores**. They are the sites of the implantation of the microtubules of the centromeres.

11.2.4.3 Secondary constriction

A chromosome may have one or two additional constrictions other than the primary constriction called secondary constrictions. Chromosomes do not bend in the region of secondary constriction during anaphase as the microtubules do not bind with them. Thus they are distinguishable from the primary constrictions. These are constant in position. Thus, secondary constrictions provide the characteristic land marking features for the identification of individual chromosomes as their size, position and number are constant. Certain secondary constrictions are concerned with the formation of nucleoli. Hence, the secondary constrictions are called **nucleolar organizers.**

11.2.4.4 Satellite or Trebent

It is a rounded knob-like body separated from the rest of the chromosome by a secondary constriction. Chromosomes bearing satellites are called **SAT** (Sine acid thymonucleinio= without thymonucleicacid or DNA) **chromosomes**. These are also constant in size and position and are hence useful in the identification of individual chromosomes.

11.2.4.5 Telomeres

The tips of chromosomes are called telomeres. They determine the polarity of the chromosomes. They prevent the fusion of chromosomes with one another by their ends. If a chromosome breaks, the broken ends can fuse due to lack of telomeres.

11.3 EUCHROMATIN AND HETEROCHROMATIN

The coiling of chromonemata is not uniform all along the length of the chromosome. Because of this uneven coiling, the chromosomes exhibit differential staining. The regions with more coils are stained darkly and such regions are called **heterochromatin** regions and are genetically inactive (Fig.11.4). The regions with less number of coils are stained lightly and are called **euchromatin** regions which are genetically active. This differential staining ability is referred to as **heteropycnosis**.

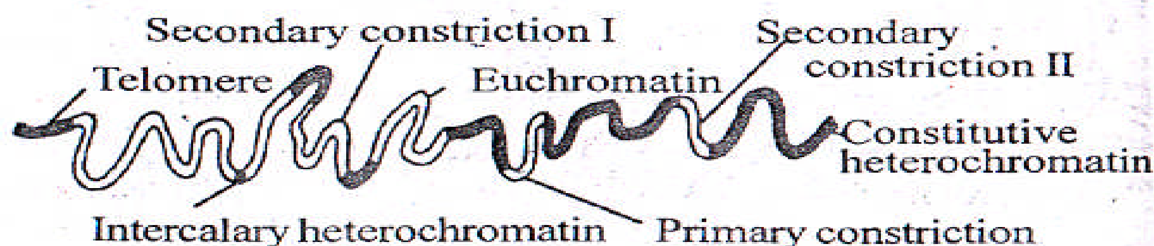

Fig. 11.4 : Structure of heterochromatin

11.4 ULTRA STRUCTURE OF CHROMOSOME

The chromosomes contain DNA and protein. Two views have been proposed to explain the association between DNA and protein in the chromosome. Multistranded model and folded fibril model.

11.4.1 Multistranded Model

This was proposed by **Ris** (1966). According to this model the 20 A^0 wide DNA molecule is associated with histone proteins to forms a 40 A^0 wide DNA fibril. Two such 40 A^0 wide DNA fibrils make up a 100 A^0 fibril called the elementary chromosome fibril. This is the smallest visible unit of the chromosome under electron microscope. Two 100 A^0 elementary chromosomal fibrils wrap around each other to form a 200 A^0 fibril. Thus each 200 A^0 fibrils are associated to form the chromatid of the chromosome. Each chromatids apparently has 16-32

elementary chromosome fibrils. This model is of historical importance and at present scientists believes that there is only single DNA molecule per chromatid.

11.4.2 Folded fibril Model

Taylor (1967) Suggested that the body of the classical chromatid is represented by a single DNA protein fiber, first coiled to form the 25-30 mm fiber and then folded back longitudionally and transversely. This model applies to both interphase and anaphase chromosome. This is also called **unineme theory**.

11.5 ORGANIZATION OF EUKARYOTIC CHROMOSOME

Chromosomes of eukaryotes are made up of chromatin. The chromatin is made of genetic material (DNA) in association with histone proteins. Hence chromosomes are chemically nucleoproteins. The histones are the basic proteins and have a fixed ratio with DNA, usually 1:1. Five classes of histones have been recognized (H2A, H2B, H3, H4 and H1). The first four classes of histones have a high content of acidic and basic amino acids. Nonhistone proteins are concerned with gene expression. RNA polymerase is a major nonhistone protein.

Electron microscopic and biochemical evidences indicate that the chromatin is like a **'string of beads**' structure. Each bead is a discrete unit and these beads were called **nucleosmoes** by **Qudet** (Fig. 11. 5). The nucleosome is an oblate particle of about 11nm diameter and 5-7 nm length. It consists of a **core particle** of histone proteins and a small **spacer DNA** or **linker DNA.** The core consists of an octamer of 8 histone molecules, having two copies each of H_2A, H_2B, H_3 and H_4. The DNA is wrapped around the core to form a super helix. DNA is wrapped around the core by 1.75 turns (146 base pairs). Part of DNA that joins two nucleosomes is called **linker DNA** or **inter-nucleosomal DNA** or **spacer DNA.** Its length is 15-400 base pairs. The H_1 histone molecule is bounded to the linker DNA.

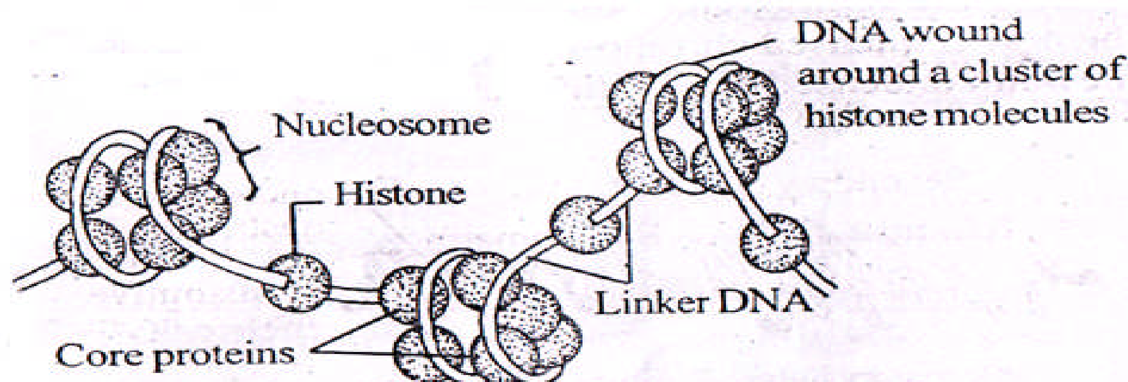

Fig. 11.5 : Beads on a string appearance of nucleoprotein

11.5.1 Arrangement or Packing of Chromatin in Chromosomes

Mitotic chromosomes are made up of a thick fiber, the unit fiber of diameter of 20 - 30 nm. This fiber arises from the folding of the nucleosome chain into a solenoid structure. Each turn of the solenoid contains about 6 nucleosomes. The solenoid is further coiled into a super solenoid structure with a diameter of 400 nm and a wall of 30 nm thick. This whole structure is stabilized by interactions between H_1 protein molecules of neighbouring nucleosomes. This super solenoid structure is the unit fiber of 20 to 30 nm which represents the chromonema of light microscopy. This model was proposed by **Finish** and **Clug**. This is the most likely form of chromatin folding in chromatids (Fig. 11.6).

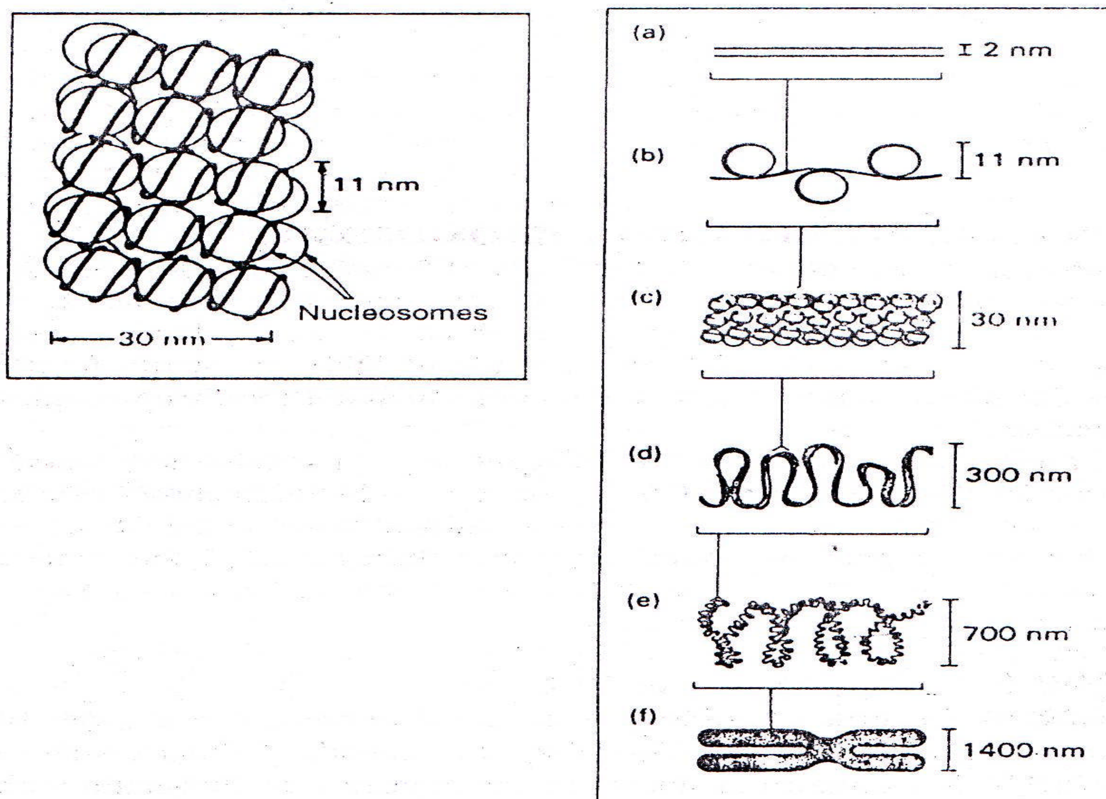

Fig. 11.6 The solenoid model for the 30 nm chromatin fiber. A summary of the different levels of DNA packaging in a chromosome : (a) naked DNA, (b) beads-on-a-string; (c) the 30 nm chromatin fiber; (d) the 30 nm fiber made up of looped 30 nm fiber made up of looped 30 nm chromatin; (e) the 70 nm supercoiled structure that comprises each arm of a metaphase chromosome.

11.6 FUNCTIONS OF CHROMOSOMES

1. Chromosomes are involved in controlling and regulating the activities of a cell
2. They are chiefly concerned with the transfer of genetic traits from one generation to the next. Hence they are referred to as **physical basis of heredity.**
3. **Sutton** and **Boveri** (1902) proposed the **chromosome theory** which states that chromosomes are the **vehicles of heredity.**

4. The heterochromatin helps in the formation of nucleolus.
5. Changes in the position, number and the structure of chromosomes lead to the formation of new species.

11.7 TYPES OF CHROMOSOMES

Normally chromosomes are of two types autosomes and allosomes.

Autosomes: They determine the somatic or vegetative activities of cell like growth, development etc., and they are more in number.

Allosomes: These are also called sex chromosomes. They determine mostly the sexual chracters of organisms. In human beings they are identified as X, Y chromosomes.

In addition to these types of chromosomes, special types of chromosomes are present. They are Polytene chromosomes, Lamp brush chromosomes and Supernumenary chromosomes.

11.7.1 Polytene Chromosomes or Salivary gland Chromosomes

These were discovered by **Balbiani** (1881) in the salivary gland cells of dipteran species like *Chironomas* larva hence they are called salivary gland chromosomes. These are giant chromosomes and larger in size. In *Drosophila melanogaster*, it is 1000 times larger than somatic chromosomes. The larger size of the chromosomes is due to the presence of many longitudional strands called chromonemata. Hence they are also called polytene chromosomes (many stranded) (Fig. 11. 7). The many strands of the giant chromosomes are due to repeated division of the chromosome without the cytoplasmic division. This is called **endomitosis**. The polytene chromosomes contain transverse bands, which consists of alternating dark and light regions. The dark bands are darkly stained and contain more DNA and less RNA. The inter bands are lightly stained and contain more RNA and less DNA. At certain places, the bands become enlarged to form **puffs** or **balbiani rings**. In the region of puffs the DNA unfolds into active loops that actively synthesize RNA.

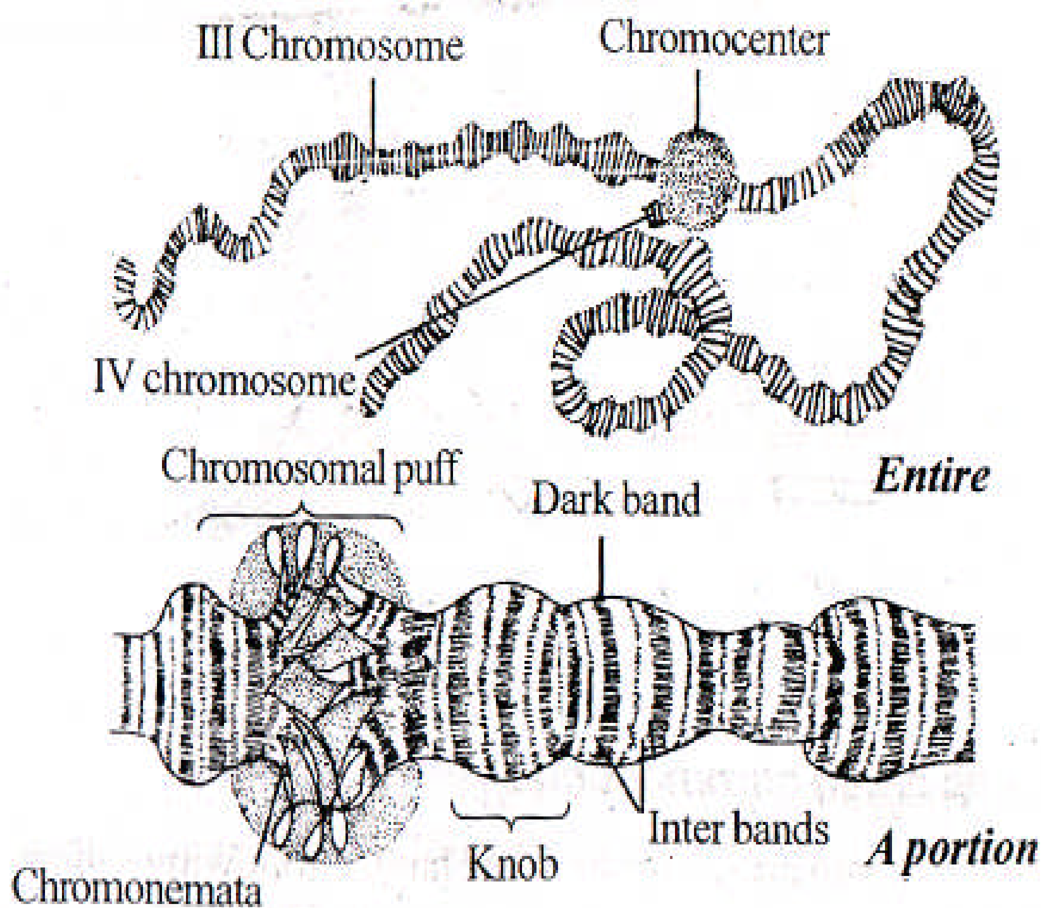

Fig 11.7 : Polytene chromosome

11.7.2 Lamp brush chromosomes

These were discovered by **Ruckert**. They look like the brushes used to clean the chimneys of oil lamps. So they were named as lamp brush chromosomes (Fig 11.8 & 11.9). These were observed at the diplotene stage of meiotic prophase in vertebrate oocytes, spermatocytes of some invertebrates. The chromosomes are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata. Each lamp brush chromosome consists of a main axis and many lateral loops. The main axis of each chromosome is formed of four chromatids. Each chromatid contains a series of thickenings called chromomeres. From each chromomere a pair of lateral loops arise, one on each side. Each loop contains DNA and matrix of ribo-nucleo protein fibrils. Synthesis of proteins takes place in lateral loops.

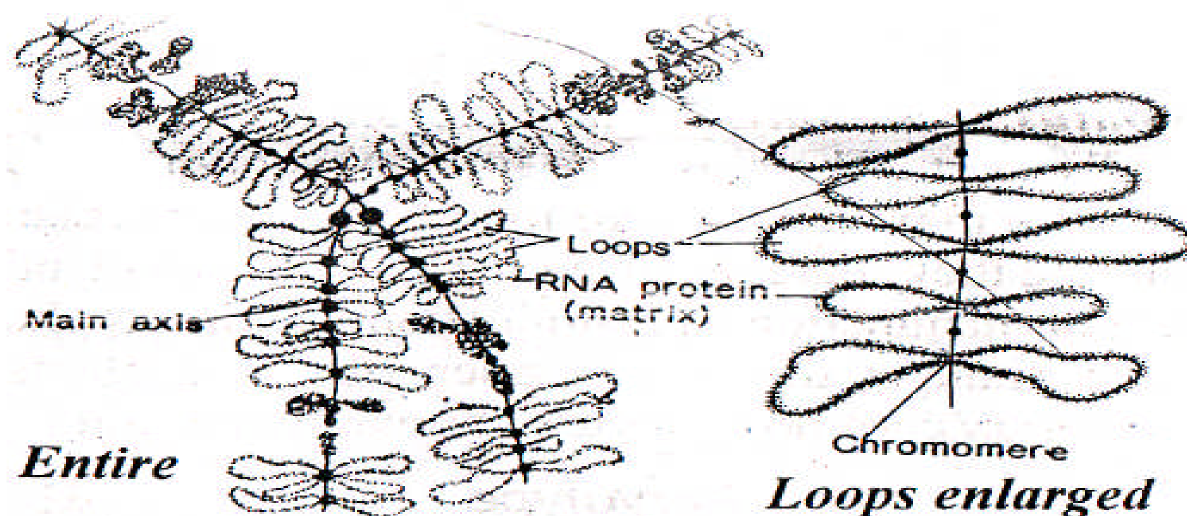

Fig. 11.8 : Lamp brush chromosomes

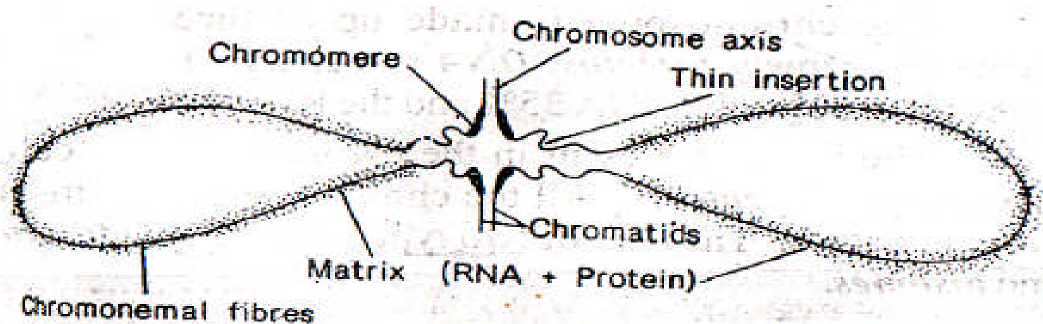

Fig.11.9 : Lamp brush chromosomes with a pair of loops

11.7.3 Supernumenary chromosomes:

The complements of some plant and animal nuclei contain one or more **accessory** or **supernumenary** or **B-chromosomes**, in addition to the normal chromosomes. In general they are smaller in size than the normal chromosomes, and genetically inert. They produce little phenotypic effect of the organism in which they are found. They are relatively unstable and they segregate irregularly at meiosis. In some plants they are entirely euchromatic, while in others they are partially heterochromatic and partially euchromatic.

11.8 SUMMARY

Chromosomes are self-replicating, thread like structures located inside the nucleus. They are popularly known as hereditary vehicles, because they are the carriers of genes. When observed under light microscope, each chromosome consists of two rod like structures called chromatids joined with each other by centromere. The shape of the chromosome is largely

governed by the position of the centromere. Accordingly the chromosomes are classified into four types namely metacentric, submetacentric, acrocentric and telocentric. Mitotic metaphase is convenient for measuring the chromosome size, since they are thick, rod shaped and reach maximum condensation during that period. The number of chromosome may vary from species to species but it remains constant for a particular species. The number of chromosomes seems as an aid in the determination of phylogenetic status, taxonomic position of plant and animal species.

A typical metaphase chromosome consists of five parts viz., chromonemata, primary constriction, secondary constriction, satellites and telomeres. Differential staining of chromatin is observed due to the presence of heterochromatin and euchromatin. The ultrastructre or chromosome can be explained by two models viz., multistrand model and folded fibril model. In eukaryotes the DNA is associated with proteins called histones. Under EM, chromatin appears to have a string of beads. These beads are called nucleosomes. Chromosomes are involved mainly in controlling and regulating the activities of a cell, transfer of genetic traits from one generation to the next etc.

Normally autosomes and allosomes are present. In addition to these special types of chromosomes viz., polytene or salivary gland chromosomes, lamp brush chromosomes and supernumenary chromosomes are present.

11.9 TECHNICAL TERMS

Chromosomes, hereditary vehicles, chromatids, centromere, metacentric chromosomes, submetacentric chromosomes, acrocentric chromosomes, telocentric chromosomes, chromonemata, primary constriction, secondary constriction, satellite, telomeres, heterochromatin, euchromatin, multistrand model, folded fibril model, histones, nucleosomes, polytene chromosomes, lamp brush chromosomes, supernumerary chromosomes.

11.10 SELF ASSESSMENT QUESTIONS

1. Write an account on structure and functions of chromosomes
2. What are the different types of chromosomes
3. Write an account on organization of eukaryotic chromosome

Short notes

1. Chromosome shapes
2. Chromosome number
3. Nucleosome
4. Polytene chromosomes
5. Lamp brush chromosomes
6. Supernumerary chromosomes
7. Euchromatin and Heterochromatin

8. Ultra structure of chromosome

9. Packaging of chromatin in chromosomes

10. Functions of Chromosomes

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LESSON 12

PHYSICO - CHEMICAL PROPERTIES OF DNA & RNA AND DNA REPLICATION

12.0 OBJECTIVES

The objectives of this topic are

- To know about the structure of nucleic acids DNA and RNA
- To study structure and functions of different DNAs and RNAs
- To Understand the mechanism of DNA replication

STRUCTURE

12.1 INTRODUCTION
12.2 STRUCTURE OF DNA
12.3 PROPERTIES OF DNA
12.4 FUNCTIONS OF DNA
12.5 TYPES OF DNA
12.6 STRUCTURE OF RNA
12.7 TYPES OF RNA
12.7.1 MESSENGER RNA
12.7.2 TRANSFER RNA
12.7.3 RIBOSOMAL RNA
12.8 REPLICATION OF DNA
12.9 SUMMARY
12.10 TECHNICAL TERMS
12.11 SELF ASSESSMENT QUESTIONS
12.12 REFERENCE BOOKS

12.1 INTRODUCTION

Nucleic acid is a phosphorous rich macromolecule with acid property, present in the nucleus of cells and cause a number of biochemical activities of cell that lead to the growth and development of organisms. The nucleic acid was first isolated in 1868 by **Frederick Miescher** in the nuclei of pus cells and he called it as **nuclein**. **Altman** (1889) coined the term **nucleic acid**.

Nucleic acids are found in all organisms such as plants, animals, bacteria and viruses. Although DNA is chiefly concentrated in the chromosomes, it can be found in traces in the chloroplast and mitochondria. Transduction experiments conducted by **Hershey** and **Chase** (1954) confirmed DNA as the genetic material and hence it is referred to as '**chemical basis of heredity**'. But in some plant viruses RNA acts the genetic material.

Nucleic acid molecule is a long chain polymer. It is composed of monomeric units called **nucleotides.** Each nucleotide consists of a **nucleoside** and a **phosphate group**. Each nucleoside consists of a **sugar** and a **nitrogenous base** (Fig. 12. 1).

SUGAR: In nucleic acids the sugar is Pentose. In RNA it is called ribose sugar, whereas in DNA it is deoxyribose sugar.

NITROGENOUS BASES: Nitrogenous bases are of two types, namely **purines** and **pyrimidines**. Purines are heterocyclic compounds having two C-N rings and pyrimidines are homocyclic compounds with a single C-N ring. In nucleic acids there are two main purine bases. They are **Adenine** and **Guanine.** Similarly three pyramiding bases are found. They are **Cytosine**, **Thymine** and **Uracil**.

PHOSPHATE GROUP: A nucleotide is derived from a nucleoside by the addition of a molecule of phosphoric acid. In D.N.A four types of nucleosides are present.

Deoxycytidine

Thymidine

Deoxyadinocine

Deoxyguanocine

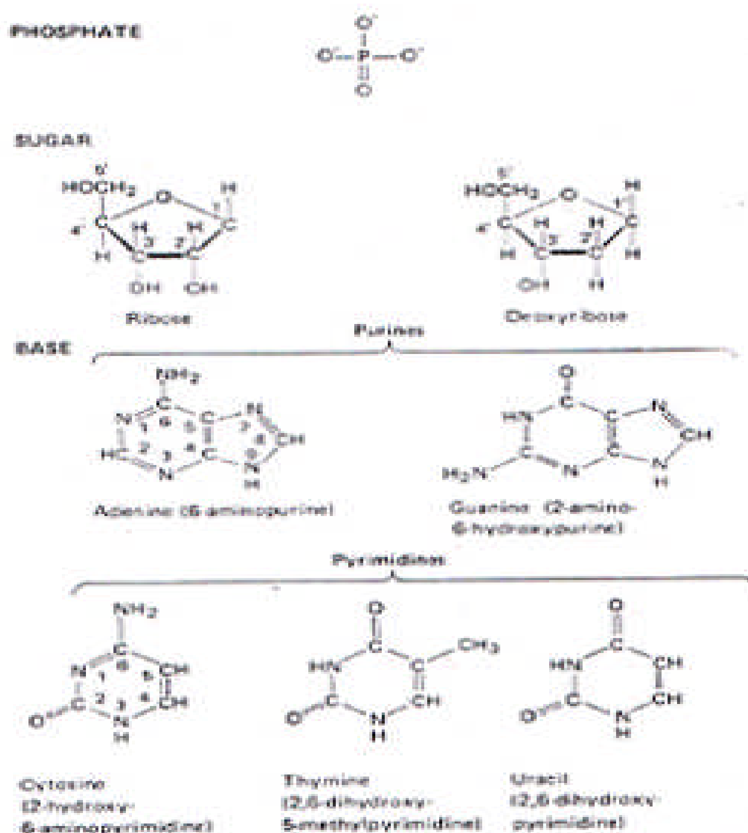
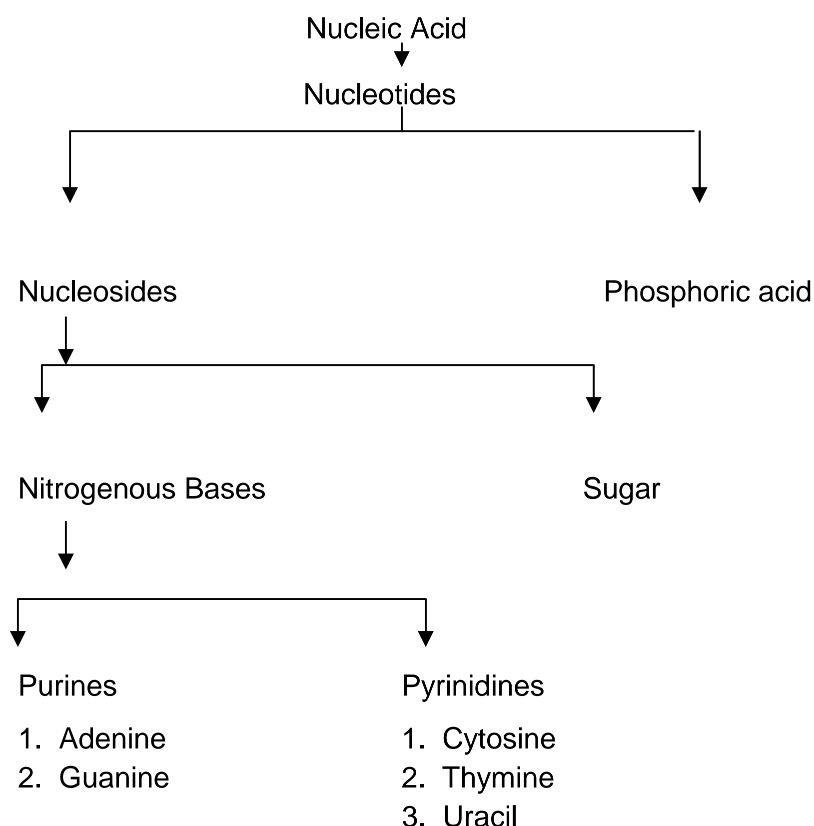


Fig. 12.1 Components of Nucleic acids



Purines
1. Adenine
2. Guanine

Pyrinidines
1. Cytosine
2. Thymine
3. Uracil

A number of nueleotiside units link with one another to form a polynucleotide chain or nucleic acid. Nucleic acids are broadly classified into two types based on the type of sugar preset in them. They are

1. Deoxyribonucleic acid
2. Ribonucleic acid

12.2 STRUCTURE OF DNA

The structure of DNA molecule (**B type**) was proposed by **J. Watson** and **F. Crick** in 1953. So it is called the **Watson and crick model of DNA**. They were awarded with Noble Prize in medicine in 1962 for this work, along with **M. Wilking** who provided X-ray crystallographic proof for the proposed DNA model. According to Watson and Crick, DNA is in the form of a double helix. The technique of X-ray diffraction (crystallography) developed by **Franklin** and **Wilking**, **Chargaff's** chemical analysis**, Pauling's** proposal of hydrogen bonds were very useful in building a model of DNA molecule.

DNA is composed of four deoxy ribonucleotides namely deoxyadenylate, deoxy guanylate, deoxycytidylate and deoxythymidylate (Fig. 12. 2).

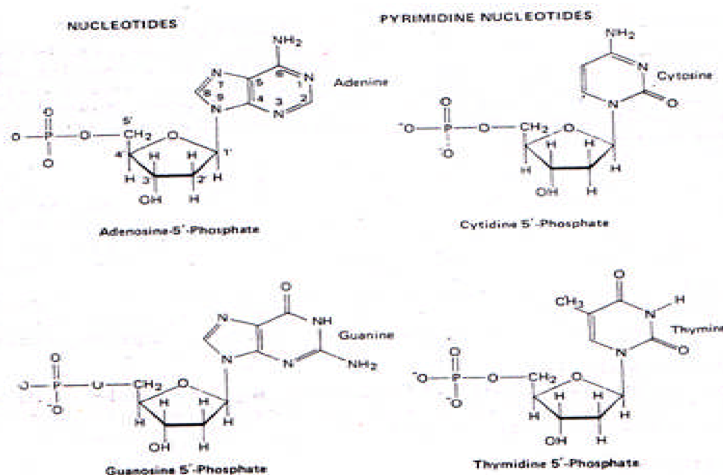


Fig. 12.2 Structure of the four Deoxyribose Nucleotides

It is composed of a two strands which are spirally coiled around one other. The strands are antiparallel to one another and are held closely by hydrogen bonds. The DNA molecule is in the form of double helix. The two polynucleotide chains are coiled around each other to form double helix. The DNA molecule is about 20 A^0 in diametir but its length is uncertain. It is coiled around its own axis and is tightly coiled inside the chromosome arm. DNA molecule looks like a twisted ladder. Each strand of DNA is a polymer of thousands of nucleotides. Hence it is called polynucleotide strand. The two strands are complementary to one another and are linked by nitrogen base pairs which appear as steps. The DNA helix has two external grooves, namely major groove and minor groove. The major groove is wide and deep. The miner groove is wide and deep.

The nitrogenous bases A, T and G, C are complementary to each other linked. **Erwin Chargaff** discovered that purines (A, G) and pyrimidines (C, T) exist in 1:1 ratio. Adenine is complementary to T and vice versce. A is lilnked to T by two weak Hydrogen bonds A=T. G is complementary to C and are linked to each other by three weak hydrogen bonds G≡C. The backbone of DNA polynucleotide strand is formed by alternately arranged phosphate and sugar groups. They are linked with each other by phosphodiester bonds (Fig. 12. 3).

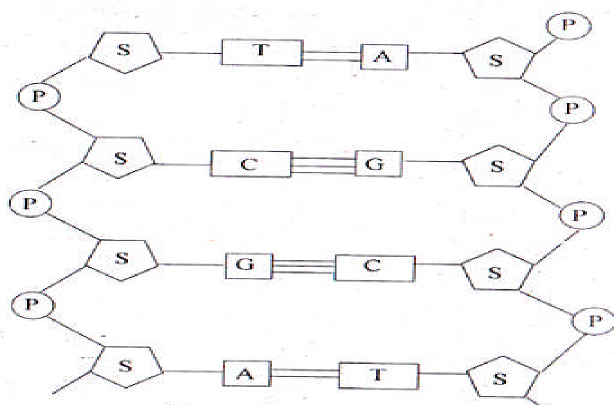


Fig. 12. 3 Structure of DNA

The nitrogen base molecule is joined to the sugar by a glycosidic bond. In DNA double helix, the length of each coil is 34 A^0 and contains 10 equally spaced base pairs. The distance between two successive base pairs is 3.4 A^0 and the angle between them is 36^0 (12. 4).

12.3 PROPERTIES OF DNA

1. **Size**: the size of the DNA molecule varies from organism to organism.

2. **Molecular weight**: It has very high molecular weight of 30,000 Daltons to several millions.

3. **Denaturation**: Denaturation refers to separation of the strands of a DNA. It is brought about by high temperature, acid or alkali. High p^H and low salt concentrations also cause denaturation of DNA.

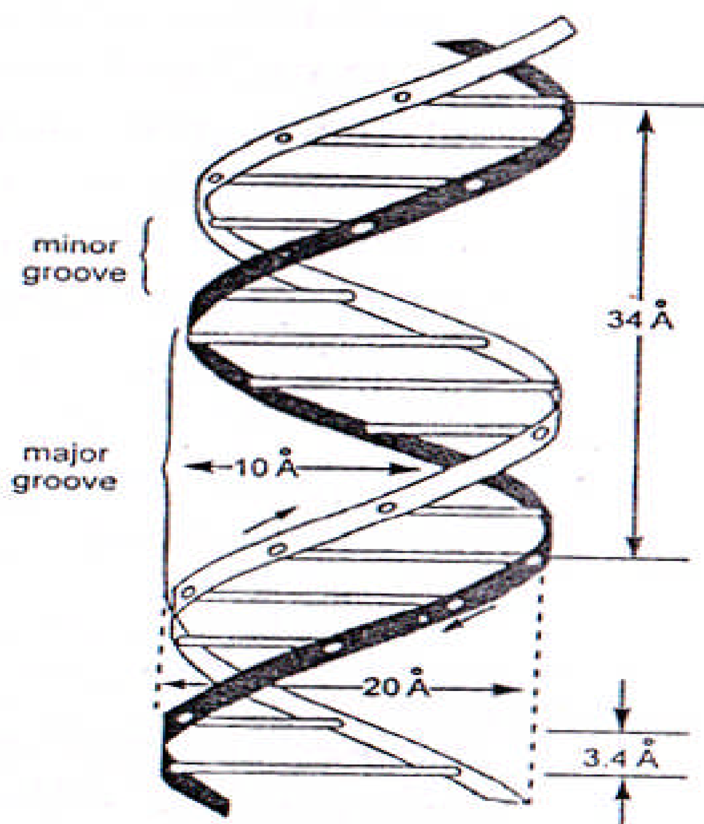


Fig. 12.4 Watson-Crick double helical DNA molecule

4. **Renaturation**: The denatured single strands of DNA can be made into double stranded DNA by coiling or by neutralizing the medium. This process is called renaturation.

5. **Stability**: The DNA is a highly stable molecule. The stability is due to two forces.

   (a) Hydrogen bonding between the bases.

   (b) Hydrophobic interaction between bases.

6. **Hyperchromatic effect**: It shows high absorption spectrum at ultraviolet light (260 nm). A denatured DNA molecule absorbs more light as its bases in single strands are exposed. This phenomenon of increased light absorption is called hyperchromatic effect. A single stranded DNA does not show the hyperchromatic effect. This phenomenon of hyper chromatic effect can be used to distinguish single or double stranded DNA in an unknown sample.

12.4 FUNCTIONS OF DNA

DNA plays an important role in all biosynthetic and hereditary functions of all living organisms.

1. DNA acts as the carrier of genetic information from generation to generation

2. DNA is a very stable macromolecule in almost all living organisms and it is immortal.

3. DNA controls all developmental processes of an organism and all life activities.

4. DNA synthesizes RNAs, which are involved in the synthesis of proteins. This is known as **heterocatalysis**.

5. A single DNA molecule duplicates into two daughter DNA molecules. It is called **autocatalysis** or **replication**.

12.5 TYPES OF DNA

DNA is classified into three types. They are A- DNA, B-DNA, and Z-DNA (Fig. 12. 5).

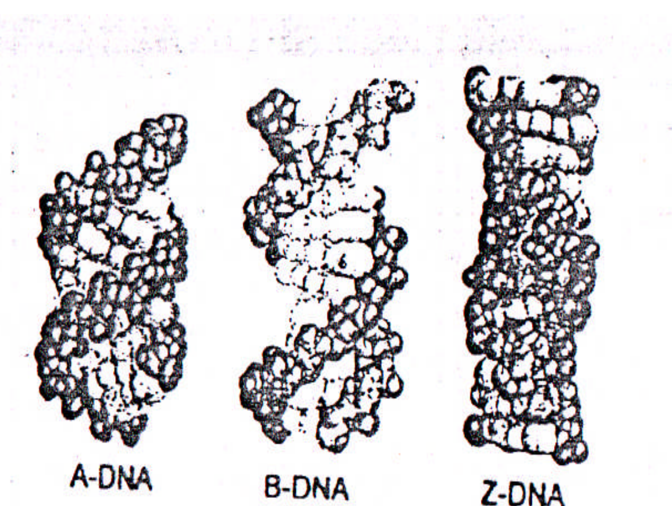


Fig. 12.5 Types of DNA

A-DNA: It is a double helical DNA having 11 residues per turn. It has a right handed helix. It is formed by the dehydration of B-DNA.

B- DNA: It is more common and has clockwise helix structure. This is the Watson and Crick double helix. It has 10 residues per turn. It shows a definite hereditary stability.

Z-DNA: It is uncommon and has an alternating clockwise and anticlockwise structure. It is a left handed helix having 12 residues per turn. The strands in it are in zig-zag orientation. So, it was named as zig-zag DNA. It has no hereditary stability. It was discovered separately by Rodley group in Newzealand and Sashisekharan group in India.

In bacteriophage ϕX 174 which attacks *E.coli*, DNA is single stranded.

12.6 STRUCTURE OF RNA

It is chiefly concentrated in ribosomes and also found in plastids and mitochondria in traces. It is synthesized inside the nucleolus but is later released into cytoplasm. RNA plays an important role in protein synthesis and is non-genetic. However, in many plant viruses (TMV) it acts as the genetic material.

RNA is made up of a single polynucleotide strand. However, in Reo virus and Wound tumour viruses, double stranded RNA is present. RNA contains ribose sugar. It consists of several units of ribo-nucleotides. The nucleotides of RNA comprise of three components called phosphate group, ribose sugar ($C_5 H_{10} O_5$) and nitrogenous bases. The nitrogen bases are Adenine, Guanine, Cytosine and Uracil. Uracil differes from Thymine of DNA in lacking a methyl (CH_3) group. These nitrogen bases do not show complementarity, hence there is no 1:1 ratio of purires and pyrimidines. The RNA molecule is normally single stranded. Sometimes the strand may be folded back upon itself and this double strand may be coiled to form a helical structure like that of DNA.

12.7 TYPES OF RNA

Three types of RNA are found in cells. They are Messenger RNA, Transfer RNA, and Ribosomal RNA.

12.7.1 Messenger RNA

It is a straight, unfolded polynucleotide molecule consisting of about few hundred nucleotides. It is synthesized from DNA template chain by a process called transcription. The molecular weight is about 5,00,000 Daltons. The life span of mRNA in bacteria is about two minutes. In eukaryotes it lives for about four hours. It carries genetic information required for the synthesis of a specific protein from DNA to ribosomes. The genetic message is present in the form of triplet codons. There are 64 codons present. Of these three codons UAA, UAG, UGA are termination codons **AUG** and **GUG** are known as **initiator codons** (Fig. 12. 6). The mRNA constitutes 5-10 % of the total cellular RNA and is highly unstable.

One end of mRNA is called 5' end, and, the other end is called 3' end. At the 5' end a cap is found in most eukaryotes and animal viruses. The cap is formed by the condensation of guanylate residues. It helps the mRNA to bind with ribosomes. The cap is followed by noncoding region I. The noncoding region is followed by coding region. The coding region contains initiation codon, actual coding region and termination codon. The coding region is followed by noncoding region. At the end of the mRNA, there is a polyadenylate sequence (Poly A).

mRNA is synthesized from a DNA strand though the action of an enzyme called RNA polymerase. The synthesis of mRNA from DNA is called transcription.

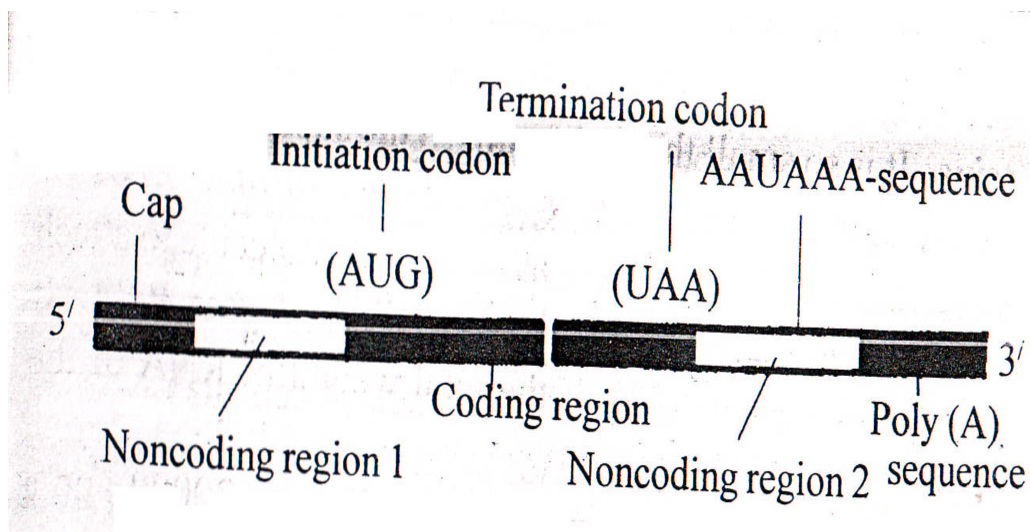


Fig. 12.6 General features of Eukaryotes mRNA

12.7.2 Transfer RNA

It is also known as '**Soluble RNA**' or `**Adaptor RNA**'. tRNA helps in bringing the amino acids to the ribosomes and plays a key role in protein synthesis. It constitutes about 15% of the total RNA of cells. It is composed of 80-90 nucleotides and has a molecular weight of about 25,000 Daltons. It is the smallest in size among the RNAs. It is composed of a single strand which is folded forming five arms, and attain the shape of clover leaf. The 5' end of the arm comes near the 3' due to folding. **Holley** (1965) proposed **clover leaf model** of tRNA molecule (Fig. 12. 7).

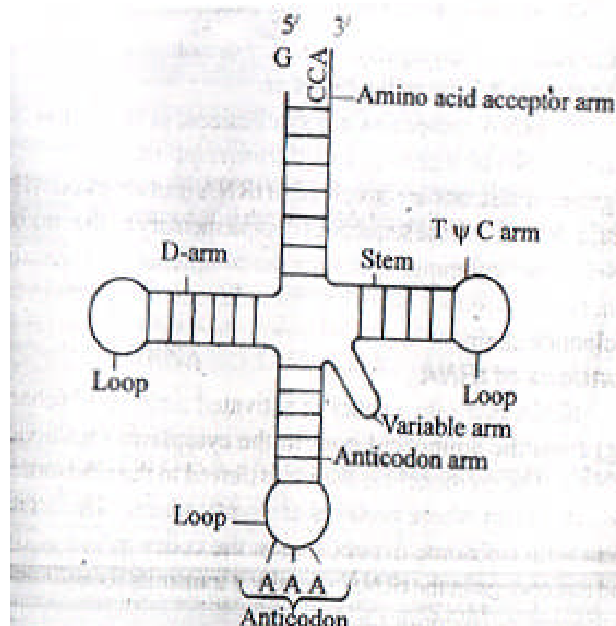


Fig. 12.7: Structure of tRNA

The five arms are amino acid acceptor arm, D arm, Articodon arm, Variable arm and TUC arm. Each arm is made up of a stem and a loop. But the amino acid acceptor arm has no loop, and variable arm has no stem. In the stem, the bases pair with each other. There is no base paining in the loops.

Amino Acid Acceptor Arm: It has no loop. The terminus has 3' end of the nucleotide chain. It has a tail with constant CCA base sequence. It is called **amino acid binding site** or **CCA end.** The 5' end terminates in G or C.

D arm: It has 3-4 bases in the stem and 7-11 unpaired bases in the loop. It helps in identifying the enzyme that activates an amino acid.

Anticodon arm: It is present opposite to amino acid acceptor arm. In this the stem has 5 paired bases and the loop has 7 unpaired bases. The middle 3 bases constitute the **anticodon**.

Variable arm: In this stem may or may not present. It has a loop with 4-5 bases.

TUC arm: It has a constant TUC sequence. Its loop has ribosome recognition site.

12.7.3 Ribosomal RNA

It is found in ribosomes and constitutes about 75-80% of the total cellular RNA. It's molecular weight is about 10,00,000 Daltons. It consisits of a single strand, which is folded at several places to form pseudohelix, where the bases show complementary pairing due to the formation of hydrogen bonds (Fig. 12. 8). Different types of rRNAs are present. 28s, 18s, 5.8s and 5s rRNAs are presnt in eukaryotes and 23s, 16s, and 5s rRNAs are present in prokaryotes.

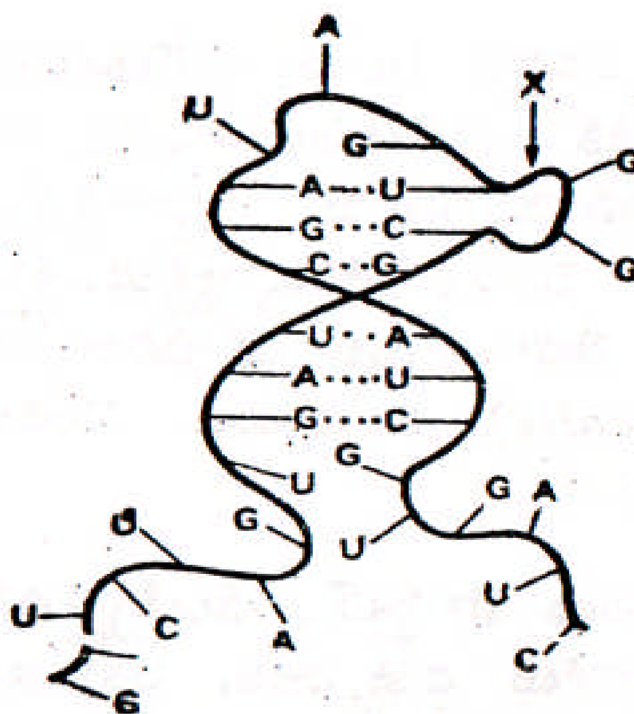


Fig. 12.8 : Structure of rRNA

12.8 REPLICATION OF DNA

Replication is the duplication process by which a single DNA molecule produces exact copies of its own structure. Replication of DNA occurs during the S subphase of interphase of cell division. It occurs inside the chromosomes. There are three hypotheses of DNA replication. They are semi conservative, conservative and dispersive methods.

Semi Conservative Method

This method is proposed by **Watson and Crick**. In this method one strand of DNA molecule serves as a template for the synthesis of new complementary strand. As one strand of the parent molecule is conserved in the daughter molecule, this method is called semi conservative method (Fig. 12. 9).

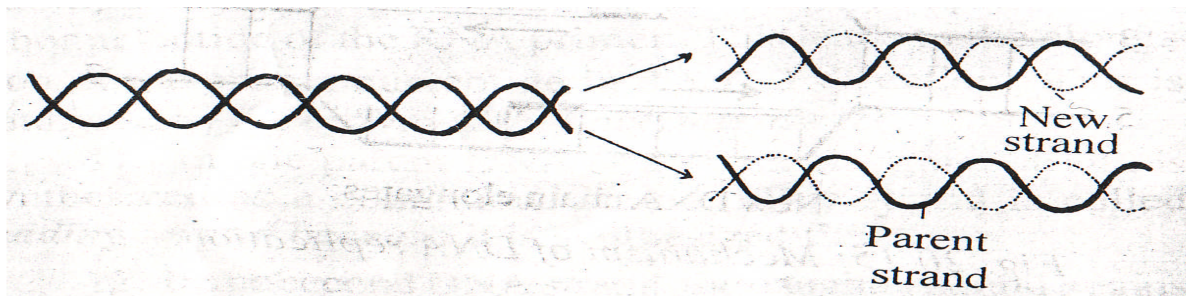


Fig. 12.9 : Semi conservative method.

Conservative method

According to this method the DNA strands do not separate. The two strands act as a template and produce a new daughter double strand. Thus, of the two double helices produced, one would be of entirely of old material and the other would be of entirely new material (Fig. 12. 10).

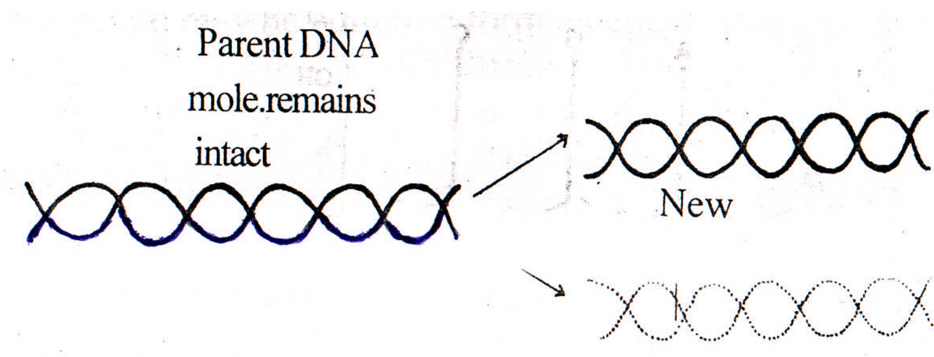


Fig.12.10: Conservative method.

Dispersive Method

According to this method, the parent DNA molecule breaks down into small pieces. Each piece synthesizes a small DNA molecule. Then the daughter DNA molecule is assembled by the linking of the old and new pieces at random. As the pieces of parent DNA molecule

remain scattered in the daughter DNA molecule this method is called dispersive method of DNA replication (Fig. 12. 11).

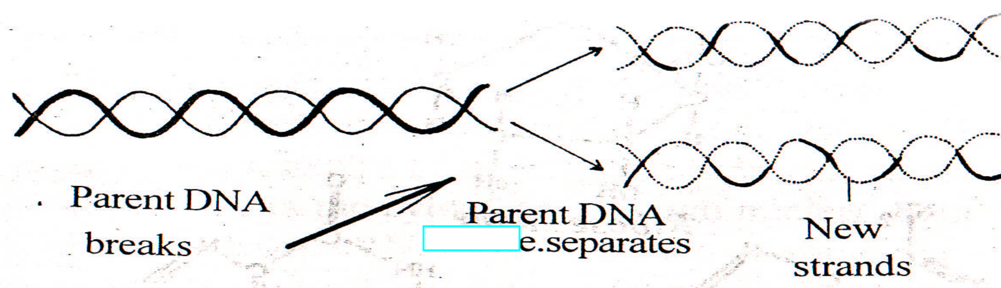


Fig. 12.11: Dispersive method.

Of these three methods of DNA replication, the semiconservative method is universally accepted and is experimentally proved by **Meselson** and **Stahl**.

Experimental evidence for semi-conservative method of DNA Replication: Meselson and Stahl's experiment: M. Meselson & FW Stahl (1958) verified the semi conservative nature of DNA replication in a series of experiments using isotopically labeled DNA and a form of isopycnic density gradient centrifugation. They cultured *Escherichia coli* cells in a medium in which the nitrogen was ^{15}N instead of commonly occurring and lighter ^{14}N.

DNA in which the nitrogen atoms are heavy (^{15}N) can be distinguished from DNA containing light nitrogen (^{14}N), because during isopycnic centrifugation, the two different DNAs band at different density positions in the centrifuge tube.

E.Coli cells grown for sometime in the presence of ^{15}N -medium were washed free of the medium and transferred to ^{14}N -containing medium and allowed to continue to grow for specific lengths of time.

DNA isolated from cells grown for one generation of time in the ^{14}N medium had a density intermediate to that of the DNA from cells grown only in ^{15}N –containing medium and that of DNA from cells grown only in ^{14}N -containing medium (control). The single band of intermediate density consisted of DNA in which one stand contained ^{15}N and the other contained ^{14}N –containing medium (control). When the incubation in the ^{14}N medium was carried out for two generations of time, two DNA bands were formed, one at the same density position as the DNA from cells grown exclusively in ^{14}N medium, and the other of intermediate density. Subsequent generations produced greater number of DNA molecules that banded at the ^{14}N -containing DNA position in the density gradient. These results are consistent only with the model of semi conservative replication. Studies using other prokaryotes as well as eukaryotes indicate the semi conservative replication of DNA is probably a universal mechanism (Fig. 12. 12).

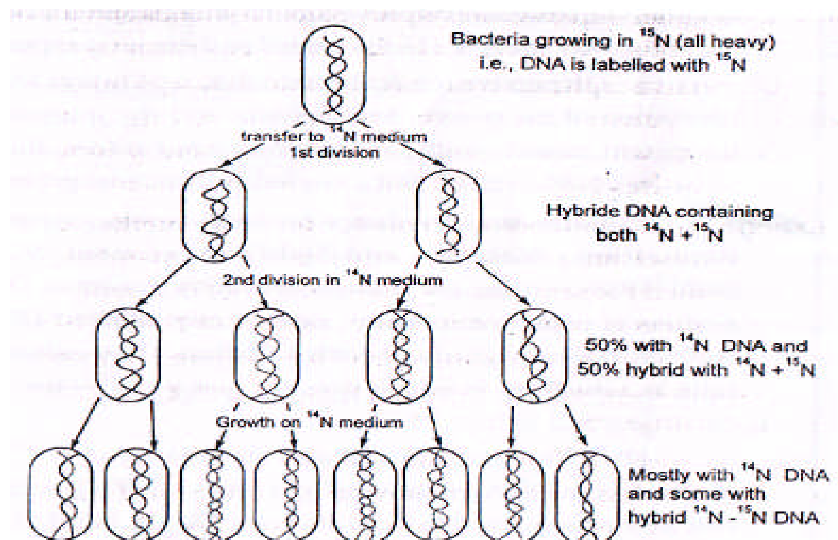


Fig. 12.12 : Meselson andStahl's experiment with *E.Coli* bacteria

Process of DNA replication: The complete process of DNA replication involves following steps in *E. coli* (Fig. 12. 13).

DNA replication starts at a specific point, called initiation point or origin. At the site of origin, the two strands separate. This separation is brought about by helicase. At the point wherethe two strands are separated, a 'Y' shaped replicating fork is formed. The duplication of DNA is brought about by the movement of the replication fork. The separated DNA strands act as templates. DNA synthesis requires a primer. Primer is a short RNA polynucleotide chain of about 50-100 nucleotides. The primer is synthesized by RNA polymerase on DNA template close to the origin of replication.

Synthesis of the new DNA strands takes place by the addition of DNA nucleotides to the 3' OH group of the last nucleotide of the RNA primer. this leads to the elongation of the primer nucleotide in the 5'-3' direction. This is catalysed by the enzyme DNA polymerase III. In one parent DNA strand the daughter strand is synthesized as a continuous strand. This strand is called leading strand because it is synthesized first. In the second DNA strand, the daughter strand synthesis begins slightly later. Hence this daughter strand is called lagging strand.

The **leading strand** of DNA is synthesized in 5'-3 direction in one strand. The **lagging strand** of DNA is synthesized in its opposite direction in short segments consisting of 1000 to 2000 nucleotides. These segments are called **Okazaki fragments**.

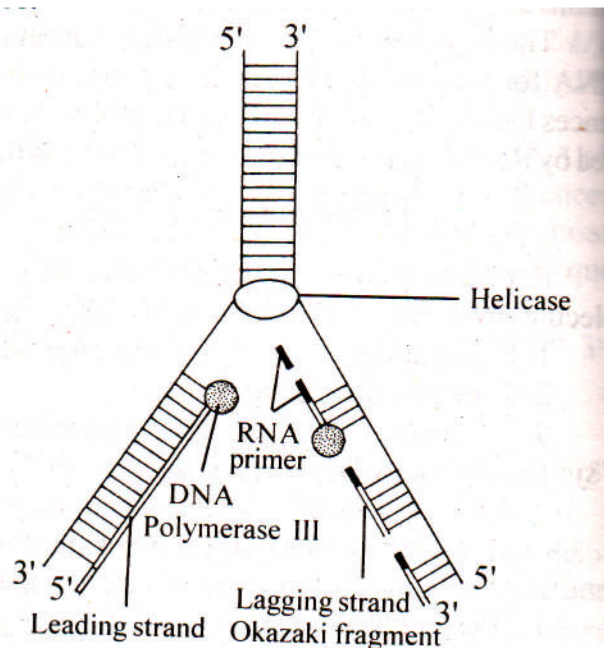


Fig. 12.13 : Summary of major steps in DNA replication.

The RNA primer is degraded at the end of replication. Replication may be unidirectional or bi-directional. The newly formed chain is exactly complementary to the template chain. In replication both strands are involved. The synthesis is occurs in the 5'-3' direction in both strands. In one strand it goes from left to right and in the other strand in the reverse direction.

DNA REPLICATION IN EUKARYOTES

In Eukaryotes cells, the mechanism of DNA replication is expected to be more complex than in prokaryotes. The replication of Eukaryotes DNA begins at multiple point of origin. Their RNA primer is formed of about 10 nucleotides and the Okazaki fragments are much shorter (100 to 150 nucleotides).

Enzymes for DNA synthesis: About 20 or more different proteins and enzymes are required during DNA replication. These collectively form DNA replication system. The main enzymes involved are DNA polymerase, polyunucleotide ligase, unwinding proteins.

DNA polymerase has three sites for attachment. One of them attaches to the template DNA, the second to the triphosphate nucleoside and the third one to the 3' –OH ends (often called primer strands). All biologically important DNA polymerases possess an important property. They will add nucleotides to a primer strand from 5' end to 3' end of the polynucleotide chain. The new strands are synthesized in fragments and these fragments are then added up by the enzyme, polynucleotide ligase.

DNA polymerase enzymes: There are three DNA polymerase enzymes that participate in the process of DNA replication. These are DNA polymerase I (Pol I, DNA polymerase II (Pol II) and DNA Polymerase III (Pol III).

DNA polymerase I: This enzyme was isolated around 1960 by **Arthur Kornberg** and was the first enzyme suggested to be involved in DNA replication (also called **Kornberg enzyme**). It is

now considered to be a DNA repair enzyme rather than replication enzyme. This enzyme is known to have five active sites, namely template site, primer site, 5'--- 3' cleavage or exonuclease site, nucleoside triphosphate site and 5' ---- 3 cleavage site or 5'--- 3' exonuclease site. Pol I is mainly involved in removing RNA primers from okazaki or precursor fragments and filling the resultant gaps due to its 5' ------3' polymerizing capacity. It has both polymerization and exonuclease activity.

DNA polymerase II: This enzyme resembles DNA polymerase I in its activity, but is a DNA repair enzyme. It brings about the growth in 5'------3' direction, using free3' – OH groups.

DNA polymerase III: Pol III plays an essential role in DNA replication. It is a multimeric enzyme having ten subunits such as alpha (α), beta (β), epsilon (e), theta (θ), tau (τ), gamma (γ), delta (δ), chi (x) and psi (ψ). All these ten subunits needed for DNA replication in vitro; however, all having different functions.

DNA ligase or polynucleotide ligase: DNA ligase enzyme helps in joining or sealing the joints of two DNA fragments by catalyzing the synthesis of a phosphodiester bond between a 3'-OH group at the end of one chain and 5'-phosphate group at the end of other chain. It is made up of single polynucleotide chain. It has following important functions.

i. It helps in joining the DNA fragments during DNA replication.
ii. It helps in repairing single stranded nicks in duplex DNA molecule.
iii. It helps in linking the ends of linear DNA duplexes to form circular DNA.
iv. It helps in joining the segments of DNA during recombination which takes place during meiosis, genetic transformation or transduction.

DNA unwinding and untwisting proteins: DNA unwinding proteins were discovered by **B. Alberts et al** in T Phage. A number of them are found in prokaryotic as well as Eukaryotes cells. They possess specific binding sites for short segments of about 8 nucleotide residues. Their molecular weight varies from 10,000 to 75,000 Daltons. Several molecules of unwinding protein bind in succession to one strand of DNA duplex in advance for the formation of replication fork.

The untwisting proteins cause nicks in one of the two strands of super coiled DNA. This allows some unwinding of DNA molecules which relieves the torsional stress. The nick is released afterwards completing the strand.

DNA replication in eukaryotes: Eukaryotes DNA replication requires two different DNA polymerase enzymes, namely DNA polymerase α and DNA polymerase δ. DNA polymerase δ synthesizes the DNA on the leading strand (continuous DNA synthesis), where as DNA polymerase α synthesizes the DNA on the lagging strand (discontinuous DNA synthesis). Besides these two enzymes, six more factors are involved in Eukaryotes DNA replication.

12.9 SUMMARY

Nucleic acid is a macromolecule present in the nucleus of cells and cause a number of biochemical activities of cell that lead to the growth and development of organisms. Nucleic acids are of two types deoxyribose nucleic acid and Ribonucleic acid. DNA is genetic material, so it is referred to as chemical basis of heredity. In some viruses RNA acts as genetic material. Nucleic acids consist of a pentose sugar, nitrogenous bases Adenine, Guanine, Cytosine,

Thymine Uracil and a phosphate group. The structure DNA was proposed by Watson and Crick. So is called Watson and Crick model of DNA. X-ray diffraction and chemical analysis are useful in building a model of DNA molecule. The DNA is double helical in nature and the two strands are complementary to one another and are linked by nitrogen base pairs which appear as steps. A is linked to T by two hydrogen bonds and G is linked to C by three hydrogen bonds. DNA shows many properties such as Denaturation, Renaturation, hyper chromatic effect etc. DNA has various functions in the cell such as carrier of genetic information form generation to generation, controlling developmental processes of organisms, synthesis of RNAs which in turn are involved in the synthesis of proteins (heterocatalysis), duplication (autocatalysis); there are different types of DNAs namely A-DNA B-DNA and Z- DNA.

RNA is chiefly concentrated in ribosomes and also found in plastids and mitochondria in traces. It plays an important role in protein synthesis. It is non-genetic, but in some viruses it is genetic. Generally it is single stranded. Its structure is similar to that of DNA except Thymine. In RNA Uracil is present in the place of Thymine. Three types of RNA are found in cells. They are messenger RNA, transfer RNA and ribosomal RNA

DNA replication occurs during the S subphase of interphase of cell division. There are three types of DNA replication. They are semi conservative, conservative and dispersive methods. But semi conservative mode is the widely accepted one.

12.10 TECHNICAL TERMS

Nucleic acid, Deoxyribose nucleic acid (DNA), Ribose Nucleic acid (RNA), Adenine, Guanine, Cytosine, Thymine, Uracil, autocatalysis, heterocatalysis, A-DNA, B-DNA, Z-DNA, DNA replication, protein synthesis, messenger RNA, transfer RNA, ribosomal RNA, complementary strand, conservative mode of DNA replication, replication fork, polymerase, leading strand, lagging strand, okazaki fragments, RNA primer.

12.11 SELF ASSESSMENT QUESTIONS

1. Give an account on structure of Watson crick model of DNA
2. Enumerate an account on different properties of DNA
3. Write an account on structure and different types of RNA
4. Elaborate about DNA replication with suitable diagram

SHORT NOTES

1. Types of DNA
2. Autocatalysis of DNA
3. Heterocatalysis of DNA
4. m RNA
5. Differences between DNA and RNA
6. t RNA

7. r RNA
8. Replication fork
9. Okazaki fragments
10. DNA polymerase
11. Semiconservative DNA replication

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- Dr. T. Srivalli

CHAPTER – 13

MENDEL'S LAWS OF INHERITANCE

13.1 OBJECTIVE :

The objective of this lesson is to study *Mendel*'s laws of inheritance. We learn how *Mendel* carried out monohybrid and dihybrid crosses and analysed the results of these crosses to formulate basic laws of inheritance.

STRUCTURE :

13.1 INTRODUCTION
13.2 REASONS FOR MENDEL'S SUCCESS
13.3 MENDEL'S EXPERIMENTS
13.4 MENDEL'S LAWS
13.5 BACK CROSS AND TEST CROSS
13.6 GENE INTERACTION
13.7 ALLELIC GENE INTERACTION
13.8 NON ALLELIC GENE INTERACTION
13.3 SUMMARY
13.4 TECHNICAL TERMS
13.5 SELF ASSESSMENT QUESTIONS
13.6 REFERENCE BOOKS

13.1 INTRODUCTION :

Every living organism reproduces its own kind, which show resemblances and variations. The phenomenon of transmission of these resemblances and variations from the parent to the offspring is known as heredity. The study of heredity is known as Genetics. *W. Bateson* in 1906 coined the word 'genetics'. *Gregor Johann Mende*l was the first man to demonstrate the way by which characters are transmitted from one generation to another. He also formulated the basic laws of inheritance. Therefore he is called the Father of Genetics. The study of *Mendel*'s principles of heredity is known as Mendelism.

Gregor Johann Mendel was a monk in the Augustinian monastery at Brunn in Austria. In 1857 he began to collect and observe numerous varieties of garden pea, whose seeds were offered for sale by seedsmen. He carried out simple crossing experiments on garden pea in the monastery gardens for seven years. In 1865, he presented the results of his experiments at two meetings of Natural History Society of Brunn and published them in the annual proceedings. But his work was overlooked by the scientists of that time. In 1900, three scientists, *De Vries* (Holland), *Carrens* (Germany) and *Von Tschermak* (Austria) independently conducted crossing experiments and obtained the same type of results as that of *Mendel*. The three scientists rediscovered *Mendel*'s

paper and proclaimed its importance. The reason for *Mendel* to succeed in discovering the laws, now bearing his name, lies in his wise choice of material and methods of study.

13.2 REASONS FOR MENDEL'S SUCCESS

1. *Mendel* selected garden pea (*Pisum sativum*) plant as experimental material and it had the following advantages.
   a. It was an annual plant, and takes only few months to complete its life cycle. So several generations can be studied within a short duration.
   b. Flowers are bisexual and predominantly self-pollinating. Artificial fertilization is possible in these flowers and was always successful.
   c. Plants belonging to two different generations when crossed, would produce fertile offspring.
   d. There are 7 pairs of well defined contrasting characters in this plant
2. *Mendel* maintained the statistical record of all the experiments and analyzed them mathematically to obtain results.
3. *Mendel* could obtain results easily because the 7 pairs of characters he chose had a sharp contrast with out any intermediate stages.
4. *Mendel* studied the inheritance of one character at a time. After establishing the genetic behaviour of one character, he studied the two characters together.
5. *Mendel* could formulate laws of inheritance because of the independent assortment of the 7 pairs of contrasting characters.

Mendel chose the following 7 pairs of contrasting characters

1. Height of the plant	tall	dwarf
2. Flowers	axillary	terminal
3. Colour of the pod	green	yellow
4. Shape of the pod	inflated	constricted
5. Shape of the seed	round	wrinkled
6. Colour of the seed coat	grey	white
7. Colour of the cotyledons	yellow	green

13.3 MENDEL'S EXPERIMENTS

Monohybrid Cross:

When *Mendel* carried crossing experiments, he confined his study first to a single character, and after establishing the genetic behaviour of a single trait (for eg: height of the plant) he then studied characters like height of the plant and colour of the flower together. So a cross, involving contrasting expressions of a single trait is known as monohybrid cross.

In order to understand the crossing experiments, the meaning of certain terms used in the explanation of the experiments must be clearly understood. In every organism the factors responsible for the expression of various characters are know as genes. Every organism acquires two genes,

one from each parent for every character for eg: Tallness is represented by TT and dwarfness is represented by tt. The genetic composition acquired by the organism from its parents is know as 'genotype' and the characters expressed by these genes in an organism such as colour, shape, structure, weight, chemical composition are known as phenotype. Genes responsible for a pair of contrasting characters, are know as alleles. If the two alleles of the same trait are similar, for example TT or tt, the organism is said to be homozygous for that particular trait. If the two alleles of the same trait are dissimilar, for eg Tt, the organism is said to be heterozygous for that particular trait. These alleles of a gene are present on same locus on homologous chromosomes. Gene symbols are used to denote these genes and alleles. Dominant genes are denoted by capital letters, and recessive genes are denoted by small letters of the English alphabet.

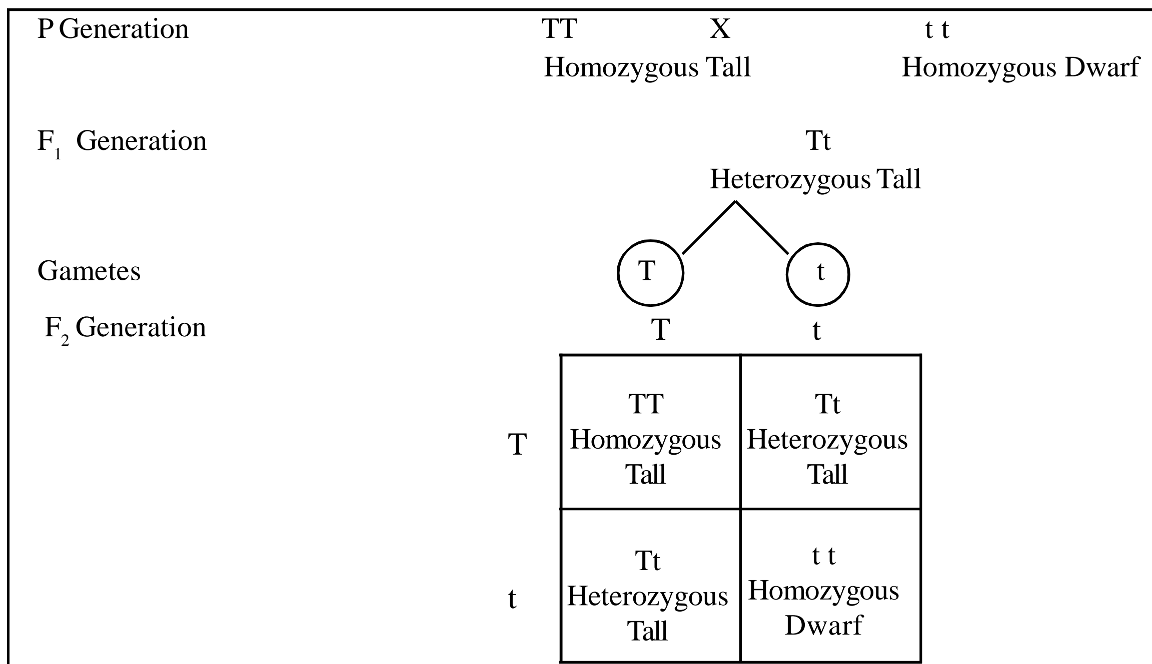


FIGURE 1

Mendel selected pure tall plants (TT) and pure dwarf plants (tt) and made a cross between these two. These plants are known as parental generation (P generation). Tall plants produce gametes with gene 'T' and dwarf plants produce gametes with gene 't'. When these two fuse, seeds with genotype 'Tt' are produced. These seeds germinate to form plants of first generation or F_1 generation. *Mendel* observed that all the plants in F_1 generation were tall. When *Mendel* allowed self-pollination among F_1 plants, the second generation plants are formed. These are known as F_2 generation. *Mendel* observed that F_2 generation had tall and dwarf plants in the ratio of 3:1.

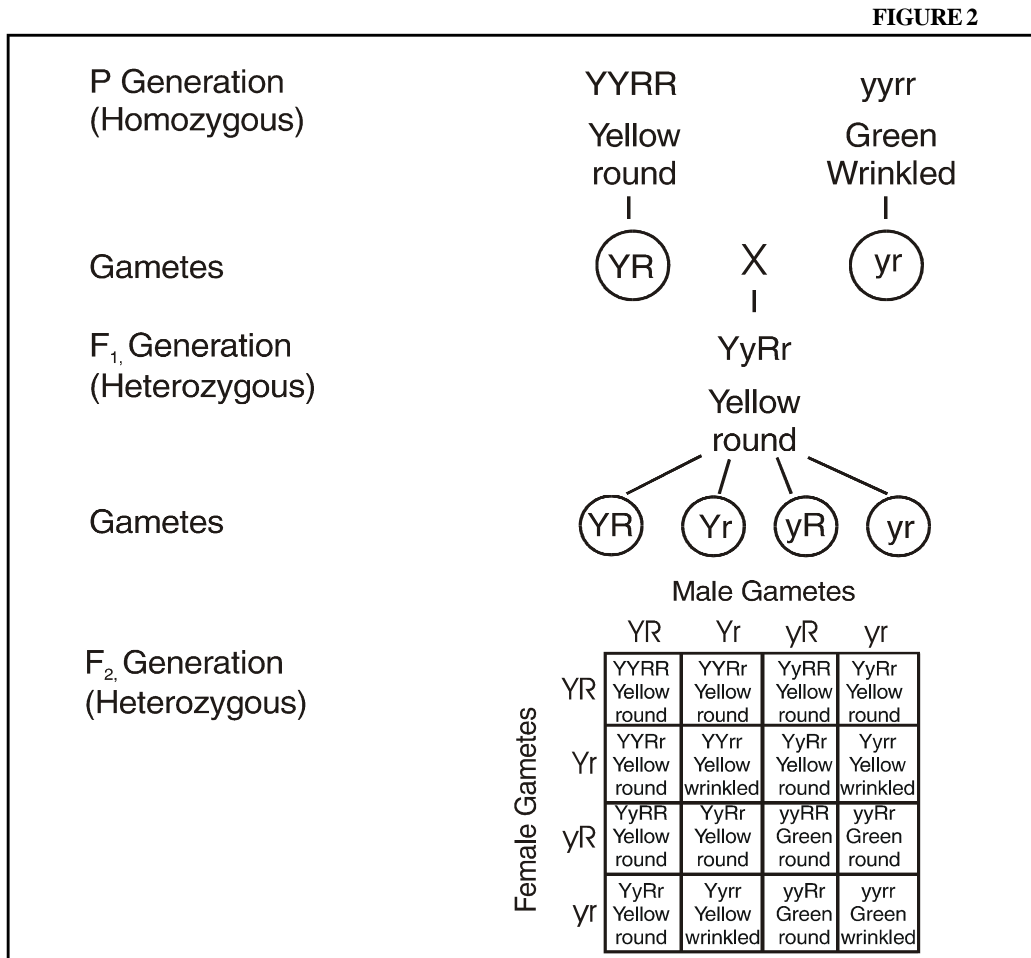
F_1 generation plants inherit gene 'T' responsible for tallness and gene 't' responsible for dwarfness and become heterozygous (Tt) for height of the plant. But *Mendel* observed that all plants were tall. Even though F_1 plants have both the genes responsible for tallness (T) and dwarfness (t), only gene 'T' is expressed, and gene 't' remained recessive. The character expressed in F_1 generation is known as 'dominant character', and the character which remained recessive is known as 'recessive character'. F_2 generation plants formed by self-polination of F_1 generation, consisted of both tall and dwarf plants. Therefore *Mendel* found that the gene responsible for dwarfness remained recessive, and the gene responsible for tallness was expressed. When *Mendel* allowed self-pollintaion among F_2 tall plants and dwarf plants separately, he found that recessive character was

pure. Of the ¾ of the dominant character, ¼ was pure. So he found that the there were three genotypes among F_2 plants that are 'TT', 'Tt' and 'tt'[see Figure 1].

Dihybrid Cross:

Mendel after establishing the behaviour of one pair of characters in pea plant, he observed the inheritance of two pairs of characters. So a cross involving two pairs of characters is known as a dihybrid cross.

FIGURE 2



Mendel crossed a plant having yellow cotyledons (YY) and round seeds (RR) with green cotyledons (yy) and wrinkled seeds (rr). In F_1 generation, plants with yellow cotyledons and round seeds are formed. When self-pollination is allowed among F_1 plants, plants with 4 types of phenotypes are formed in F_2 generation. They are as follows.

1. Yellow cotyledons, round seeds
2. Yellow cotyledons, wrinkled seeds
3. Green cotyledons, round seeds
4. Green cotyledons, wrinkled seeds

The results of dihybrid F_2 generation can be known with the help of *Punnet*'s checker board. This shows the possible ways of fusion of gametes and the number of recombinations. It is known

from the *Punnet*'s checker board, that there are 4 types of phenotypes in the ratio of 9:3:3:1. This is known as dihybrid ratio. *Punnet*'s checker board also shows that there are 9 genotypes in the ratio of 1:2:2:4:1:2:1:2:1 [see Figure 2].

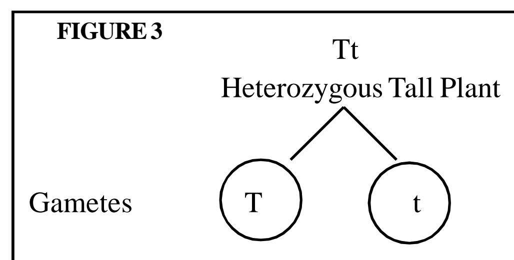
The phenotype of dihybrid F_1 plants reveals that, round and yellow character is dominant over wrinkled and green character. So *Mendel* observed that, yellow and round are dominant characters and green and wrinkled are recessive characters. During the formation of gametes in F_1 plants, one gene from each pair of contrasting characters, enter the gametes independent of other genes.

13.4 MENDEL'S LAWS

Basing on the above crossing experiments, *Mendel* formulated the following laws of inheritance.

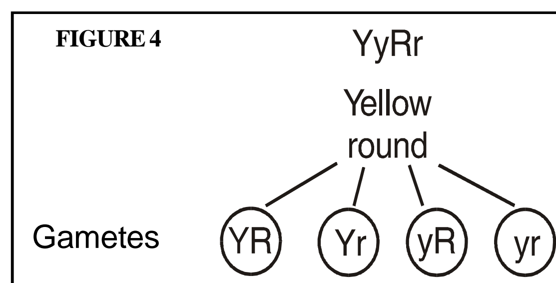
1. Law of Segregation: Even though there are two factors for a particular character in an individual, they separate or segregate during the formation of gametes. This is known as Law of Segregation. This means that gametes possess either dominant character or recessive character but not both. For eg. Plants having the genotype 'Tt' forms gametes with gene 'T' and gene 't'. So the gametes will be pure for the given character. Hence this law is also known as Law of Purity of Gametes [see Fig.3].

FIGURE 3



2. Law of independent assortment: According to this law, characters (genes) of one pair (round & yellow) segregate or separate independent of characters (genes) of another pair (green, wrinkled). For example, during the formation of gametes in F_1 hybrid plant (Rr Yy), 2 allelic genes Rr and Yy enter gametes independent of each other. Here each gene behaves as a separate unit. So this law is also known as Law of Unit Characters [see Fig.4].

FIGURE 4



13.5 BACK CROSS AND TEST CROSS :

Mendel used Back cross and Test cross to differentiate homozygous dominant (TT) plants from heterozygous dominant (Tt) plants.

Back Cross: A cross involving F_1 hybrid and its parent is known as back cross[see Figure 5].

When an F_1 hybrid plant was crossed with its dominant parent, all the plants that were formed had the same phenotype. Checker board shows two types of genotypes –TT, Tt

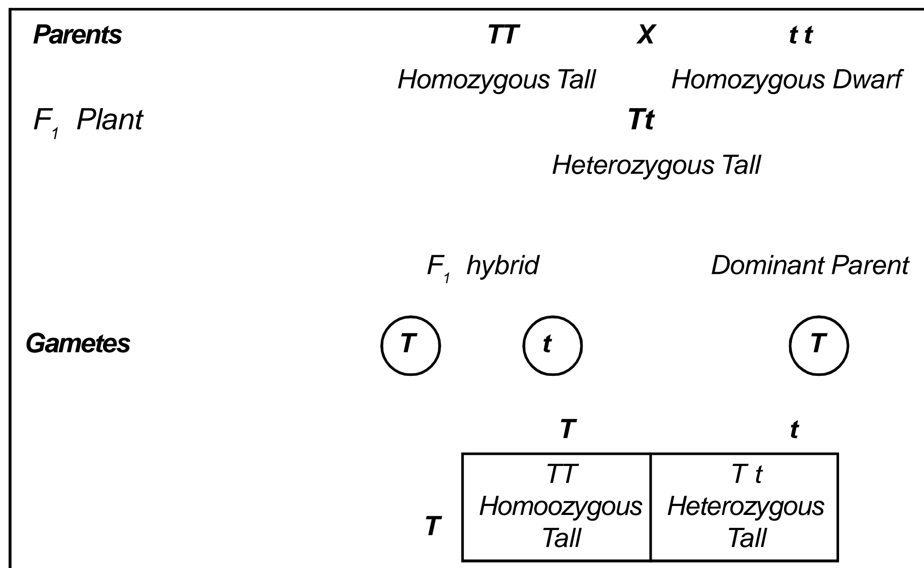


FIGURE 5

Test cross: A cross involving an F_1 hybrid plant and its recessive parent is known as 'test cross'.

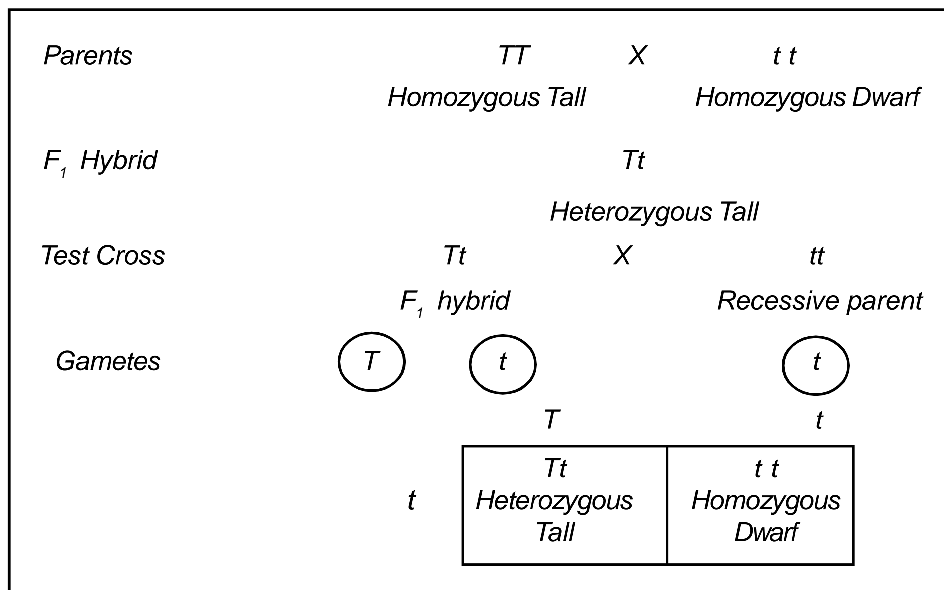


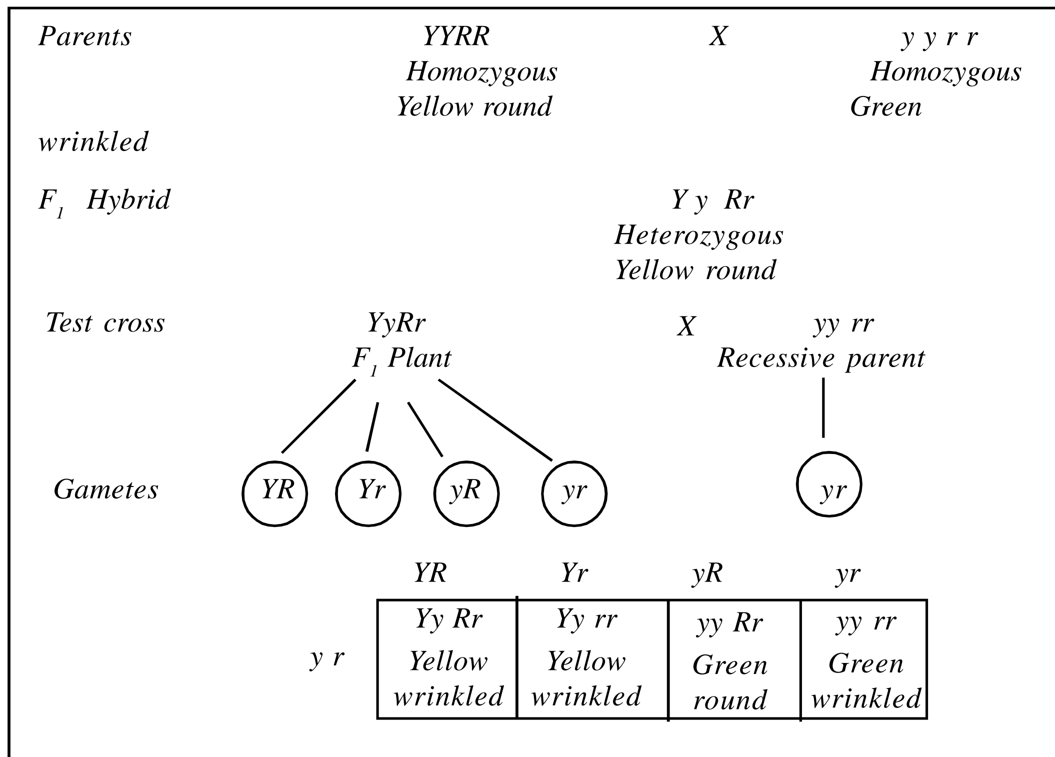
FIGURE 6

A test cross yields phenotypically dominant and recessive offspring, in the ratio of 1:1. The character that remained recessive in an individual will be expressed in a test cross. From this, one can know whether an individual is heterozygous or homozygous [see Figure 6].

Dihybrid Test Cross

A test cross between an F_1 plant and its double recessive parent yields 4 types of phenotypes in the ratio 1:1:1:1. [see Figure 7]

FIGURE 7



13.6 GENE INTERACTION :

Although Mendelian inheritance is confirmed through the experiments carried out after the rediscovery of *Mendel*'s work in 1900, it was not confirmed that *Mendel*'s laws are applicable universally to all organisms. Some experimental results differ from those established by *Mendel*. These variations were as follows.

(1) In some organisms incomplete dominance is observed in the F_1 generation. This means intermediate characters are seen instead of parental characters.

(2) Various genes were found to possess more than two alleles.

(3) It was found that a trait is governed by the interaction of two or more genes. This resulted in F2 phenotypic ratios deviating from the typical Mendelian ratios.

Depending upon the form of interaction Mendelian phenotypic ratios are modified in various ways. Interaction of genes is of two types.

1. Allelic gene interaction

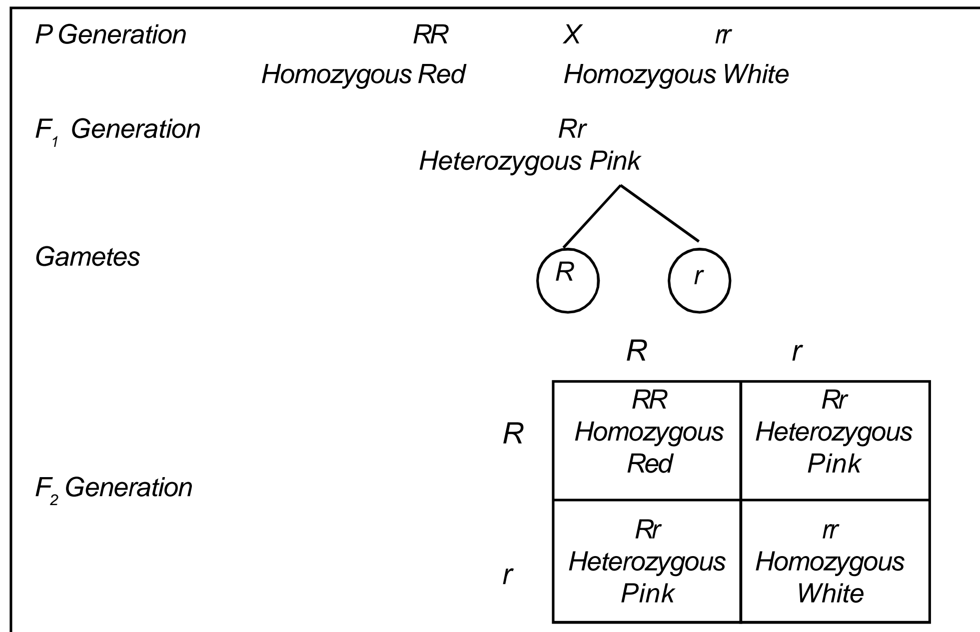
2. Non allelic gene interaction.

13.7 ALLELIC GENE INTERACTION

In this two allelic genes (present on homologous chromosomes) interact to bring about new phenotype.

1:2:1 ratio: Eg: Snapdragon (*Oenothera lamarkiana*) flower colour.

FIGURE 8



In Snapdragon, when red flowered variety was crossed with white flowered variety, pink coloured flowers are obtained in F1 generation. When F_1 plants are self-pollinated the F_2 progeny showed individuals of three phenotypes in the ratio of 1 white: 2 pink: 1red.

If the above cross is observed, in F_1 generation, the parental characters (red, white) were not expressed but phenotype with new colour (pink) was formed. When F_1 plants were self pollinated, F_2 generation showed both parental characters (red and white) and a new (pink) character. In this, each genotype had a distinct phenotype i.e., "RR" (homozygous) had red phenotype, rr (homozygous) had white phenotype, and Rr (heterozygous) had pink phenotype. They do not resemble either of the parents and showed intermediate phenotype. So both dominant gene "R" and recessive gene "r" had about equal effect on the phenotype (flower colour) [see Figure 8].

Co-dominance: Eg: Human blood alleles.

There are four phenotypic classes of human blood types. They are A, AB, B and O. These blood types are due to the action of three alleles of the blood group gene "I". The alleles I^A and I^B are dominant over the allele I^O. A cross between I^AI^A and I^BI^B will give AB blood group which is different from the blood groups A and B. Here a gene is having more than two alleles. In this I^A and I^B show co-dominance and the third allele I^O is recessive to I^A I^B.

13.2.8 NON-ALLELIC INTERACTION OF GENES :

In this, interaction of two or more genes present on different chromosomes (non-homologous chromosomes) takes place to effect the same trait.

Interaction of two gene pairs influencing the same trait: Eg: Comb shape in fowls (chickens)

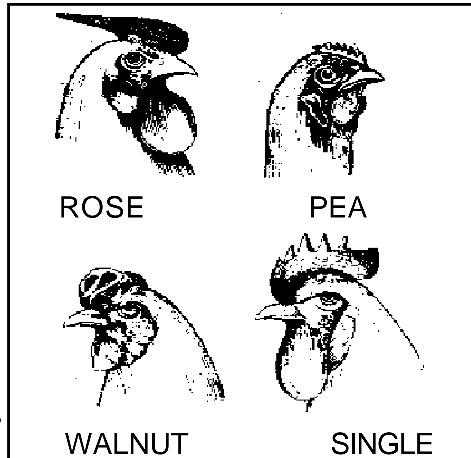
In fowls, different breeds differ in their comb shape. The Wyandottes have "rose" type, the Brahmas have "pea" type and the Leghorns have "single" type combs. When "Pea" type is crossed

with "rose" type in F_1 generation, a new type of comb called "walnut" appeared. In pea type comb, gene "P" is dominant and in rose type cpmb gene "R" is dominant. These two genes P and R are present on different chromosomes (non- homologous chromosomes). Single type is recessive to these two types. The genotypes of these three are as follows.

Pea type : PPrr

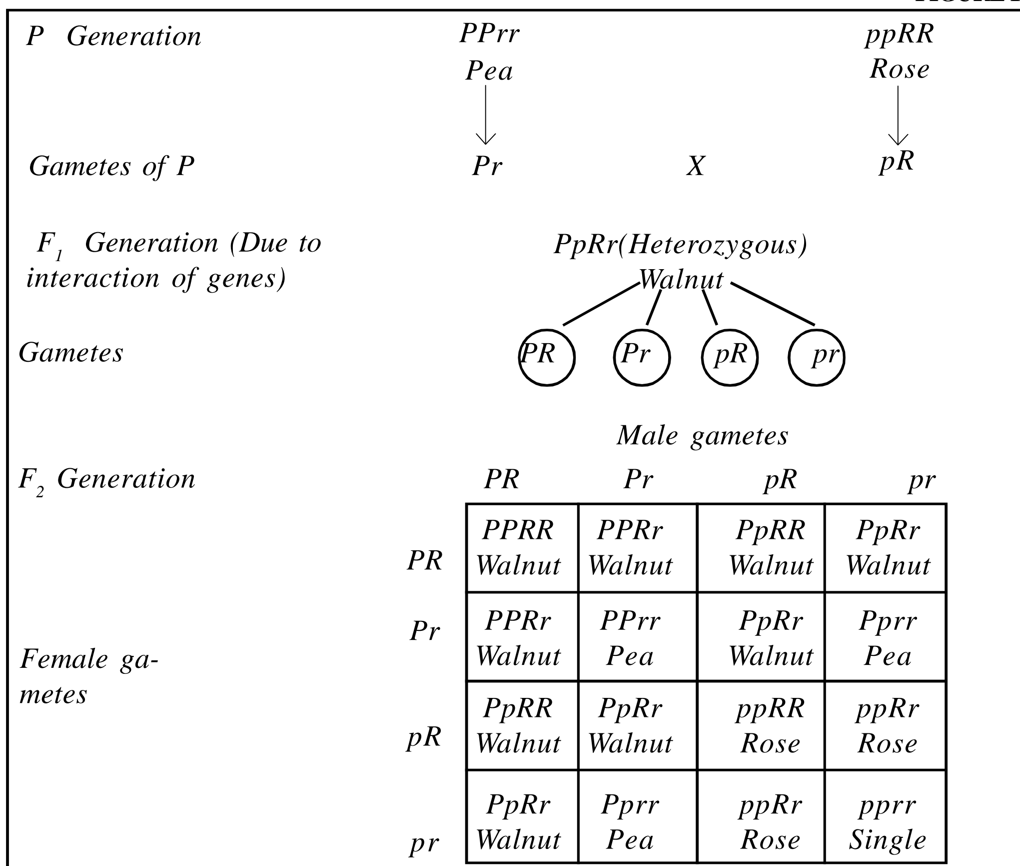
Rose type : RRpp

Single type : pprr



Comb shapes in
FIGURE 9

FIGURE 10



	PR	Pr	pR	pr
PR	PPRR Walnut	PPRr Walnut	PpRR Walnut	PpRr Walnut
Pr	PPRr Walnut	PPrr Pea	PpRr Walnut	Pprr Pea
pR	PpRR Walnut	PpRr Walnut	ppRR Rose	ppRr Rose
pr	PpRr Walnut	Pprr Pea	ppRr Rose	pprr Single

When the pea and rose types are crossed, the F_1 generation will have the genotype PpRr. Because of the influence of the two dominant genes P and R, a new type of phenotype "walnut" comb was produced. The F_1 walnut will produce four kinds of gametes – PR, Pr, pR, pr. In F_2

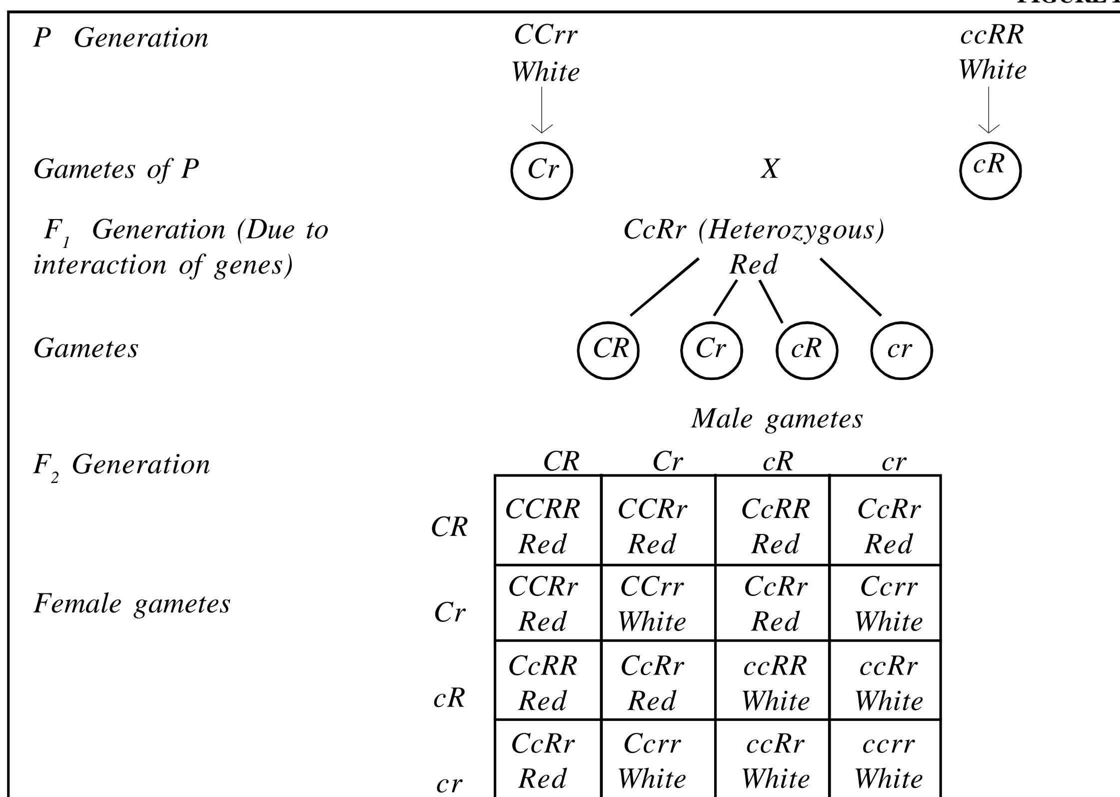
generation, walnut, pea, rose and single are produced in the ratio of 9:3:3:1. The checker board shows 9 classes with both P and R genes resulting in walnut phenotype. Pea type is formed in 3 classes where the dominant gene “P” is present, Rose type is formed in 3 classes where the dominant gene “R” is present, and single type is formed where the recessive genes “r” and “p” are present [see Figure 10]

Complementary genes: *9:7 ratio :*

Sometimes a trait is produced by the interaction of two or more different genes present on separate chromosomes, which complement one another. *Bateson* and *Punnet* found that there are two different white flowered varieties of sweet pea plants. When such two different white varieties are crossed, red coloured flowers are formed in the F_1 generation. When the F_1 plants are self pollinated, the F_2 generation showed red flowered and white flowered plants in the ratio of 9:7. This is a modification of *Mendel*'s dihybrid ratio (9:3:3:1). Here the red colour of the flower is due to the production of anthocyanin pigment. The formation of this pigment depends on two independent factors. Two genes “C” and “R” are necessary for the production of red colour. These two genes cannot produce red colour separately. Of these two genes, one gene (C) seems to produce a colour base, while the second and gene (R) produces an enzyme which activates the colour base to produce the red colour.

The checkerboard shows 9 classes having both C and R and so the phenotype is red. The remaining 7 classes have either C or R and so the phenotype is white. Therefore the complementary action of C and R is responsible for the production of red colour in flowers of sweet pea plant [see Figure 11].

FIGURE 11



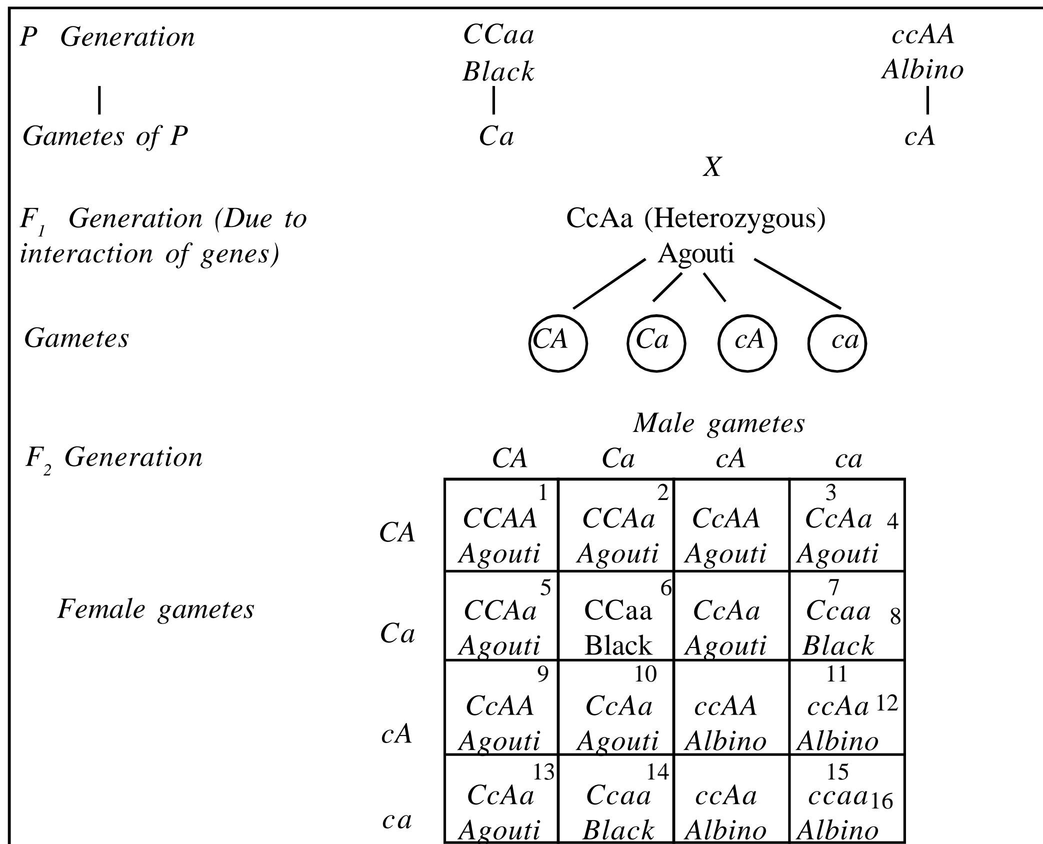
Supplementary Genes: ***9:3:4 ratio***

Supplementary genes are two independent pairs of genes influencing the same trait. Here, one dominant gene will produce its effect whether the other gene is present or not; but the second dominant gene can produce its effect only in the presence of the first dominant gene.

In rats the coat colour black (C) is dominant over albino (c). Besides these two, there is another type known as agouti (wild). In agouti type, the individual hairs are black with yellow band near the tip. The gene 'A' responsible for agouti type is dominant. When true breeding black rat (Ccaa) is crossed with albino (ccAA), all the F_1 off spring are of agaouti type. In F_2 generation, agouti, black and albino are formed in the ration of 9:3:4.

The checker board shows that the phenotype of 3 classes (6,18,14) which consist of dominant gene 'C' is black; the phenotype of 4 classes (11,12,15,16) which consist of recessive gene 'c' is albino. Here, even though the dominant gene 'A' is present in these 4 classes, albino trait is expressed because of the absence of the dominant gene 'C'. In the remaining classes, agouti trait is expressed because of the presence of both the dominant genes A & C [see Fig.12].

FIGURE 12



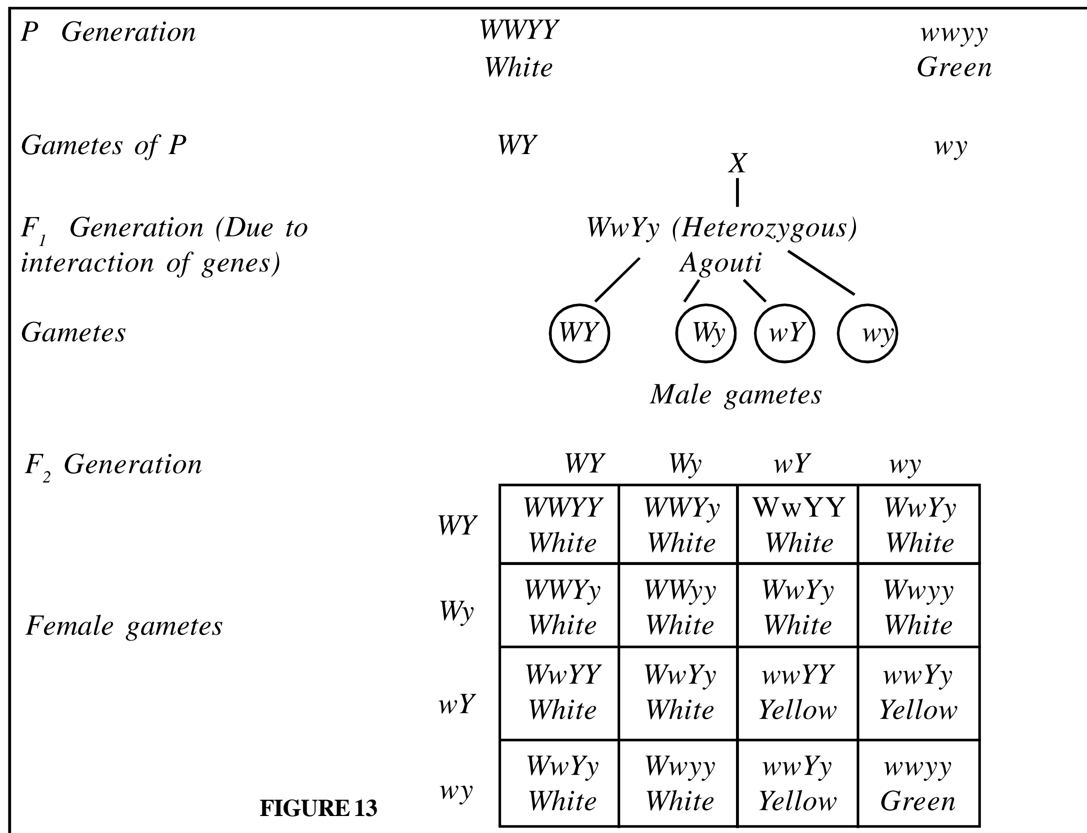
	CA	Ca	cA	ca
CA	1 CCAA Agouti	2 CCAa Agouti	3 CcAA Agouti	4 CcAa Agouti
Ca	5 CCAa Agouti	6 CCaa Black	7 CcAa Agouti	8 Ccaa Black
cA	9 CcAA Agouti	10 CcAa Agouti	11 ccAA Albino	12 ccAa Albino
ca	13 CcAa Agouti	14 Ccaa Black	15 ccAa Albino	16 ccaa Albino

Epistasis: ***12:3:1 ratio***

Sometimes, two independent genes affect the same trait in an organism. Here, one gene masks the effect of the other. This effect is known as 'epistasis'. The gene that masks the effect of another is known to be 'epistatic' and the gene whose expression is prevented is known to be 'hypostatic'.

Eg: Summer squash (*Cucurbita pepo*)

In this, gene ‘Y’ responsible for yellow colour of fruit is dominant over gene ‘y’ responsible for green colour. Another dominant gene ‘W’ is responsible for white colour and is epistatic to gene ‘Y’. This means that white colour gene ‘W’ masks the effect of yellow colour gene ‘Y’. So when the gene ‘W’ is present, the fruit colour will always be white irrespective of the presence of the genes of other colours (Y or y). In the absence of the gene ‘W’, the fruit colour will be yellow if ‘Y’ is present, and green if ‘Y’ is absent [see Fig.13].



	WY	Wy	wY	wy
WY	WWYY White	WWYy White	WwYY White	WwYy White
Wy	WWYy White	WWyy White	WwYy White	Wwyy White
wY	WwYY White	WwYy White	wwYY Yellow	wwYy Yellow
wy	WwYy White	Wwyy White	wwYy Yellow	wwyy Green

FIGURE 13

Duplicate Genes: ***15:1 ratio :***

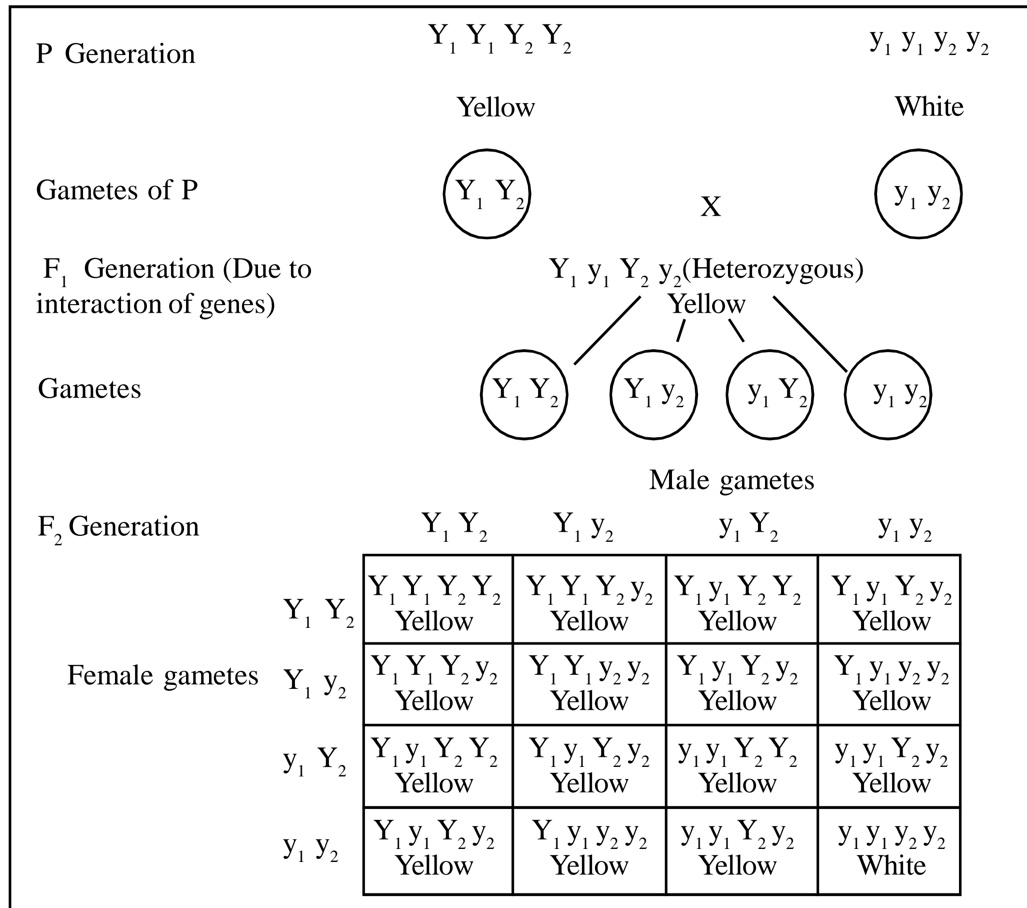
Two or more independent genes present on different chromosome pairs have an identical effect on a trait. These are called duplicate genes. For example, in Maize, when a cross is made between a plant with yellow endosperm and a plant with white endosperm, F_1 plants are formed with yellow endosperm. F_1 plants on self pollination, give plants with yellow and white endosperms in the ratio of 15:1. This ratio suggests that the above cross is a dibybrid cross and not a monohybrid cross. Yellow colour of the endosperm is due to two independent dominant genes Y_1 and Y_2. The presence of either or both of these genes results in a yellow endosperm, whereas in the absence of both these genes, a white endosperm is produced [see Fig.14].

Inhibitory factors: ***13:3 ratio :***

An inhibitory factor is one, which can produce no phenotypic effect by itself, but when present in a dominant form prevents or inhibits the expression of another independent dominant gene present

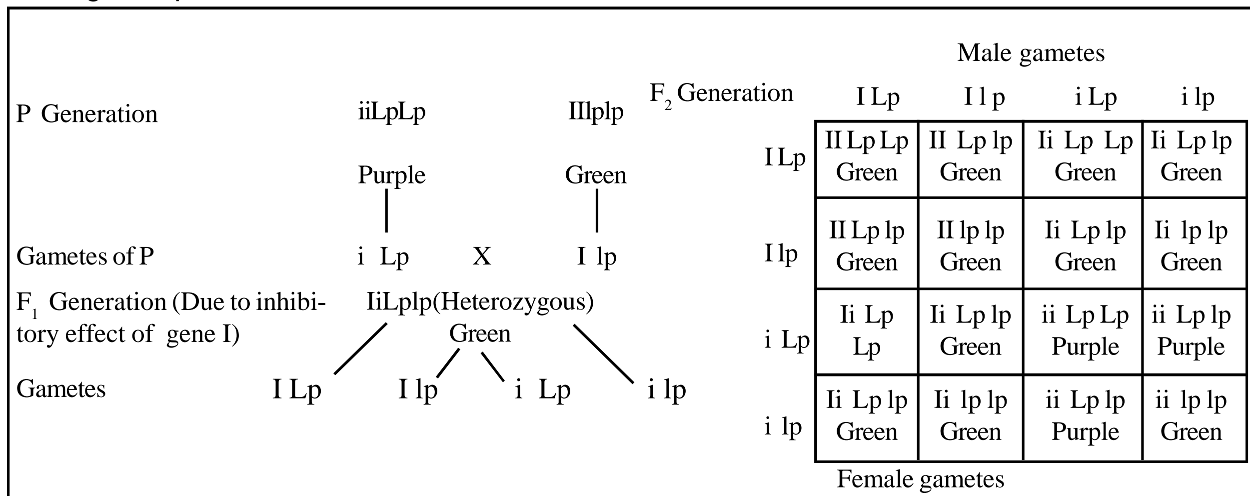
on another chromosome. This may be illustrated by the inheritance of leaf colour in rice. In rice leaf, purple pigment gene Lp is dominant

FIGURE 14



to green colour gene lp. There is another factor 'I', which inhibits the effect of Lp and prevent the formation of purple colour, and so the leaves become green. This inhibitory factor has no effect in its recessive form 'i', and so the leaves will be purple or green based on the presence or absence of the gene Lp.

FIGURE 15



13.3 SUMMARY :

In this chapter we study *Mendel*'s crossing experiments. *Mendel* confined his study first to a single character, and after establishing the genetic behaviour of a single trait, he then studied the second character along with the first character. A cross involving a single character is known as monohybrid cross. Basing on the results of monohybrid cross, he formulated the law of segregation. A cross involving two characters is known as dihybrid cross. Basing on the results of dihybrid cross, he formulated the law of independent assortment. *Mendel* used back cross and test cross to differentiate homozygous dominant (TT) plants from heterozygous dominant (Tt) plants.

13.4 TECHNICAL TERMS :

Genes, genotype, phenotype, alleles, homozygous, heterozygous, locus, monohybrid cross, dihybrid cross, back cross, test cross, incomplete dominance, co-dominance, complementary genes, supplementary genes, epistasis, duplicate genes, inhibitory factor.

13.5 SELF ASSESSMENT QUESTIONS :

Essay type questions:

1. Why did *Mendel* choose Pea as the experimental material in his experiments? Give an account of monohybrid cross.
2. Explain in detail the laws of *Mendel*.
3. What does gene interaction mean? Give an account of complimentary and supplementary genes with suitable examples.

Short answer type of Questions:

1. Dihybrid cross
2. Reasons for *Mendel*'s success
3. Back cross
4. Test cross
5. Epistasis
6. Allelic gene interaction

13.6 REFERENCE BOOKS :

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- P.M. VASANTHA KUMARI

Lesson – 14

LINKAGE AND CROSSING OVER

14.0 OBJECTIVE :

The objective of this lesson is to know about linkage and crossing over. In linkage, we learn about linkage group, arrangement of linked genes, kinds of linkage and significance of linkage. In crossing over, we learn about exchange of chromosomeal segments between non sister chromatids, kinds of crossing over, the mechanism of crossing over and the significance of crossing over.

STRUCTURE :

14.1 INTRODUCTION
14.2 BATESON AND PUNNET'S EXPERIMENTS
14.3 LINKAGE GROUP
14.4 KINDS OF LINKAGE
14.5 ARRANGEMENT OF LINKED GENES
14.6 SIGNIFICANCE OF LINKAGE
14.7 CROSSING OVER
14.8 MECHANISM OF CROSSING OVER
14.9 KINDS OF CROSSING OVER
14.10 HUTCHINSON'S EXPERIMENTS
14.11 FACTORS CONTROLLING THE FREQUENCY OF CROSSING OVER
14.12 SIGNIFICANCE OF CROSSING OVER
14.13 SUMMARY
14.14 TECHNICAL TERMS
14.15 MODEL QUESTIONS
14.16 REFERENCE BOOKS

14.1 INTRODUCTION :

According to *Mendel*'s Law of assortment, factors for different pairs of contrasting characters assort independently from each other. But *W.Bateson* and *R.C. Punnet* (1906) observed exception to the above law in sweet pea plant.

14.2 BATESON AND PUNNET'S EXPERIMENTS :

Bateson and *Punnet* crossed a pea plant with blue flowers and long pollen (BBLL) with a plant having red flowers and round pollen (bbll). These were true breeding homozygous parents. All the F_1 offspring showed blue flowers and long pollen (BBLL). So blue colour and long pollen are dominant over red colour and round pollen. When the F_1 hybrid (BbLl) was test crossed with the recessive parent (bbll), the following 4 types of phenotypes were formed.

1. Blue flowers, long Pollen
2. Blue flowers, round Pollen
3. Red flowers, long pollen
4. Red flowers, round pollen

The above 4 types of plants were separated and counted. These were in the ratio of 7:1:1:7. This ratio differed from *Mendel*'s test cross ratio of 1:1:1:1.

In *Mendel*'s garden pea plant, seven pairs of genes responsible for seven contrasting pairs of characters were present on 7 different homologous chromosomes. These genes segregated along with the chromosomes during the formation of gametes. Because of this, 4 types of phenotypes are formed in the ratio of 1:1:1:1.

But in *Bateson* and *Punnet*'s experiments of sweet pea, two dominant genes, gene "B" for blue colour and gene "L" for long pollen were on the same chromosome. Recessive genes, gene "b" for Red colour and gene 'l' for round pollen were present just opposite to the dominant genes on the second homologous chromosome. The results of Bateson and Punnet experiments indicate that 4 types of gametes from F_1 hybrid plants are not formed in equal ratio. More gametes are formed without crossing over between the genes B, L and b,l. Less number of gametes are formed with crossing over between the two genes B, L and b, l. This tendency of two dominant genes or two recessive genes to remain together is known as *coupling*. Because of this, parental combinations are more in number than the recombinations in offspring of test cross. This resulted in 7:1:1:7 ratio in *Bateon* and *Punnet*'s experiments.

In another experiment, *Bateson* and *Punnet* crossed a plant having blue flowers and round pollen (BBll) with a plant having red flowers and long pollen (bbLL). The F_1 hybrids were blue and long (BbLl). When the F_1 plant was test crossed with the recessive parent (bbll), the F_2 progeny were in 1:7:7:1 ratio instead of normal 1:1:1:1 ratio. The F_2 progeny had the following four types of phenotypes.

1. Blue and Long : 1
2. Blue and round : 7
3. Red and long : 7
4. Red and round : 1

The above results show that the two dominant alleles or two recessive alleles repelled with each other because they came from different parents. The gametes with genotypes Bl and bL were formed more in number. Hence the blue round and red long plants were produced in more

numbers. So, if two dominant alleles or two recessive alleles are coming from different parents, these alleles tend to remain separated. This phenomenon is known as repulsion.

Bateson and *Punnet* could not give the exact reasons for coupling and repulsion. But *Morgan* (1910) observed these two phenomena i.e., coupling and repulsion in *Drosophila* and explained that these two phenomena are essentially the two aspects of the same phenomenon, "Linkage". He also explained that genes are arranged in a linear formation in the chromosome. During gamete formation, the genes present on a chromosome tend to remain together, instead of assorting independently. This phenomenon is known as Linkage. Because of this phenomenon, genes located in the same chromosome are inherited together by off spring.

The degree of linkage is determined by the distance between the two genes. Linkage strength is inversely proportional to the distance between the two genes. Closely related genes show strong linkage. Hence there will be less possibility of crossing over, resulting in more number of parental combinations and less number of recombinations. Widely located genes show weak linkage, and therefore there is possibility of crossing over, resulting in more number of recombinations. Therefore in a test cross, the percentage of recombinations is like a measuring rod for Linkage.

14.3 LINKAGE GROUP :

All the genes located on the same chromosome constitute a linkage group. The total number of linkage groups in an organism is equal to the number of chromosomal pairs. For example, there are 4 chromosomes in *Drosophila*, which means that there are 4 linkage groups. So also there are 7 chromosomes in garden pea, which means that there are 7 linkage groups.

In *Drosophila melanogaster*, there are four pairs of chromosomes. Of these, one pair is sex chromosomes. In *Drosophila melanogater*, *Morgan* and co-workers found numerous characters many of which were associated together in groups. For example, sex linked characters such as miniature wings, yellow colour body, singed bristles and so on which are present on sex chromosome tended to be inherited together. This shows that above genes are strongly linked and form a linkage group. Similarly, autosomal genes which are present on other three chromosomes form three linkage groups and tend to be inherited together.

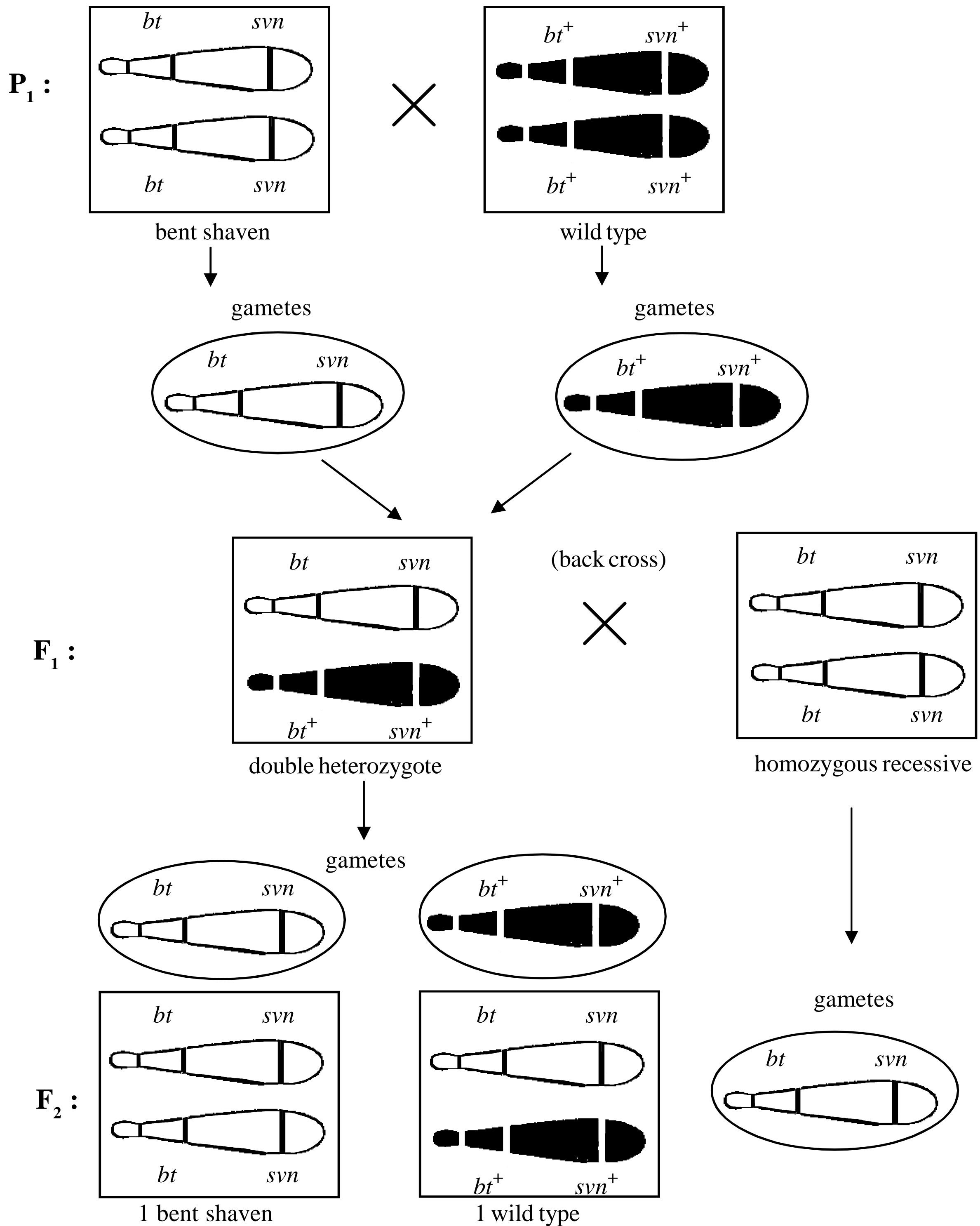
14.4 KINDS OF LINKAGE :

Morgan and his associates conducted experiments in *Drosophila* and other organisms and found out two types of linkage.

1. Complete linkage: In this, two or more genes are inherited together for a number of generations. Here genes are closely associated and tend to be inherited together. As a result, offspring receive only parental characters. Eg: *Drosophila* genes which control the bent wings and the genes which control the shaven bristles.

T.H. Morgan and co workers studied the phenomenon of linkage and crossing over in *Drosophila melanogaster* and found complete linkage in male *Drosophila* fly. The following experiment explains this finding.

In *Drosophila melanogaster*, all the genes present on fourth chromosome show little or no assortment during transmission. For example, when a fly with bent wings and shaven bristles (bt svn / bt svn) is crossed to a fly with normal wings and normal bristles (bt+ svn+ /bt+ svn+), the resulting F1 flies were heterozygous and show normal wings and normal bristles (bt+ svn+ / bt



Complete linkage in *Drosophila melanogaster*

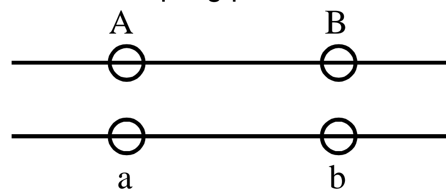
svn). When such F1 flies are crossed to a flies with homozygous bent wings and shaven bristles (bt svn /bt svn) flies, the offspring were either flies with normal wings and normal bristles, or with bent wings and shaven bristles. These are parental combination. Expected recombinations of normal wings - shaven bristles, and bent wings – normal bristles did not appear. This shows the absence of independent assortment and complete linkage of these two gene pairs.Complete linkage is noted in all the four linkage groups when *Drosophila* male is used as a heterozygous parent in a test cross.

2. Incomplete linkage: Linked genes do not always remain together. During meiosis the homologous nonsister chromatids exchange segments. So the linked genes separate because of the crossing over of chromatid segments. These genes, which separate during the process of crossing over, are known as incompletely linked genes and the phenomenon is known as 'incomplete linkage'. It is very common and has been observed in maize, sorghum, pea etc.

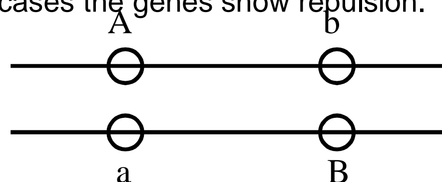
14.5 ARRANGEMENT OF LINKED GENES :

Based upon the arrangement of genes in a chromosome, linkage can be classified into two types.

1. CIS arrangement: The dominant genes (A and B) are located in one member of the chromosome pair and the two recessive genes (a and b) are located in the other chromosome. This type of arrangement (AB/ab) with two dominant genes on the same chromosome is called CIS arrangement. The heterozygotes with such arrangement are known as CIS heterozygotes. In such cases the genes are said to be in coupling phase.



2. TRANS arrangement: In this type, one dominant gene (A) and one recessive gene (b) are located in one chromosome and other dominant gene (B) and recessive gene (a) are present in second homologous chromosome. The heterozygotes with such arrangement (Ab/aB) are known as Trans heterozygotes. In such cases the genes show repulsion.



14.6 SIGNIFICANCE OF LINKAGE :

1) Linkage helps in transmitting parental combinations to the next generation

2) It inhibits the formation of recombinations

3) It helps in raising hybrid varieties, which resemble their parents.

4) Sometimes genes controlling qualitative characters like leaf shape, colour of the stem, colour of the fruit etc. are linked to the genes controlling quantitative characters like size of the fruit, weight of the grain, crop yield etc. Because of this, we can estimate quantitative characters on the basis of qualitative characters easily in the plants raised by hybridization.These genes which express the qualitative characters are known as marker genes.

Eg: Anthocyanin pigment (qualitative) in leaves– Crop yield (quantitative) in rice

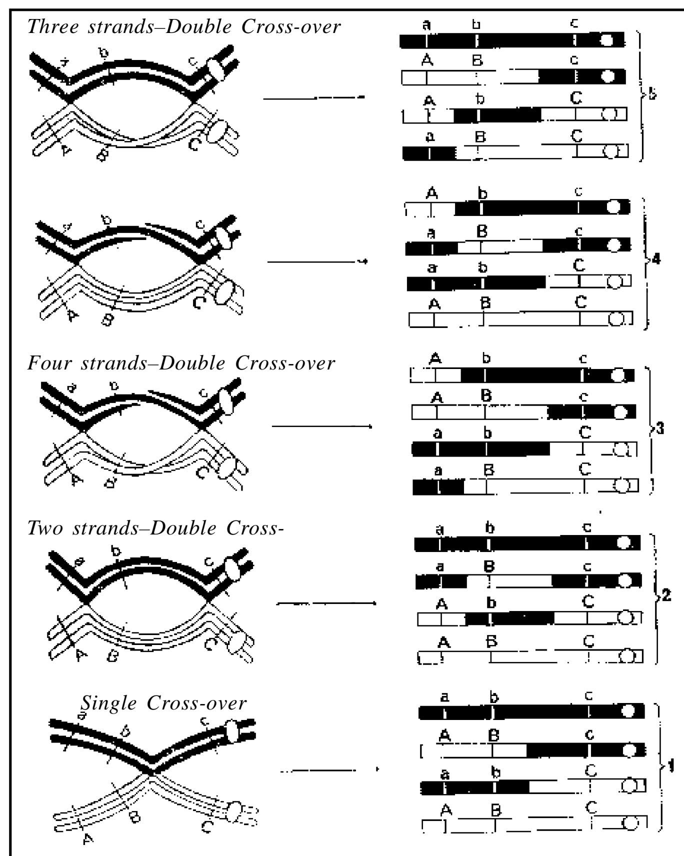
Colour of tomato (qualitative) fruit – Weight of tomato fruit (quantitative)

14.7 CROSSING OVER :

Crossing over may be defined as the exchange of chromosomal segments between non-sister chromatids of a pair of homologous chromosomes. *Morgan* and *Castle* (1912) were the first to introduce the term 'Crossing over' for this exchange of genetic material.

14.8 MECHANISM OF CROSSING OVER :

During the second substage i.e. Zygotene of prophase-I of Meiosis I, the homologous chromosomes get paired. In this stage, two homologous chromosomes attract and come close together to form pairs. This pairing is very precise and the paired chromosomes are known as a 'diad' or 'bivalent' and the process is known as 'synapsis'. In Pachytene stage, each chromosome of a bivalent splits longitudinally to form two sister chromatids. Thus the bivalent now consists of four chromatids and is known as a 'tetrad'. During this stage, the nonsister chromatids of homologous chromosomes twist over each other.



These points of contact are known as chiasmata. At each chiasma, the chromatids break by the activity of an enzyme called endonuelease. The broken segment of one chromatid fuses with the other chromatid. This is brought about by another enzyme known as ligase. This exchange of chromatid segments between non-sister chromatids is called crossing over. After the completion of crossing over, the non-sister chromatids start repelling each other. The chromatids separate

progressively from the centromere towards the chiasma. The chiasmata start moving in zipper-like fashion towards the ends of the tetrad. This movement of chiasmata is known as 'terminalization'. The chromatids get shortened and the homologous chromosomes separate except at their ends.

Theories Explaining the Mechanism of Crossing Over :

There are two theories which explain the mechanism of crossing over.

(1) Copy choice theory (2) Breakage and exchange theory

Copy choice theory: This was proposed by *Belling* in 1931. According to this theory, crossing-over and recombination occur during the synthesis of new chromatids. After pairing of homologous chromosomes, the parent chromatids of each chromosome begin to replicate. It is assumed that a newly synthesized daughter chromatid is formed by copying of one chromosome up to a certain distance and then switching over to the other chromosome for copying the remaining distance. The new chromatids thus carry information from both parental chromatids.

Breakage and Exchange theory: This theory is widely accepted. According to this theory the non sister chromatids of pachytene tetrad break into segments. The broken segments of non-sister chromatids exchange and rejoin to form new combinations.

There is a controversy regarding the breakage of chromatid segments. Following are the different theories which explain the breaking of chromatid segments.

(1) The Contact first theory: This theory was proposed by *Serebrovsky*. According to him, the non sister chromatids of homologous chromosomes first touch and cross each other. Then the breakage occurs at the points of contact of the chromatids. The broken segments rejoin to form new combinations.

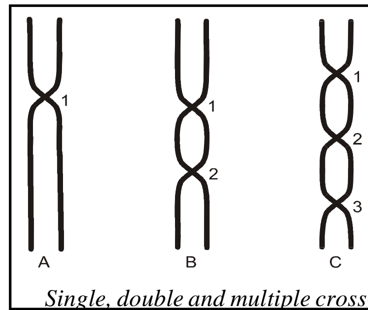
(2) The breakage first theory: This was proposed by *Muller*. According to this theory, the chromosomes first break in to two or more segments. Then the broken segments exchange and rejoin to form new combinations.

(3) The torsion theory: This was proposed by *Darlington*. According to this theory, the two homologous chromosomes coil spirally around each other and develop a torsion among the chromatids which are involved in crossing over. Because of this, breakage occurs in non-sister chromatids and the broken segments rejoin forming new combinations.

14.9 KINDS OF CROSSING OVER :

Depending upon the number of chiasmata formed, crossing-over may be of the following kinds.

Single Crossing-over: In this type, only one chiasma is formed all along the length of a chromosome. Only one chromatid of each chromosome is involved in crossing-over. It produces two non-cross-over gametes, and two cross over gametes.



Single, double and multiple cross-

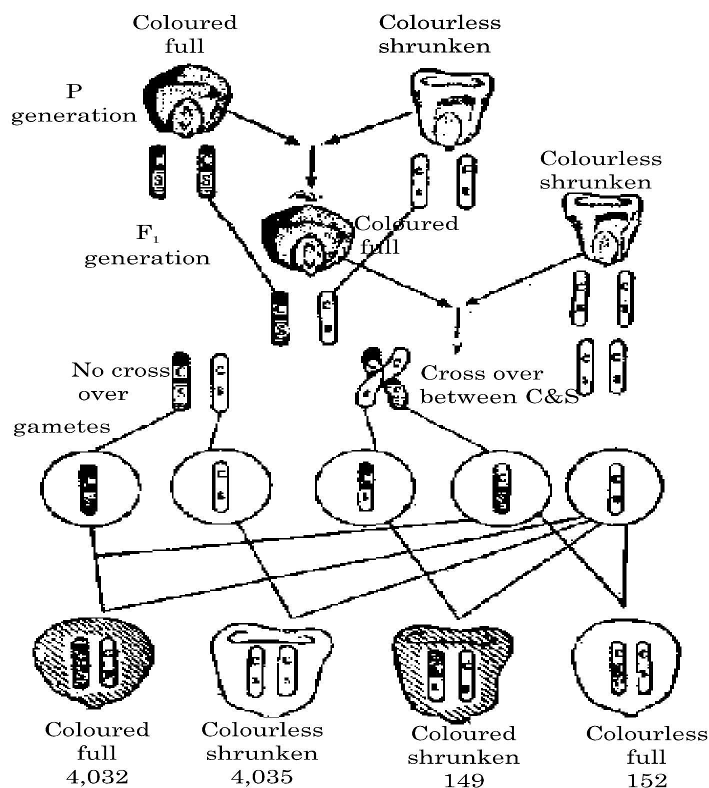
Double crossing-over: In this type, two chiasmata are formed along the entire length of chromosome. Both the chiasmata may be between the same chromatids or between different chromatids. Thus two or three or all the four chromatids may be involved in the process of double cross over. This occurs less frequently.

Multiple Crossing-over: In this type, crossing over occurs at three, four or more points between any two given points in the chromosome pair. Multiple Crossing-over does not occur frequently due to interference in crossing-over.

14.10 HUTCHINSON'S EXPERIMENTS :

Hutchinson carried crossing experiments in Maize which help us to understand linkage and crossing over.

Hutchinson considered colour of the seed and shape of the endosperm in Maize. He crossed a plant with coloured seed (C) and full endosperm (S), with a plant with colourless seed (c) and shrunken endosperm (s).



In F1 generation all plants had coloured seeds and round endosperm (CcSs). When F_1 plants were test crossed with the double recessive parent, it yielded the following number of plants with four different genotypes.

CcSs (Coloured, full) plants	:	4032
Ccss plants (Coloured, shrunken)	:	149
ccSs plants (Colourless, full)	:	152
ccss plants (Colourless, shrunken)	:	4035

The results of the above experiments show that parental combinations are formed more in number than recombinations. This shows that the gene for colour of the seed and the gene for shape of the endosperm are

strongly linked. Recombinations which are formed in less numbers indicate that crossing over took place between these two pairs of genes.

14.11 FACTORS CONTROLLING THE FREQUENCY OF CROSSING OVER :

1. High and low temperatures increase the frequency of crossing-over.
2. X-rays increase the frequency of crossing-over.
3. The frequency of crossing over decreases with increasing in age in female Drosophila.
4. Some gene mutations are known to decrease the frequency of crossing-over.
5. Inversions of chromosome segments suppress crossing-over.

14.12 SIGNIFICANCE OF CROSSING OVER :

1. Crossing-over provides a direct evidence for the linear arrangement of genes in the chromosomes.
2. Frequency of crossing-over is very useful in the construction of chromosome maps.
3. Crossing-over increases frequency of genetic variations.
4. Crossing-over plays an important role in raising new varieties in plant breeding methods.

14.13 SUMMARY :

Bateson and *Punnet* crossing experiments on sweet pea explained two phenomena known as coupling and repulsion. *Morgan* explained that these two phenomena are the two aspects of the same phenomenon called linkage. He also explained that genes are arranged in a linear fashion in a chromosome. During gamete formation, genes present on a chromosome tend to remain together instead of assorting independently. This phenomenon is known as linkage. All the genes located on the same chromosome constitute a linkage group. The total number of linkage groups in an organism is equal to the number of chromosomal pairs. The degree of linkage is determined by the distance between the two genes. Linkage strength is inversely proportional to the distance between the two genes. *Morgan* and his associates conducted experiments in *Drosophila* and other organisms and found out two types of linkage namely complete linkage and incomplete linkage. Linkage helps in transmitting parental combinations to the next generation.

Crossing over may be defined as the exchange of chromosomal segments between non sister chromatids of a pair of homologous chromosomes. Crossing over occurs during the second sub-stage i.e., Pachytene of Prophase I of Meiosis I. Depending upon the number of chiasmata formed, crossing over is of 3 types namely, single crossing over, double crossing over and multiple crossing over. Copy choice theory, and Breakage and exchange theory explain the mechanism of crossing over. Frequency of crossing over is very useful in the construction of chromosome maps.

14.14 TECHNICAL TERMS :

Linkage, coupling, repulsion, linkage group, linked genes, crossing over, parental combinations, recombinations, synapsis, bivalent, non-sister chromatids, sister chromatids, chiasmata, terminalization.

14.15 SELF ASSESSMENT QUESTIONS :

Essay type questions:

1. Explain the phenomenon of linkage with suitable examples.
2. Describe the mechanism of crossing over and mention its significance.

Short answer type of Questions:

1. Coupling and repulsion
2. Linkage group
3. Significance of crossing over
4. Kinds of crossing over

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- P.M. VASANTHA KUMARI

LESSON 15

GENE EXPRESSION - TRANSCRIPTION, TRANSLATION, (PROTEIN SYNTHESIS), GENETIC CODE, GENE REGULATION IN PROKARYOTES Eg - LAC OPERON

15.0 OBJECTIVES

- The objectives of this topic are
- To know about basic mechanism of protein Synthesis
- To learn elaborately about genetic code
- To study gene regulation in prokaryotes with reference to Lac operon.

STRUCTURE

15.I PROTEIN SYNTHESIS

15.1 INTRODUCTION

15.2 TRANSCRIPTION

15.3 TRANSLATION

15.3.1 ACTIVATION OF AMINO ACIDS

15.3.2 TRANSFER OF ACTIVATED AMINO ACIDS TO THE SITE OF ROTEIN SYNTHESIS

15.3.3 INITIATION OF POLY PEPTIDE CHAIN

15.3.4 CHAIN ELONGATION

15.3.5 CHAIN TERMINATION

15.II GENETIC CODE

15.4 INTRODUCTION

15.5 DISCOVERY OF GENETIC CODE

15.6 PROPERTIES OF GENETIC CODE

15. III GENE REGULATION IN PROKARYOTES

15.7 INTRODUCTION

15.8 LAC OPERON

15.9 SUMMARY

15.10 TECHNICAL TERMS

15.11 SELF ASSESSMENT QUESTIONS

15.12 REFERENCES

15.I PROTEIN SYNTHESIS

15.1 Introduction

Proteins are macromolecules, which are made up of several proteins. In molecular biology the central dogma was proposed by **Crick**, 1958. According to this the genetic information flows form nucleic acids to proteins.

DNA ⟶ RNA ⟶ Proteins

The first phase in central dogma is known as **transcription** and second phase is known as **translation**. According to this the transfer of genetic information is unidirectional. **Baltimore** and **Temin** discovered RNA dependent DNA polymerase enzyme called **reverse transcriptase**. This enzyme synthesizes double stranded DNA by using single stranded RNA template. This exciting finding in molecular biology gave rise to the concept of **central dogma reverse**.

The basic mechanism of protein synthesis is same in prokaryotes and in eukaryotes with only few minor differences. Protein synthesis occurs on ribosomes. Each ribosome consists of two subunits. The subunits of ribosomes remain associated during protein synthesis and become dissociated after protein synthesis. The mechanism of protein synthesis in *E coli*, prokaryote is given below. Protein synthesis is broadly divided into two stages. They are transcription and Translation.

15.2 TRANSCRIPTION

In this the genetic information in the nucleotide sequence of DNA molecule is transferred to a complementary sequence of RNA. The process of formation of RNA from DNA is called **transcription**. It occurs in the nucleus. Transcription requires DNA strand; ribonucleoside triphosphates such as ATP, GTP, UTP, CTP; RNA polymerase; divalent metal ions such as Mn^{++} or Mg^{++}; Protein factors such as rho (p). For the synthesis of RNA, the DNA double helix temporally unwinds. The three types of RNA i.e., rRNA, tRNA and mRNA are synthesized on DNA template. Trancription involves 3 main steps viz., Initiation of transcription, Elongation of RNA strand and Termination of transcription. During transcription uncoiling of two strands of DNA takes place, and a bubble like structure is formed which is known as **transcription bubble**. One strand acts as a template for RNA. This strand is called **coding strand .** The other strand that is not transcribed is called **anticoding strand**. Thymine of DNA is replaced by Uracil in RNA. Except this, RNA is equivalent to DNA.

The template strand has a special region called **promoter**. This region is located near the 5' end. The RNA synthesis starts from the promoter. The promoter region has a set of sequences which actually initiates RNA synthesis. This sequence is called **initiation site**. During transcription, the sigma factor (a) combines with the core enzyme to form RNA polymerase. RNA polymerase moves near the template DNA. The RNA polymerase recognizes

the promoter site of DNA and gets attached with it. The recognition is actually done by sigma factor. In this region, the DNA unwinds and a gap is formed. The nucleotide complementary to the first base of the initiation site of the template DNA gets attached to the RNA polymerase. The first nucleotide triphosphate to be incorporated into the RNA chain is usually either ATP or GTP. Hence, the first base of a gene will be a pyrimidine, either T or C. Then a second nucleotide triphosphate is attached to first nucleotide triphosphate through the RNA polymerase. In this way, nucleotide triphosphates are added one by one and the RNA chain grows and elongates. The elongating RNA has a base sequence complementary to that in DNA. When the elongating RNA chain is about 10 bases long, the sigma factor dissociates from the core enzyme and is available to bind to free core enzyme to initiate a new round of transcription. As transcription proceeds, the unwound part of the DNA strand reforms its original double helix. The transcription is stopped at a particular site in the DNA molecule called **transcription termination site or pause site**. This site has the sequence poly A ie AAAAAA. When the RNA polymerase reaches this site, the addition of nucleotides to the RNA is stopped. The stop sequence on the DNA strand is recognised by the RNA polymerase with the association of some protein factors such as rho factor (p) (Fig. 15. 1).

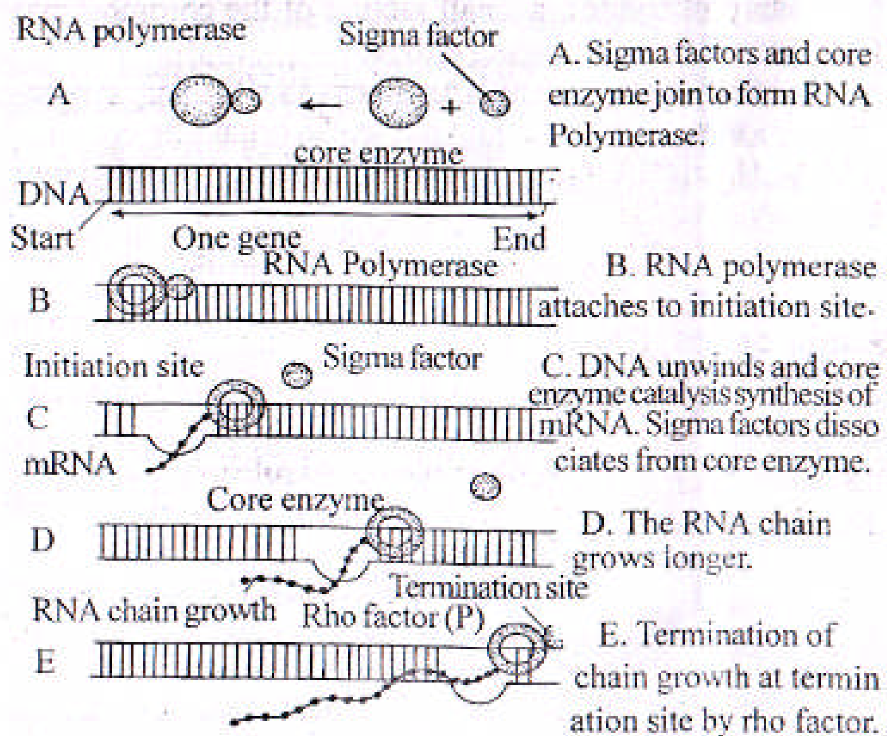


Fig. 15.1 : Scheme of RNA synthesis

After transcription, RNA synthesized is pulled away from the DNA with the help of ribosome. In prokaryotic cells the RNA synthesized is directly released into the cytoplasm. Whereas in eukaryotes, this is a complex process. mRNA synthesis occurs in the nucleus. The primary transcriptions of other RNAs are present in the nucleolus. After post transcriptional modifications they are released into the cytoplasm . The mRNA is short lived. After translation it is destroyed by the enzymes present in the cytoplasm.

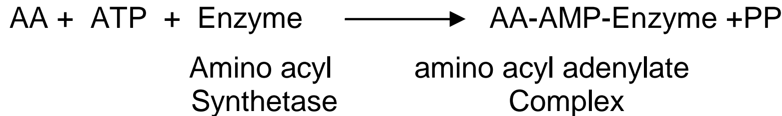
15.3 TRANSLATION

The synthesis of a polypeptide chain by binding specific amino acids in a sequence based on the codons of mRNA is called **translation**. It takes place on ribosomes. These are five steps involved in translation. They are

1. Activation of amino acids
2. Transfer of activated amino acids to the site of protein synthesis
3. Initiation of Polypeptide chain
4. Chain elongation
5. Chain termination

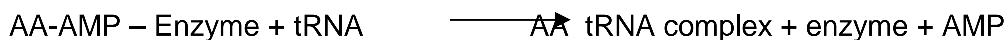
15.3.1 Activation of Amino Acids

The building blocks of proteins are amino acids. Amino acids are scattered in the cytoplasm and exist in inactive state. Depending on the codons of mRNA, specific amino acids get activated. This requires **amino acyl synthetase enzymes** and energy in the form of ATP. Each amino acid has its specific amino acyl synthetase enzyme and tRNA. The amino acyl synthetase enzyme consists of two binding sites, one for the amino acid and the other for tRNA. The enzyme first combines with the amino acid and forms **amino acyl adenylate complex.** During this process pyrophosphate is released.



15.3.2 Transfer of activated amino acids to the site of protein synthesis

This is also a specific process. The amino acyl adenylate complex combines with the specific tRNA and transfers the amino acid to tRNA. All tRNAs have -CCA towards 3' end. This adenine of -CCA replaces the adenylate group of AMP in the aminoacyl adenylate complex and forms aminoacyl tRNA complex. The amino acyl synthetase enzyme and AMP are released.



The amino acid attached to tRNA is said to be **charged.** The charged or activated amino acid moves to the ribosomes. The tRNA with attached amino acid is called **amino acyl tRNA** (Fig. 15. 2).

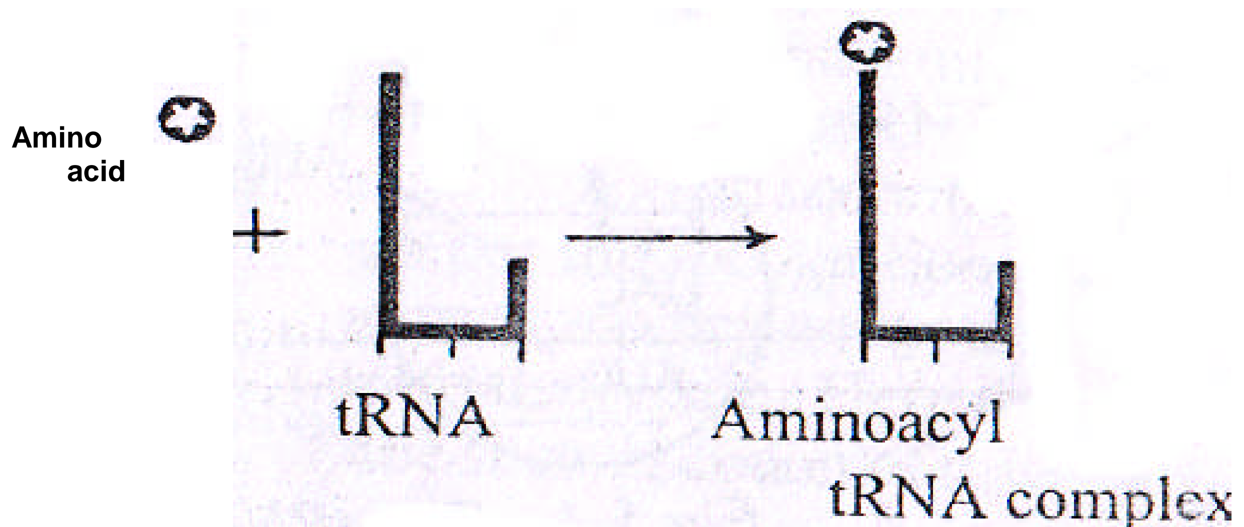


Fig. 15.2 : Attachment of amino acid to tRNA

15.3.3. Initiation of polypeptide chain

AUG is the initiation code of mRNA. It codes for the amino acid methionine. In prokaryotes, methionine enters ribosomes as formyl methionine. The tRNA bringing formyl methionine is thus called as **f- met tRNA** (Formyl methionyl tRNA) and it acts as the initiator aminoacyl tRNA. As the anticodons of tRNA are complementary to the codons of mRNA, they can develop weak hydrogen bonds with the codons of mRNA. The larger subunit of ribosomes has two sites for tRNA. They are amino **acyl site** or **acceptor site** (**A site**) and **peptidyl site** or **polymerization site** or **donor site** (**P Site**). Except initiator tRNA, all aminoacyl tRNAs first bind to A site of ribosomes and move to P site. But the initiator tRNA first binds to P site. The initiator aminoacyl tRNA combines the subunits of ribosomes in the presence of mRNA to form a complex called **initiation complex**. This requires energy in the form of GTP and the **protein factors** called **IF1, IF2 and IF3**. First, the smaller subunit of ribosome combines with IF3 and then to IF1 to form a complex. IF2 complexes with GTP and combines with the above complex. Now, the mRNA is attached to it and then to initiator aminoacyl tRNA. Finally, the whole complex combines with the larger subunit to form initiation complex. In this fmet tRNA is attached to P site and A site is free. During this process, GTP is hydorlysed to GDP and P. As a result, the initiation factors IF1, IF2 and IF3 are released. They can be used again and again (Fig. 15. 3).

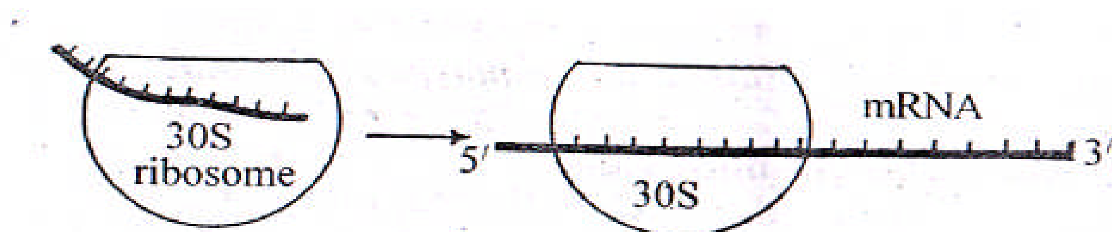


Fig. 15.3 (a) : 30S ribosomal subunit is attached to the 5’ end of mRNA

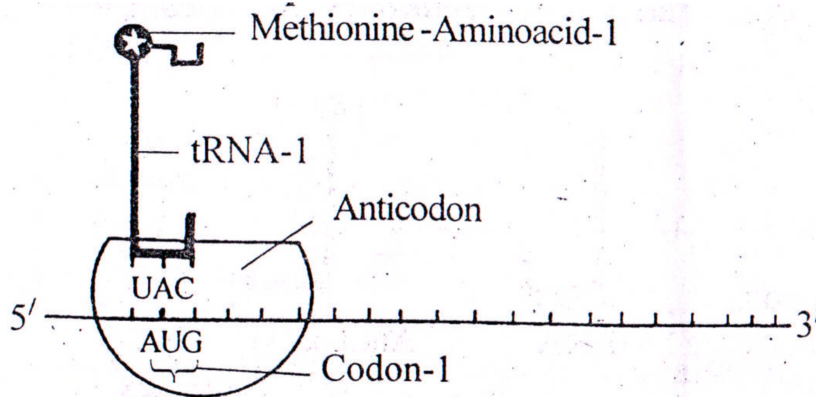


Fig.15.3 (b) : The tRNA having the anticodon UAC, attaches to the mRNA, 30S complex to form 30S pre-initiation complex.

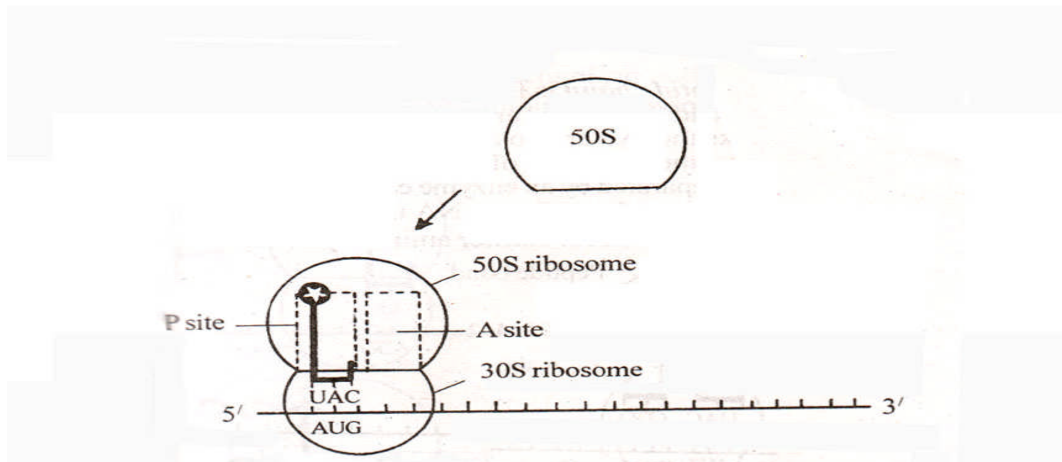


Fig.15.3 (c) : The ribosomal subunit 50S joins with the 30S pre-initiation complex to form initiation complex. The met-tRNA gets attached to the P-site of 70S ribosome.

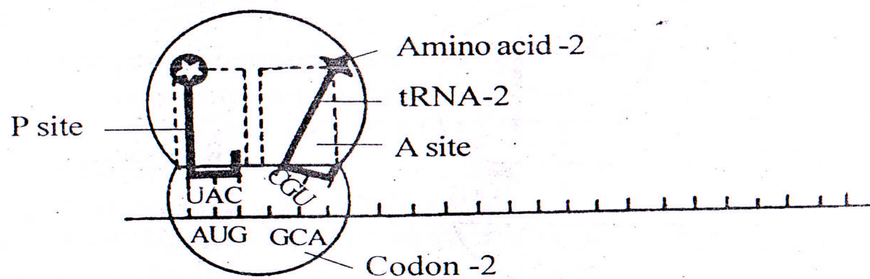


Fig.15.3 (d) : The second aminoacyl –tRNA fits into the A site of 70S ribosome.

15.3.4 Chain elongation

Chain elongation takes place by the establishment of peptide bonds between the amino acids as the ribosomes move along the mRNA, one codon at a time from the initiation codon to

the termination codon in 5' ⟶ 3' direction. This is facilitated by the **elongation factors EF-T** and **EF-G**, GTP, **peptidyl transferase**.

Depending on the second condon of the mRNA, the tRNA forms a complex with the corresponding activated amino acid (**Aminoacyl adenylate complex**). This complex moves to the ribosomes and binds at the A Site. This is facilitated by the protein factor EF-T and GTP. Hydrolysis of GTP releases EF-T and helps in its recycling.

Formyl methionine in 'P' site establishes a peptide bond with the second amino acid at the 'A' site. A molecule of water is released. Peptide bond formation is catalysed by the enzyme **peptidyl transferase**. As a result dipeptidyl tRNA complex is present in the A site, and uncharged tRNA is present in the P site. After peptide bond formation, three types of movements will occur. They are

The uncharged tRNA at the P site leaves from the ribosome.

Dipeptidyl tRNA complex move form A Site to the P Site

Ribosomes move to the next codon of m-RNA in 5' ⟶ 3' end direction.

This movement of ribosomes relative to m-RNA is called **translocation**. Translocation requires a protein factor EF-G (elongation factor G) or **Translocase** and GTP. Hydrolysis of GTP help in the release of elongation factor-G and its recycling. Therefore, the amino acyl tRNA enters 'A' site moves to 'P' site after developing peptide bond. The amino acids are added one by one as per the codon in the mRNA and a polypeptide chain is formed.

This process of binding of incoming amino acyl tRNA at 'A' Site, peptidle bond formation and translocation takes place several times until one of the termination codons occupies 'A' Site (Fig. 15.4).

As the ribosome reaches about the 25th codon on the mRNA, a new ribosome gets attached to the initiation codon and starts synthesizing another polypeptide chain. In this way many ribosomes are moving on an mRNA. The clustger of ribosomes on an mRNA is called a polyribosome or polysome. Each ribosome carries a growing polypeptide chain.

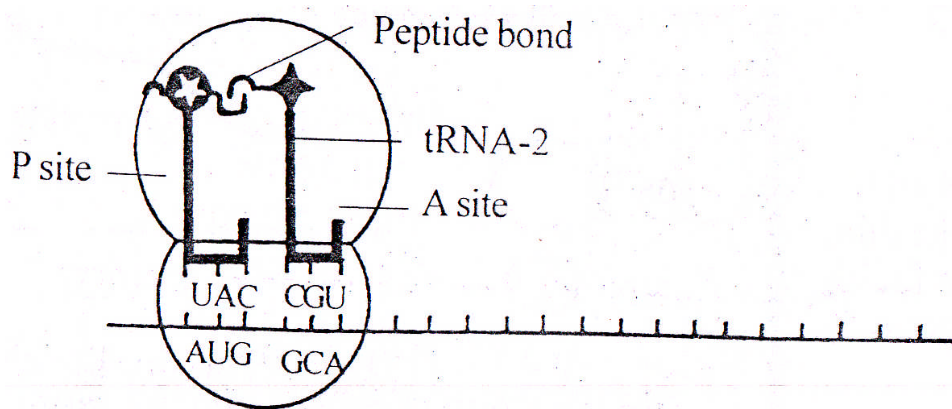


Fig.15.4 (a) : The first aminoacid and the second aminoacid are linked together by apeptide bond to produce a dipeptide.

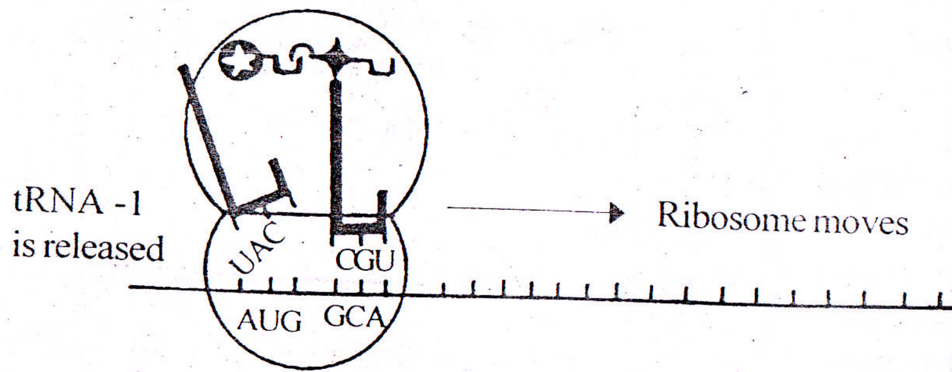


Fig. 15.4 (b) : Release of first t RNA from P site into the cytoplasm

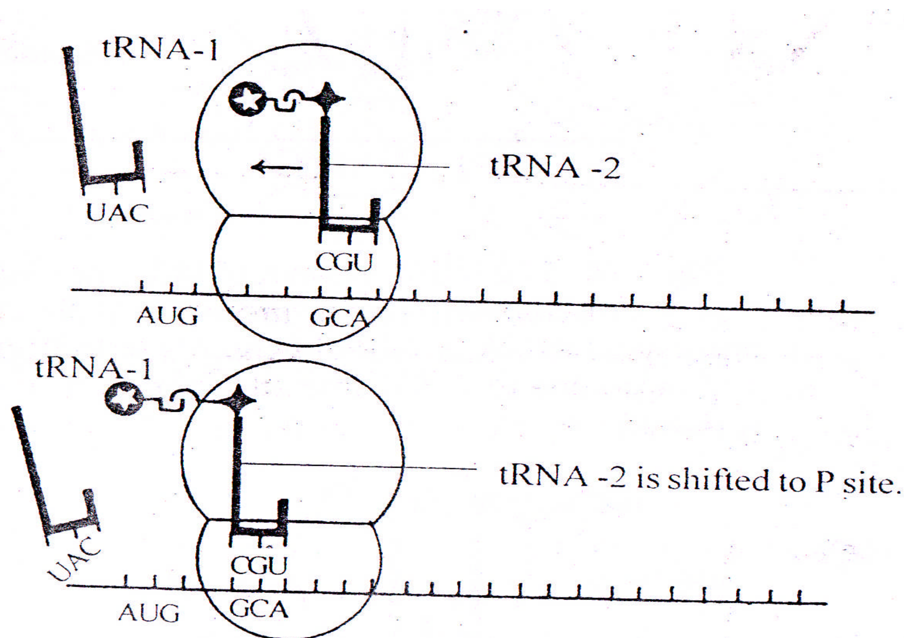


Fig.15.4 (c) : The ribosome moves on the mRNA in the 5' ⟶ 3' direction

The first codon goes out the second codone moves to P site and the third codon comes to lie in the A site. The second tRNA moves from A site to P site.

15.3.5 Chain Termination

When one of the **termination codons** or **nonsense codons** (**UAA, UAG** or **UGA**) is present in mRNA, polypeptide synthesis stops. They do not code for any amino acid and hence no amino aid enters 'A' Site. Thus they act and as **termination codons** or **stop signals** (Fig. 15.5).

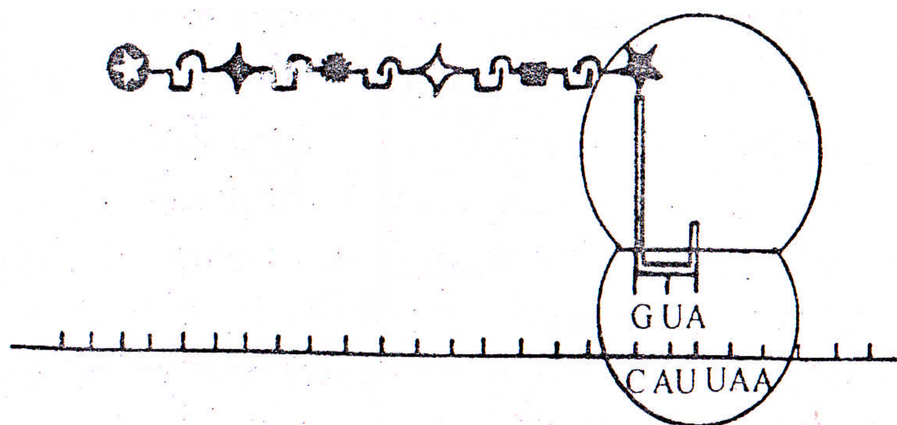


Fig. 15.5 : The ribosome reaches the termination codon.

In prokaryotes, the protein factors called **release factors RF1, RF2, RF3** are present. RF1, RF2 help in recognizing the termination codon and RF3 helps in releasing RF1 and RF2. The enzyme **peptidyl transfrase** helps in the release of polypeptide chain from 'P' site. After this, the tRNA is released from the 'P' site. As translation stops, the two ribosomal subunits are separated.

15.II GENETIC CODE

15.4 INTRODUCTION

DNA is the genetic material and carries genetic information from cell to cell and generation to generation. Gene is a segment of DNA molecule consisting of a linear sequence of nucleotide pairs.

DNA transcribes an mRNA strand which in turn translates a polypeptide chain. Though hundreds of proteins are present in an organism, all of them are not present throughout its life. DNA controls, regulates and determines the nature of proteins to be synthesized in a cell at a given time. Twenty amino acids are involved in the synthesis of proteins present in the living organisms. The number, nature and sequence of amino acids is determined by the nature and sequence of nucleotides in DNA. DNA consists of only four types of nitrogenous bases Adenine, Guanine, Thymine, and Cytosine. The group of nucleotides that specify one amino acid is known as **codon**. The relationship between the sequence of nitrogen bases in DNA / RNA and the sequence of amino acids in a polypeptide chain is called the **genetic code**.

First letter	U	C	A	G	Third (3′) letter
	Second letter				
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA *Ochre* (terminator)	UGA *Opal* (terminator)	A
	UUG Leu	UCG Ser	UAG *Amber* (terminator)	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ileu	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ileu	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ileu	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met (initiator)	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

15.5 : Discovery of genetic code:

The existence of triplet code was discovered by **Nirenberg** and **Matthaei** in 1961. They synthesized artificial mRNA using only one base Uracil. It was named as poly uridylic (poly U molecule). When such ply-U (RNA) was used for cell free synthesis of polypeptide, only polyphenyl alanine was synthesized. It shows UUU is the codon for polyphenyl alanine. Subsequently poly-A m RNA gave polylysine and ply-C m RNA gave polyproline. Therefore codon AAA was assigned to lysine and CCC to praline. After establishing the codons of different homopolymers Nirenberg and his associates tried to establish the nature of codon formed by two or more bases. These were named as copolymers. For instance if only A and C are used, poly AC with consists of ACA, CAA and CCA.

Contribution of Khorona:

Indian born biochemist H.G. Khorona, artificially synthesized mRNA with known repeating sequence of nucleotides. For this valuable work he was awarded Nobel prize in 1970. Using synthetic DNA, Khorana and his co-workers could prepare polyribonucleotides (RNA) with known repeating sequences. A repeating sequence means that if CU are two bases, these will be repeatedly present throughout the length as follows:

CU CU CU CU CU CU

In a similar manner, if CU are three bases they will be repeatedly present as follows:

ACU ACU ACU ACU ACU

Such polymers will direct the incorporation of aminoacids in a manner which can be theoretically predicated. For instance if $(CU)_n$ = (CUC/UCU/CUC/UCU) only two condons are possible and these are CUC and UCU. As these condons are present in alternating sequences the polypeptide formed would have only two aminoacids (lelucine and serine) in alternating sequence.

Assignment of codons with the help of copolymers having repetitive sequences of two bases.

Copolymer	Codons	Aminoacids	Codons
(CU)n	CUC/UCU/CUC	Leucine/serine	CUC/UCU
(UG)n	UGU/GUG/UGU	Cysteine/valine	UGU/GUG
(AC)n	ACA/CAC/ACA	threonine/histidine	ACA/CAC

Similarly if we consider a repeating sequence of three bases (ACG)n the following three kinds of homopolypetides are expected.

Assignment of codons with the help of copolymers having repetitive sequences of three bases = (ACG)n

Codons	Homopolypeptide	Codon assignment
ACG/ACG/ACG/ACG/ACG/ = Poly (ACG)	(threonine)n	ACG= threonine
A/CGA/CGA/CGA/CGA/CGA = Poly (CGA)	(arginine)n	CGA = arginine
AC/GAC/GAC/GAC/GAC = Poly (GAC)	(aspartic acid)n	GAC = asparticacid

To find out which condon codes for which amino acid depends on the previous information available regarding the composition of bases in different codons, coding for different aminoacids. On the basis of above techniques, a complete genetic code dictionary could be

prepared. Two codons namely AUG and GUG are designated as initiation codons and three codonbs namely UAA, and UAG and UGA are designated as termination condons.

15.6 PROPERTIES OF GENETIC CODE

1. The code is a triplet code.

Singlet code (a code of one nitrogenous base) is not sufficient to specify 20 amino acids. A doublet code (a code with two nitrogenous bases) is also inadequate to code for 20 amino acids as it forms a total of 16 codons (4 x 4) only. Triplet code could form 64 codons, which would be enough to code for 20 amino acids. In triplet code, three nitrogenous bases specify one amino acid. For example UUU codes for phenyl alanine, GGG for lysine (Fig. 15. 6).

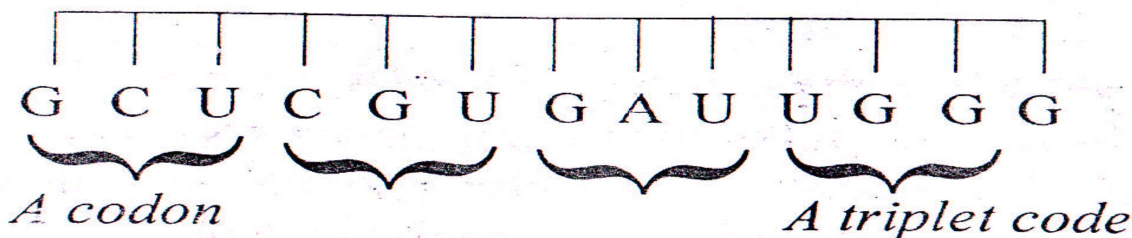


Fig.15.6 : mRNA with codons

2. The code is non-overlapping

Since DNA molecule is a long chain of nucleotides, it could be read either in an overlapping or non-overlapping manner. Thus the genetic code may be overlapping or non-overlapping. However, it was established that one codon does not overlap the other one. Hence each letter is read only once.

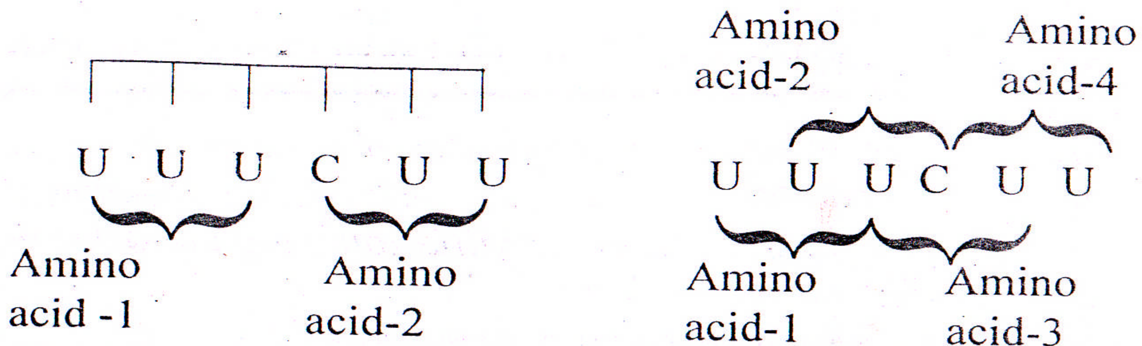


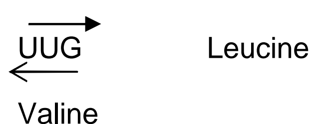
Fig.15.7 : Colinearity of mRNA and protein

3. The code is commaless

The genetic code is read in an uninterrupted manner from one end of the nucleic acid chain to the other. No punctuations between any two codons. After one amino acid is coded, the second amino acid will be automatically coded by the next three bases, so that no bases are wasted.

4. The code has polarity

If a gene is to specify the same protein repeatedly, it is essential that the code must be read between fixed start and end points. These points are **initiation codons** and the **termination codons** respectively. It is also essential that the code must be read in a fixed direction i.e., the code must have a polarity. If the code is read in opposite direction it would specify two different proteins since codons would have reversed the sequence.



Thus if the message said above is read from left to right, the codon UUG would specify Leucine. If it is read from right to left the codon is GUU would specify Valine. The available evidence indicates that the message in the mRNA is read from 5' to 3' direction. The polypeptide chain is synthesized in the amino terminal to carboxyl terminal end (N ⟶ C).

5. The code is degenerate

In a triplet code, 64 codons are possible. They will recognige 20 amino acids. Thus each amino acid is coded by more than one condon. This is called **degeneracy** and such codons are called **degenerate codons**. For example Serine is coded by UCU, UCC, UCA, UCG; Proline by CCU, CCC, CCA, CCG; Lysine by AAA and AAG.

6. Initiating codons

The codons which initiate the process of polypeptide chain formation are referred as **starting codons** or **initiating codons**. **AUG** is the starting codon in most cases which codes for Methionine. In some coses **GUG** is the starting codon which codes for Valine.

7. Termination Codons

The triplet code which is at the end of mRNA and does not specify for any amino acid and serves for termination of polypeptide synthesis, is the termination codon. The codons **UAA, UGA** and **UAG** are the termination codons. UAA is called **ochre**, UAG **amber** and UGA as **opal** or **umber.**

8. The code is universal

The genetic code has been found to be universal i.e., the same code applies in all kinds of living organisms. Eg-UUU codes for Phenyl alanine in all living organisms.

9. The code is non ambiguous

A particular codon will always code for the same amino acid, whereever it is found. The same amino acid can be coded by more than one codon, but the same condon shall never code for 2 different amino acids.

10. Wobble hypothesis

The triplet code is degenerate as there are many more codons than the number of amino acid types. This degeneracy of code was first explained by **Crick** and it is called as **Wobble hypothesis**. Generally in DNA strand, a purine canot pair with another purine and a pyrimidine cannot pair with another pyrimidine. According to Wobble hypothesis only the first two positions of a triplet codon on mRNA have a precise pairing with the bases of the RNA antiodon. The paining of the third position base of the codon may be ambiguous, and varies according to the nucleotide present in this position. Thus a single tRNA type is able to recognize two or more codons differing only in the third base. For example, the anticodon UCG of Serine tRNA recognizes two codons AGC and AGU. The bonding between the UCG-AGC is the usual Watson-crick paining pattern. In UCG-AGU pairing, hydrogen banding takes place between G and U. This is the unusual base pairing and it is called Wobble base pairing and the non-specific third base is called Wobble base (15.8).

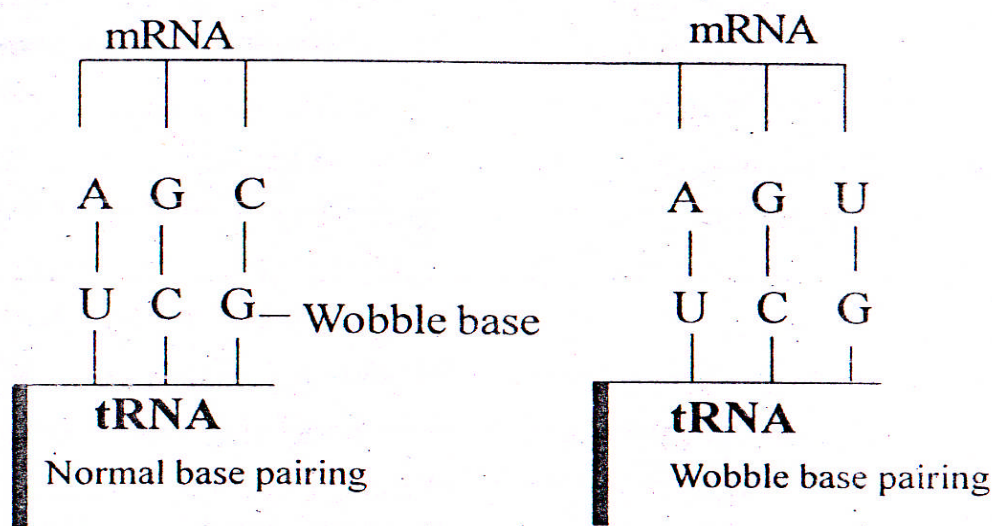


Fig.15.8: Wobble hypothesis.

5.III GENE REGULATION IN PROKARTOTES:

15.7 INTRODUCTION

DNA molecule has genetic information for almost all the proteins, which may be required by an organism in specific amounts at certain definite times of the life cycle. But all the genes (Cistrons) of the DNA molecule cannot make polypeptides all times. Only one or few genes will be functioning in any adult. The other genes will not function, and synthesize proteins. The

genes which are functioning are said to be **switched on**, and the genes which are not functioning are said to be **switched off**. Therefore, the flow of information from DNA (gene) to protein is regulated in some manner. Gene activity is regulated primarily at the level of transcription. To explain the regulating mechanism of expression at transcriptional level in prokaryotes, **Jacob** and **Monad** put forwarded a hypothesis called **Operon hypothesis**.

15.8 REGULATION IN BACTERIA-OPERON MODEL, LAC OPERON

Lac operon is a set of genes responsible for the metabolism of lactose in *E. coli.* The lac operon was discovered by F. **JACOB** and J. **MONAD** in 1961. For this they were awarded the Nobel prize in 1965. They proposed the **OPERON MODEL** to explain the regulation of genes coding for the enzymes required for lactose utilisation in *E. coli*. If bacteria are grown in media containing glucose, enzymes for the breakdown of lactose are present in very low levels. If they are grown in media without glucose but with lactose, the level of lactose enzymes increases a thousand fold. Jacob and Monad selected this system as a clear example of gene regulation based on metabolic needs of the cells. The Lac operon consists of three structural genes, three control genes and an inducer molecule (Fig. 15. 9).

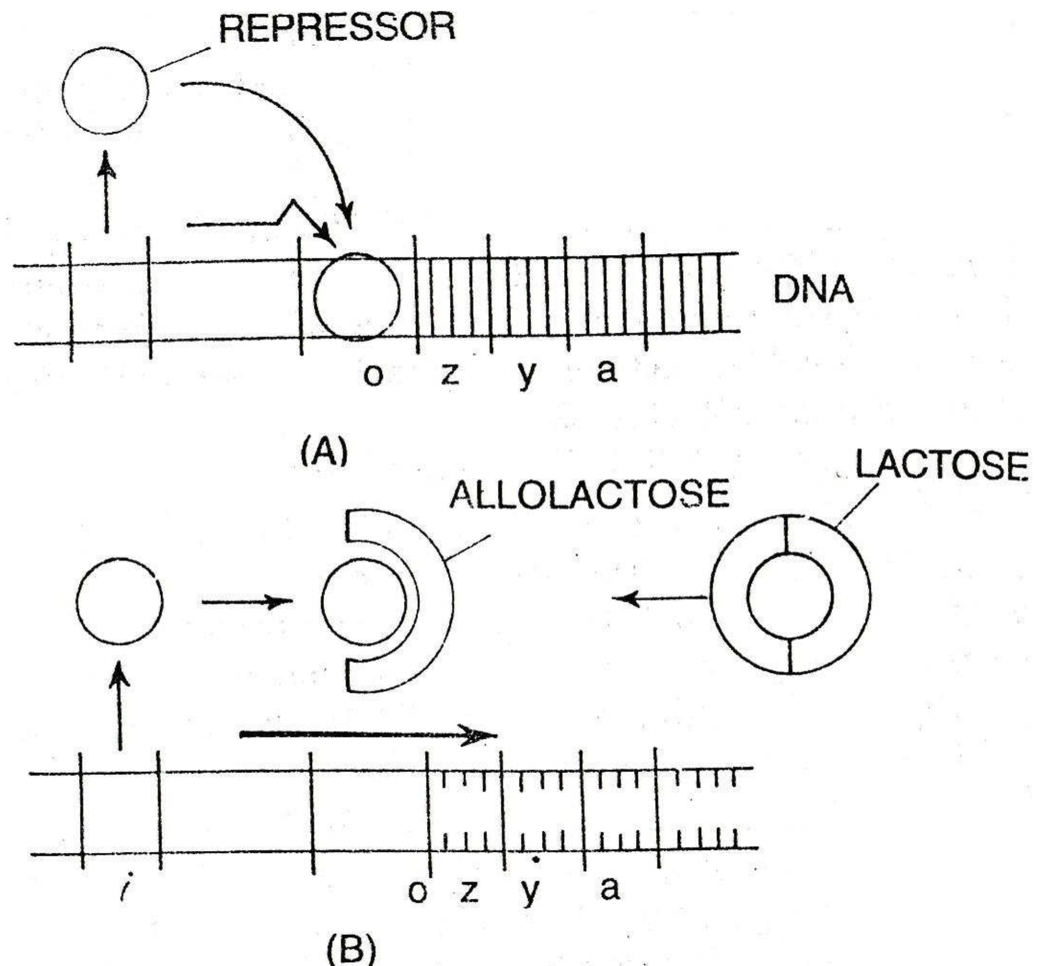


FIG 15.9 Structure of Lac operon

Structural genes: Jacob and Monad identified three genes which determine the structure and synthesis of the three relevant enzymes, which take part in the breakdown of

Lactose. These three genes were named the structural genes and were designated "z" (b-galactosidase) "y" (β-galactoside permease) and "a" (b – galactoside transacetylase).

Control genes: The DNA near the structural genes were found to have regulatory function controlling transcription of structural genes. Three control genes are present promotor gene, operator gene and regulator gene.

The promoteo gene (p) represents a sequence of bases that are recognised by enzyme RNA polymerase. The promotor – polymerase binding initiates transcription of the neighbouring structural genes of a operon. The operator gene (o) is closely linked to the first structural gene 'z'. When the operator gene is active, the structural genes synthesize the enzymes. The operator and promotor of an operon are under the control of yet another segment of DNA called regulatory gene (i), which is situated apart from the promotor, operator and structural genes of an operon. The activity of
operator gene is decided by a repressor protein synthesized by the regulator gene. When the repressor binds to the operator gene, the operator gene is made non-functional. This state of the operator gene is called repressed state and the phenomenon is called repression.. During repression, the enzymes are not synthesized.

Inducer or Effector molecule: An inducer or effector molecule is classically a small molecule. It may be a sugar, an amino acid or may be a nucleotide, that can bind to a regulator protein and thereby change its ability to interact with an operator or promotor.

In the lac operon system, the lactose functions as an inducer for the synthesis of the three enzymes. Hence the lac operon system is called an inducible system. When lactose is introduced into the medium it diffuses into the cell and binds to the repressor protein to form an inactive inducer repressor complex. The inactive inducer repressor complex cannot bind to the operator gene and the operator genes are set free to do the function. This state of the operator gene is called derepressed state and the phenomenon is called derepression. When the operator gene is derepressed, the RNA polymerase binds to the promotor gene. This initiates the transcription of structural genes. The transcription of structural genes leads to the synthesis of the three enzymes namely B-galactosidase, galactoside permease and thiogalactoside transacetylase (Fig. 15. 10). These three enzymes bring about the metabolism of lactose. B-galactosidase splits lactose into glucose and galactose. Galactoside permease facilitates the entry of lactose into the cells. The function of thiogalactoside transacetylase is not known.

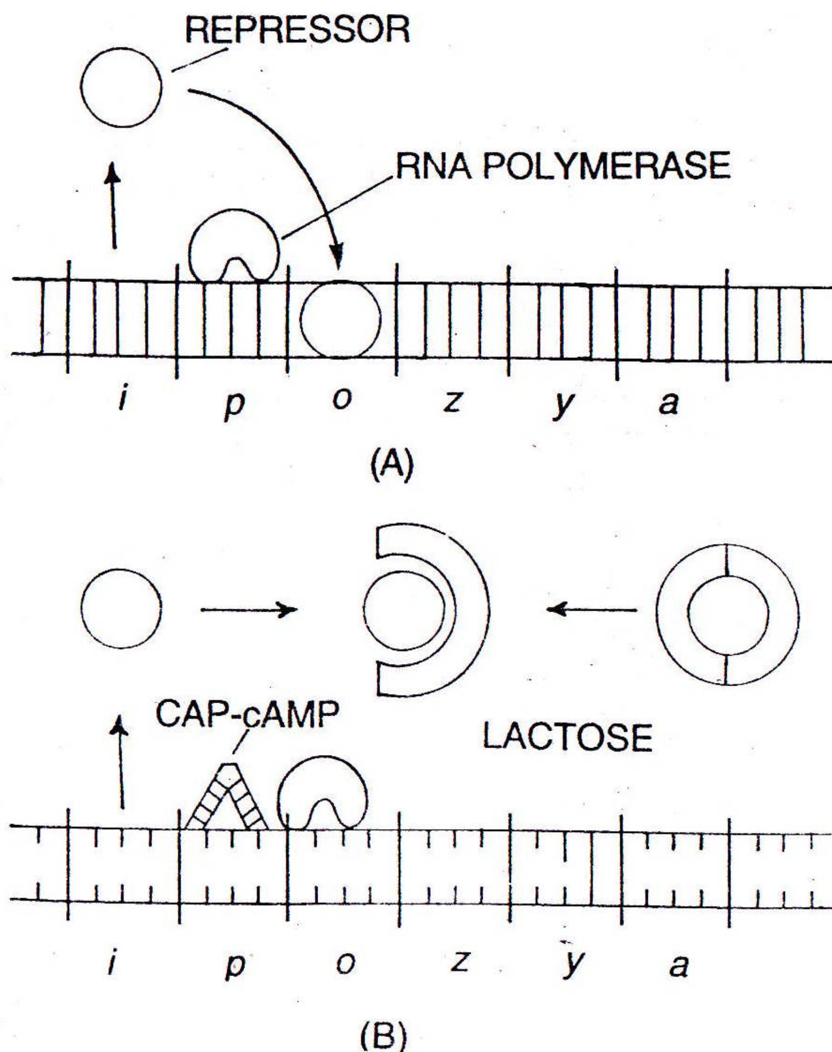


Fig. 15.10 (A) LACTOSE PRESENT (B) LACTOSE ABSENT

When *E. coli* is grown on a glucose medium in the absence of inducer i.e., lactose, the repressor protein is synthesized by the repressor gene. The repressor protein binds to the operator gene, so that the transcription of the structural genes is blocked and the three enzymes are not produced. The lac operon is a system of negative regulation. In negative regulation, the regulator protein repressor prevents the gene transcription.

It is found that a complex protein called catabolic activator protein, CAP binding with cyclic AMP (cAMP) must interact with part of the promotor region, which is nearest to the ‘i’ gene. This interaction is important for RNA polymerase to interact with the part of the promotor nearest the operator and thus initiate transcription of structural genes. The CAP-cAMP interaction with the promotor must occur for transcription, even if there is no repressor molecule binding to the operator region. Thus the CAP-cAMP complex acts as a positive regulation (Fig. 15. 11). The presence of glucose in medium is known to reduce the level of cAMP in bacteria. The fewer the cAMP molecules are, the less CAP-cAMP complex that is formed. The result is a decrease in positive regulation and a decrease in the transcription of lac enzymes.

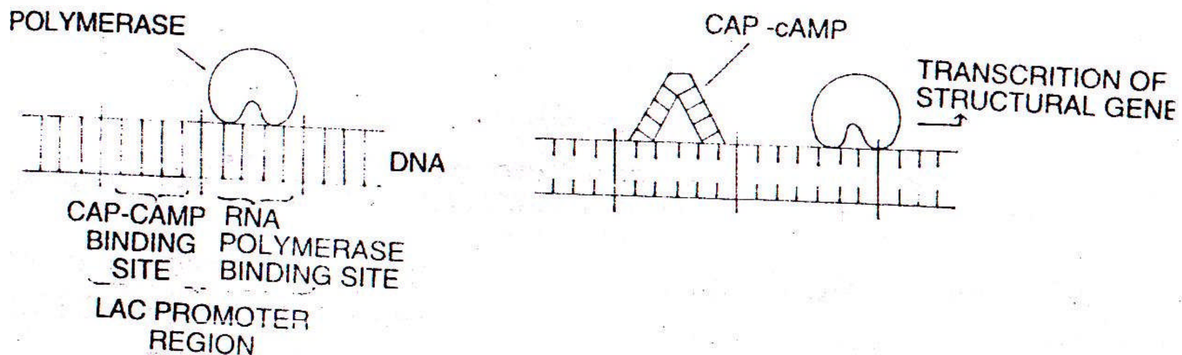


Fig 15.11 c AMP - CAP

Thus a dual control over the synthesis of enzymes for the breakdown of lactose exists. The repressor-operator and CAP-cAMP promotor interactions. Both controls are determined by the presence or absence of glucose and presence of lactose. The lac operon acts independently both positively and negatively. The repressor protein acts as a negative regulator and the CAP-cAMP protein acts as a positive regulator.

15.9 SUMMARY

The basic mechanism of protein synthesis is same in prokaryotes and in eukaryotes with few minor differences. The protein synthesis occurs on ribosomes. Protein synthesis is broadly divided into two stages, transcription and translation. During transcription the genetic information in the nucleotide sequence of DNA molecule is transferred to a complementary sequence of RNA. This process occurs with the help of the enzyme RNA polymerase or transcriptase.

The synthesis of a polypeptide chain by binding specific amino acids in a sequence based in codon of mRNA is called translation. It occurs on ribosomes and involves five steps. They are activation of amino acids, transfer of activated amino acids to the site of protein synthesis, Initiation of polypeptide chain, chain elongation and chain termination.

Twenty amino acids are involved in the synthesis proteins present in the living organisms. DNA consists only four of nitrogenous bases Adenine, Guanine Thymine and Cytosine. The amino acids are determined by nucleotide sequence in DNA. The group of nucleotide that specify one amino acid is known as codon The relationship between the sequence of nitrogen bases in DNA/RNA and the sequence of amino acids in the polypeptide chain is called the genetic code. The genetic code has many properties such as it is triplet code, non-overlapping, commaless, has polarity, degeneracy, has initiating codes, has termination codon, universal, non-ambiguous etc.

DNA molecule has genetic information for almost all the proteins. But all are not expressed at one time only. One or few genes are functioning in any adult organism. Therefore, the flow of information from DNA (gene) to protein is regulated in some manner. Gene activity is regulated primarily at the level of transcription. Jacob and Monad proposed Operon

hypothesis to explain gene regulation in prokaryotes. Lac operon is a set of genes responsible for the metabolism of lactose in *E. coli.* Lac operon consists of structural genes, and control genes. The control genes are promoter gene, regulator gene and operator gene. In this system, the lactose functions as an inducer for the synthesis of structural genes, hence it is called inducible system.

15.10 TECHNICAL TERMS

Gene expression, protein synthesis, transcription, translation, RNA polymerase, Transcriptase, amino acids, codon, genetic code, triplet code, polarity degeneracy, initiating codons, termination codons, non-ambiguous, wobble hypothesis, gene regulation, operon hypothesis, Lac operon, structural genes, control genes, promotor gene, regulator gene, operator gene, inducible system.

15.11 SELF ASSESSMENT QUESTIONS

1. Write an account on the mechanism of Transcription
2. Write an account on translation
3. Enumerate the properties of genetic code
4. Write an essay on mechanism of gene regulation is prokaryotes using lac operon

SHORT NOTES

1. Wobble hypothesis
2. Lac operon
3. Transcription
4. Positive Regulation
5. Negative Regulation
6. CAP
7. Promoter
8. Termination of Transcription
9. Polysome
10. Discovery of Generic Code
11. Khorana
12. Degeneracy
13. Termination Codons

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- Dr.T.Srivalli

Lesson – 16

GENE MUTATIONS

16.0 OBJECTIVE :

The objective of this lesson is to study gene mutations. In this chapter we learn about kinds of mutations classified according to the nature, origin, degree of magnitude of mutations and molecular basis of gene mutations.

16.2 STRUCTURE :

16.1 INTRODUCTION

16.2 KINDS OF MUTATIONS

16.3 MOLECULAR BASIS OF GENE MUTATIONS

16.4 TRANSPOSONS

16.5 SUMMARY

16.6 TECHNICAL TERMS

16.7 MODEL QUESTIONS

16.8 REFERENCE BOOKS

16.1 INTRODUCTION :

The term 'mutation' was first used by *Hugo DeVries* to describe sudden, drastic and heritable changes in populations. *De Vries* worked on *Oenothera lamarckiana* and observed variations in 837 plants out of 54, 343 plants. Earlier *Darwin* also observed these sudden changes and called them sports or saltations. *Bateson* called them discontinuous variations. *Mendel*'s experiments revealed that genes are responsible for expression of various characters. *T.H. Morgan* worked on *Drosophila melanogaster* and concluded that genes are arranged in a linear fashion in chromosomes. DNA replicates and forms exact copies. But some times mistakes occur during replication. Because of this, gene which is made up of DNA, may either loose or gain some nucleotides. This brings in a change in the structure of the gene. So a change in the gene causes a change in the phenotype. This is called mutation, or gene mutation. At present geneticists are considering only gene mutations as true mutations. These are also called point mutations or micro mutations.

16.2 KINDS OF MUTATIONS

According to the nature, origin and degree of magnitude of mutations, mutations are classified into various types.

Somatic mutations: Mutations that occur in somatic cells are called somatic mutations. They cannot be inherited and disappear with the death of an organism. But the beneficial mutations can be reproduced by vegetative reproduction through budding, grafting etc.

Eg: Famous golden delicious apple, Navel orange, Emporor seedless grapes, *Mirabilis jalapa*, Snap dragon etc.

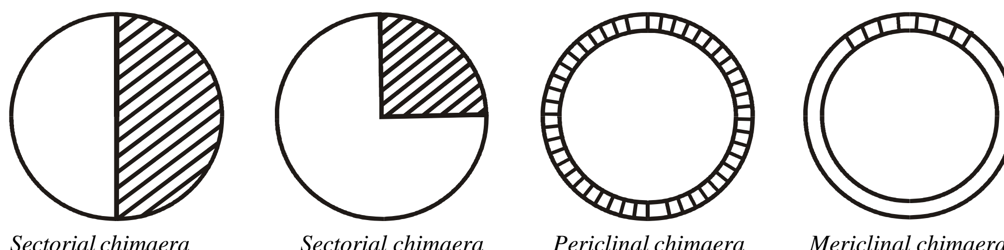
The entire vegetative bud gets mutated, if somatic mutation occurs in its early stages of development. These are called bud mutations Eg; Varieties in Potato - Cobbler, Pontiac, Barbank, Red trymph. If mutation occurs during the later stages of development of a vegetative bud, only a few cells of the bud get mutated. The organ that develops from this mutated bud consists of both mutated cells as well as non mutated cells. Such an organ containing these two types of tissues is called "Chimaera". Chimaeras occur spontaneously in nature and are of three types.

(a) Sectorial chimaera: In this, mutated cells and non mutated cells are present in segmental arrangement from epidermis to centre, in root, stem and leaves. Eg: Leaves of *Sorghum* and Maize.

(b) Periclinal chimaera: In this, two genetically different tissues are present one around the other. Eg: Golden wonder potato.

(c) Mericlinal chimaera: In this, mutated tissue is present incompletely in the epidermis. These may be seen in grafted plants.

Eg: Graft of *Solanum* and *Lycopersicon*.



Sectorial chimaera *Sectorial chimaera* *Periclinal chimaera* *Mericlinal chimaera*

Germinal mutations: These occur in germ cells or reproductive cells. These are heritable and are of great significance.

Eg: Short legged Ancon sheep

Short legged Dachshund dog

According the stage of development of germ cells, mutations are classified into

(a) Gametic mutaions

(b) Zygotic mutaions

All gametes show mutated characters, if the mutation occurs during the development of gametes. Mutated characters are seen in one half of the organism, if mutation occurs in a zygote after first division.

According to the direction of path of a mutation, mutations are of two types.

(a) Forward mutation: A mutation which changes a wild phenotype into an abnormal phenotype is called a forward mutation.

Eg: Vestigial wings of *Drosophila*.

(b) Reverse mutation: A mutation which changes back to abnormal phenotype into a wild phenotype is called reverse mutation. It occurs very rarely.

Eg: Sensitivity to Streptomycin in *E. coli*.

According to the magnitude of effect seen in the phenotype of an organism, mutations are of 4 types.

(a) Dominant Mutation: When a mutation produces a dominant gene, it is called dominant mutation. A dominant mutation expresses itself in the immediate next generation. Eg: Eyes without cornea in man.

(b) Recessive Mutation: If mutated genes are recessive to the wild type, such a mutation is called recessive mutation. It is expressed only when the mutant gene occurs in a homozygous condition, in second or following generations.

Eg. Most of the natural mutations are recessive in nature.

(c) Iso alleles: Iso alleles are mutated genes which cause small changes in the phenotype of organisms. These phenotypical changes are detected only by special methods. Iso alleles cause same type of phenotype in both homozygous as well as heterozygous conditions.

(d) Lethal Mutation: This is a mutation which is lethal to organisms in homozygous condition but not in heterozygous condition.

Eg: Death of seedlings in *Pennisetam typhoidium*, *Oryza sativa*, *Zea mays*, due to non production of chlorophyll.

Spontaneous Mutations: These mutations occur in nature due to intramolecular changes or changes in biochemical processes.

Eg: *Oenothera*, bacteria, maize, man, etc

Induced Mutations: These mutations are caused by external factors called mutagenic agents or mutagens. Mutagens are of three types namely (1) Physical mutagens (2) Chemical mutagens (3) Temperature

(1) Physical mutagens: These are of two types.

(a) Ionising radiations: X-rays, alpha rays, beta rays, gamma rays, neutrons and protons are ionising radiations which are fast moving particles that have high penetration power. So they are used to induce mutations in seeds to bring about changes. X-rays can be obtained from an X-ray machine, gamma rays from radio active isotopes like Cobalt 60, Beta rays from Phosphorus 32 and neutrons from a nuclear reactor.

(b) Non ionising radiations: Ultra violet rays are non ionising rays which have low penetrating power and are used to induce mutations in Pollen grains and bacteria and cells of other microorganisms. U.V. rays can be obtained from U.V. lamps. U.V. rays bring about structural changes in purine - pyrimidine bonds. Nitrogen bases which are affected by U.V. radiations are known as photo products. Pyrimidines are more affected than purines, by U.V. radiation. The effected pyrimidine, pairs with another pyrimidine which is closer to it, through a covalent bond. This pair of pyrimidines is called a Dimer and the process is known as ‘Dimerization’. As a result of dimerization, wrong pairing of nitrogen bases takes place, resulting in mutation of a gene.

(2) Chemical mutagens: C.Auerbach was the first to find that mutations can also be induced due to certain chemicals. Some of the common chemical mutagens are Nitrous acid, Hydroxylamine, alkylating agents like ethyl methane sulphonate, ethyl ethane sulphonate, nitrogen mustard etc.

Nitrous acid and nitrogen mustard directly react with DNA molecule and modify its structure. Base analogues such as 5-Bromo Uracil and aminopurine, incorporate themselves into the DNA molecule during its replication, and bring about mutation. This type of mutation is known as copy error.

(3) Temperature: With the increase in temperature, rate of mutation increases. *Muller* and *Altenberg* reported that in *Drosphila*, the increase of temperature from 17°C to 27°C, caused 2.5 times increase in the rate of mutation. Some times, low temperatures also cause mutations. Eg: Paddy

16.3 MOLECULAR BASIS OF GENE MUTATIONS :

Mutations occur due to molecular level changes in DNA which is a macromolecule. Mutated genes cause changes in the genetic code, which is transmitted to the next generation.

The changes in nucleotides which effect the sequence of nitrogen bases, take place by two ways

(1) Substitution (2) Frame shifts

Substitution: Replacement of one nucleotide by another nucleotide is known as substitution. Substitution is of two types – a. transitions b. transversions

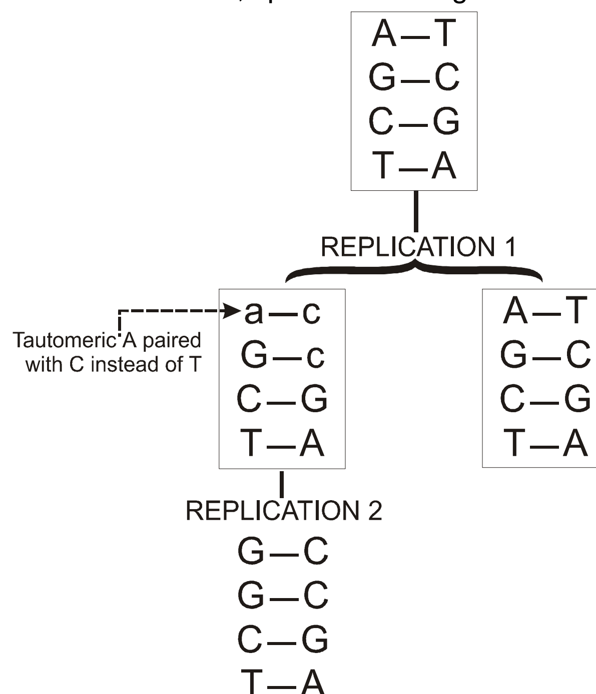
(a) Transition: In this, substitution of one purine for another purine or one pyrimidine for another pyrimidine, takes place. Thus four different transitions are possible.

ADG or CDT

In this purine - pyrimidine orientation remains the same.

Transitions occur due to tautomerism, action of base analogues and deamination.

Tautomerism: Nitrogen bases also occur as tautomeric forms. Tautomeric forms occur due to rearrangement of hydrogen atom between 1,6 positions in ring structures of nitrogen bases during replication of DNA.

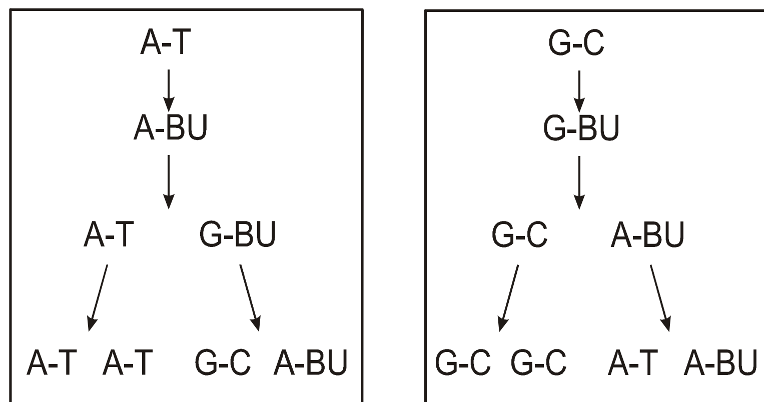


Tautomerism – Gene Mutations

During DNA replication, tautomeric forms pair with a base different from a normal base. For example, Tautomeric adenine pairs with cytosine instead of thymine. Cytosine, which entered DNA strand instead of thymine, pairs with guanine in the next replication. In this way A-T base pair changes to G-C base pair. This sort of mutation which occurs during replication is known as copy error.

Base Analogues: Some chemicals such as base analogues like 5-bromouracil, 2-amino purine, have molecular structures similar to that of normal bases. These base analogues also have tautomeric forms and

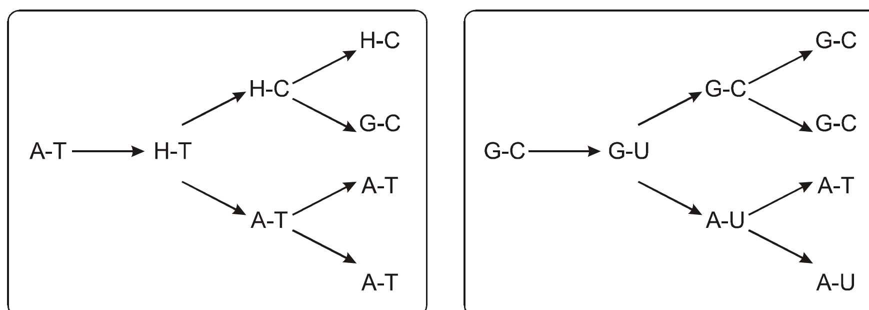
Base Analogue – Gene Mutations



ionizing forms. During DNA replication, 5-bromo uracil replaces thymine, and pairs with adenine. Tautometric 5-bromouracil pairs with guanine. Because of this, A-T base pair is replaced by G-C base pair, in successive replications.

De Amination: Nitrous acid reacts with amino group of nitrogenous base, and brings about deamination by replacing an amino group (NH_2) with a hydroxyl (-OH) group. For example, cytosine undergoes deamination to form uracil. Normally, uracil pairs with adenine. So adenine enters the complementary chain of DNA wherever uracil is present. In the next replication, adenine chooses thymine in its complementary chain. In this way A-

Deamination



Base Analogue – Gene Mutations *gene mutaions*

T base pair replaces G-C base pair. Similarly, adenine undergoes deamination to form Hypoxanthine. Hypoxanthine pairs with cytosine in the next replication. After two replications, an A-T pair changes to a GC pair.

TRANSVERSIONS:

Transversions were first discovered by *Frease* (1959). Transversion involves substitution of a purine for a pyrimidine or pyrimidine for a purine. A change occurs in the orientation of purine-pyrimidine bond, in transversion. Transversions bring 8 possible changes.

ADTDGDCDA

Transitions and transversions are induced by certain alkylating agents such as nitrogen mustard, ethyl ethane sulphonate, ethyl methane sulphonate etc. These bring about alkylation in nitrogen bases. In this, hydrogen atom is replaced by an alkyl group. Because of this, guanine-7 becomes alkylguanine which either pairs with thymine or gets separated from DNA strand .As result of alkylation, base-sugar bond undergoes hydrolysis and nitrogen bases get separated from DNA chain. The loss of base produces gap in the DNA strand. This gap may be filled either with a correct base or wrong base. If it is a wrong base, it may produce either transversion or transition, during DNA replication. For example, during replication, if purine enters opposite to the gap, pyramidine (Cytosine, guanine) enters the complementary strand. In this way purine-pyrimidine pair become pyrimidine-purine pair.

Frame shift Mutations: If chemical mutagens are introduced into the DNA molecule, addition or deletion of nitrogen base occurs in the DNA molecule. This brings about a frame shift mutation. Dyes like acridine orange and proflavin, increase the distance from $3.4A^{o}$ to $6.8A^{o}$ between any two closely placed purines. These dyes which get inserted in between two bases, are known as 'inter calating substances'. This results in a shift of the reading of the frame, during protein synthesis. Frame shift mutations generally lead to nonfunctional proteins. Because of the frame shift mutations, m-RNA either lacks one nitrogen base or has one nitrogen base in addition. This causes a change in the sequence of amino acids, which results in a change in the structure of a protein. Eg: Addition of Guanaine in between U and C changes the sequence of amino acids.

UAU CCA UAU CCA UAU

Tyrosine Proline Tyrosine Proline

Tyrosine

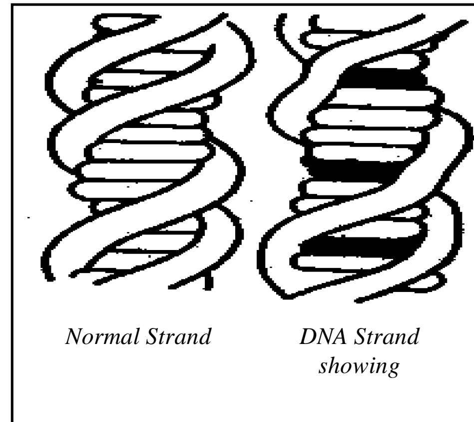
UAU GCC AUA UCC AUA

Tyrosine Alanine Isoleucine Serine

Isoleucine

Changes occur in protein structure due to substitution of a middle base for another base, in a codon. For example, if GAA becomes GUA (substitution of A with U), valine enters into hemoglobin instead glutamic acid. This causes sickle cell anaemia. This type of mutation is known as 'missense mutation'. Synthesis of polypeptide chain stops if the last base is substituted with another. For example, UAU codes for tyrosine. If last base U is replaced by G or A, the codon becomes UAG or UAA. These are terminating codons which terminate the chain. These mutations are known as 'nonsense mutations'. But in some mutations, there will be no change in the sequence of amino acids. For example, CCU, CCA, CCG, CCC code for proline. The last base makes all the difference. So the change in the last base, does not cause any effect. These are known as

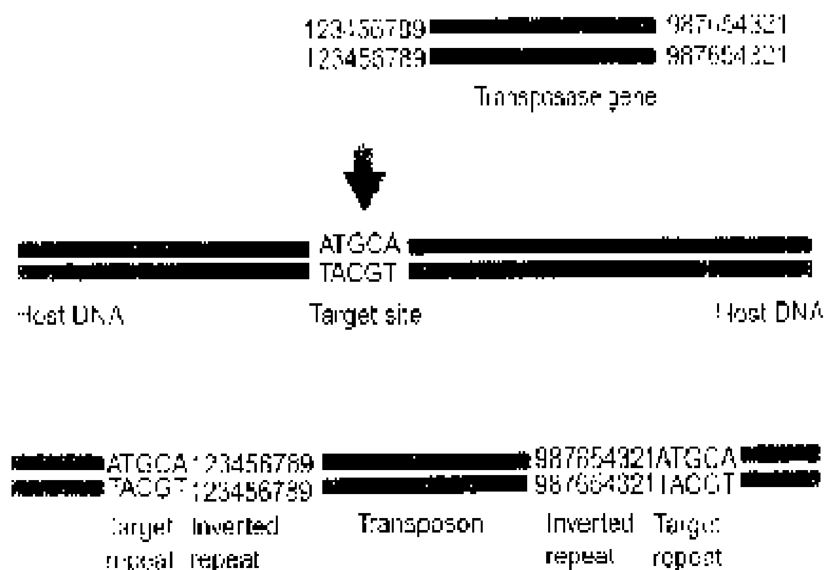
'silent mutations'. Sometimes, many amino acids get substituted due to mutations in the polypeptide chain. This brings a change in the structure of protein.These mutations are known as 'gibberish mutations'.



16.4 TRANSPOSONS :

Transposon is a term used in 1974 by *R.W. Hedges* and *A.E. Jacob*, for a DNA segment or a genetic element which could move from one molecule to another. But this was originally discovered by *Marcus Rhoades* and *Barbara McClintock* in maize plant. These genetic elements in maize were found to be responsible for turning on or turning off the expression of genes and hence the name controlling elements. *Barbara McClintock* (1950) found that the presence of a genetic factor *Ds* (dissociation) caused chromosome breakage where the particular genetic factor appears. This property of *Ds* was dependent on another unlinked genetic factor *Ac* (activator). *McClintock* could not map these genes because different plants showed their position on different chromosomes or on different positions in the same chromosome. This suggested that *Ds* keeps on jumping positions, a property which was dependent on the presence of *Ac*. So these are known as transposable elements. Since *McClintock's* discovery, transposable genetic elements have been found to be responsible for a wide variety of mutational changes.

Transposons have inverted repeats and generate target repeats



These transposable elements can integrate their genetic material into different chromosomal positions. These were first identified at the molecular level in the form of spontaneous insertions in bacterial operons. These are called insertion sequences (IS elements). Such an insertion prevents transcription and/or translation of the gene in which it is inserted. Many different types of transposable elemts have been characterised. The simplest transposons are called insertion sequences. They are named as IS elements followed by a number (IS1 IS2 IS3….). An IS element shows similar sequence of bases at each end but in reverse order known as *inverted repeats*. Insertion of transposon at the target site causes the host DNA to duplicate in such a way that the transposon DNA is flanked by short repeats of base sequences. An IS element therefore displays a characteristic structure in which its ends are identified by the inverted terminal repeats, while the adjacent ends of the flanking host DNA are identified by the short direct repeats. When observed in a sequence of DNA, this type of organisation is taken to be diagnostic character of a transposon. Transposons possess a gene that codes for an enzyme known as transposase. This enzyme fecilitates transposon insertion into possible target sites.(see figure). Some transposons carry genes for drug ressistence in addition to the gene for transposage. These transposons are named Tn followed by a number, Some transposons are large and are known as composite elements. These have genes for drug resistance at the central region flanked by IS elements.

Transposons may provide the major source of mutations in the genome. Transposable elements can promote gene mutations as well as rearrangements of the genome directly or indirectly. Transposition of genes results in deletions, insertions, inversions or translocations. The increased resistance to antibiotics in bacteria may be due to these transposons which carry these genes from plasmid to plasmid and from plasmid to chromosome. Among Eukaryotes there is evidence that genes have not only jumped within and between chromosomes, but also between all three cellular DNA enclosures namely, nuclei, mitochondria and chloroplast. Transposons can be considered as natural agents for genetic engineering because of their ability to transfer genes from one locus to another.

16.5 SUMMARY :

The term *mutation* was first used by *Hugo De Vries* to describe sudden drastic and heritable changes in populations. A change in the structure of a gene causes a change in the phenotype. This is known as gene mutation. According to the nature, origin and degree of magnitude of mutations, mutations are classified into various types namely somatic mutations, germinal mutations, forward mutations, reverse mutation, dominant mutation, recessive mutation, iso alleles, lethal mutations, spontaneous mutations and induced mutations. Gene mutations occur due to molecular level changes in DNA molecule. Mutated genes cause changes in the genetic code which are transmitted to the next generation. The changes in nucleotides which affect the sequence of nitrogen bases take place by two ways namely 1. Substitution 2. Frame shifts. Transposon is a DNA segment or a genetic element which could move from one molecule to another.

16.6 TECHNICAL TERMS :

Mutation, replication, mutant gene, mutagens, dimerization, codon, transposon.

16.5 MODEL QUESTIONS :

Essay type questions:

1. What is mutation? Give an account of various types of mutations.
2. Write about molecular basis for gene mutations.

Short answer type of Questions:

1. Mutagens
2. Substitution
3. Somatic mutations
4. Frame shift mutations
5. Transposons

16.6 REFERENCE BOOKS :

1. A.V.S.S. Sambamurthy, 1999 – Genetics – Narosa Publishing House, New Delhi.
2. Monroe W. Strickberger, 1985 –Genetics - Prentice – Hall of India Private Limited, New Delhi.
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- P.M. VASANTHA KUMARI

Lesson – 17

STRUCTURAL AND NUMERICAL ALTERATIONS OF CHROMOSOMES

17.0 OBJECTIVE :

The objective of this chapter is to study the structural and numerical alterations in chromosomes. In this chapter, we will learn about alterations in the structure and number of chromosomes which bring about a change in the phenotype. These are called chromosomal mutations which are of two types namely 1. Alterations in chromosome number 2. Alterations in chromosome structure.

STRUCTURE :

17.1 INTRODUCTION

17.2 VARIATIONS IN CHROMOSOME STRUCTURE

17.3 ALTERATIONS IN NUMBER OF CHROMOSOMES

17.4 ALTERATIONS IN THE NUMBER OF SETS OF CHROMOSOMES (EUPLOIDY)

17.5 ALTERATIONS IN THE NUMBER OF CHROMOSOMES (ANEUPLOIDY)

17.6 SUMMARY

17.7 TECHNICAL TERMS

17.8 SELF ASSESSMENT QUESTIONS

17.9 REFERENCE BOOKS

17.1 INTRODUCTION :

Alterations in the structure and number of chromosomes bring a change in the phenotype. These are called chromosomal mutations.

Chromosome mutations like gene mutations occur spontaneously in nature. These are of two types.

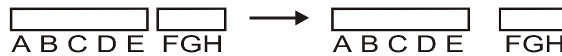
1. Alterations in chromosome number
2. Alterations in chromosome structure

17.2 ALTERATIONS IN CHROMOSOME STRUCTURE

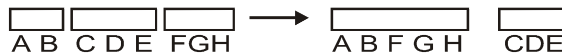
The alterations that occur in the structure of chromosome are also known as chromosomal aberrations or chromosomal rearrangements. These are of four types.

1. *Deletions or Deficiencies*
2. *Duplications*
3. *Translocations*
4. *Inversions*

Deletions or Deficiencies: Sometimes chromosomes or chromatids break into small segments and get separated from the chromosomes. So the deficiency or deletion is the loss of chromosomal segment from any chromosome. If the break occurs near the end of a chromosome, it is called terminal deficiency.

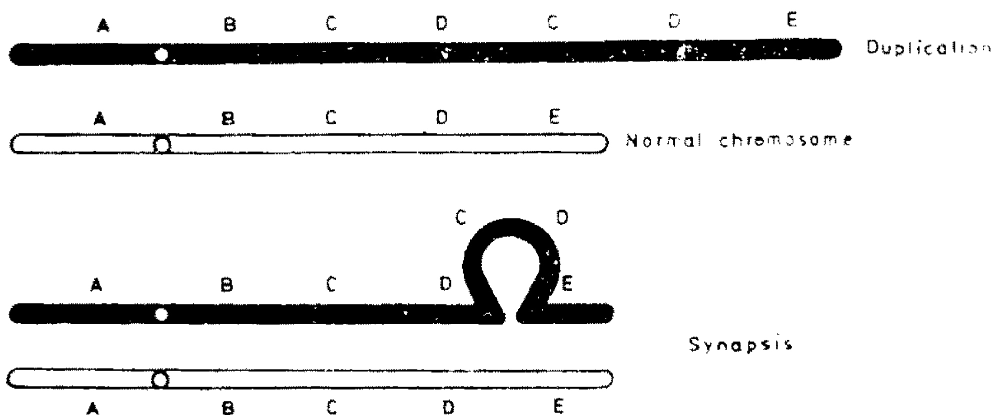


Sometimes two breaks occur at any two points, separating an intercalary segment. The broken ends of original chromosome rejoin. This is known as intercalary deficiency.



If the separated segment lacks centromere, it cannot reach any of the poles and gets digested by the action of nuclease enzymes.

Deletions which affect both the homologous chromosomes of a pair are called homozygous deletions. These are usually lethal and cause death of an organism. Deletion which occurs only in one member of the homologous pair, is known as heterozygous deletion. When such unequal chromosomes unite in synapsis, the unpaired portion of the normal chromosome projects to one side as a loop. If the genes present in the loop are recessive, they behave as dominant genes and get expressed in the next generation. This phenomenon is known as pseudo dominance. Eg: Notch mutation in female *Drosophila*.



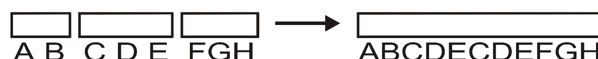
(2) Deletions cause mice to move like waltzes.

(3) *Lejeune* reported that deletion in fifth chromosome causes human babies to cry like cats.
(4)Deletion in 21st chromosome in man, causes leukemia.

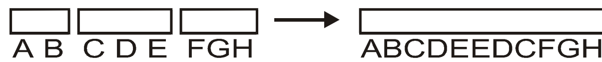
Duplications: When a segment of chromosome is represented two or more times, it is known as duplication. In this, part of a chromosome gets deleted and attaches to its homologous chromosome or non homologous chromosome. Duplications are of four types.

1. Tandem duplication: Chromatid segment which gets separated from one chromosome gets aligned to its homologue, just beside the similar gene sequence.

Because of this the chromosome which undergoes duplication, is longer than its homologue, and forms loop during synapsis.

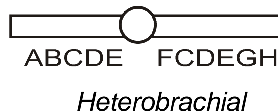


2. Reverse tandem duplication: The sequence of the repeat is just the reverse of the original alignment.



3. Displaced Duplication: It is of two types, homobrachial duplication and heterobrachial duplication. Alignment of duplicated segment to the left of the centromere is known as homobrachial duplication. Alignment of duplicated segment to the right of the centromere is known as heterobrachial duplication.

Homobrachial



Heterobrachial

4. Transposed duplication: In this, duplicated segment gets aligned to non homologous chromosome.

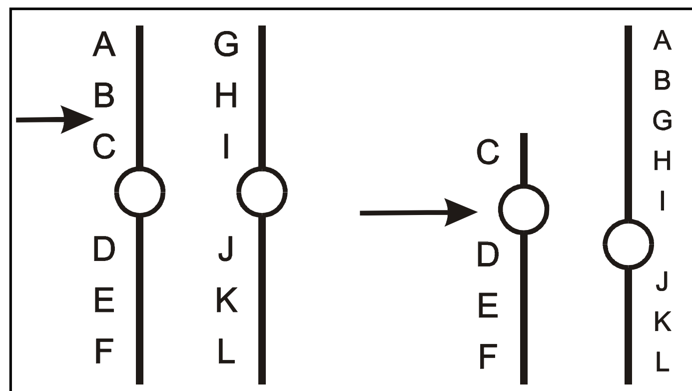
Heterozygous duplication: In a zygote formed by the union of a normal gamete with one carrying a duplication, there will be three doses of certain genes as compared to two doses of the normal zygote.

Homozygous duplication: When both the gametes which unite to form a zygote carry the same duplication in homologous chromosomes, the zygote will have four doses of certain genes and the condition is known as homozygous duplication.

Duplications occur more frequently than deletions. These are not harmful to organisms and play a very important role.

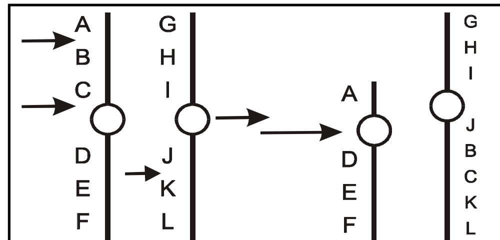
Translocations: When a segment of one chromosome is transferred to a non homologous chromosome, it is called translocation. Translocation is of three types.

(a) Simple translocation: A small segment of chromosome gets deleted and is aligned to the end of some other chromosome (non-homologous). This type of translocation is very rare in nature.



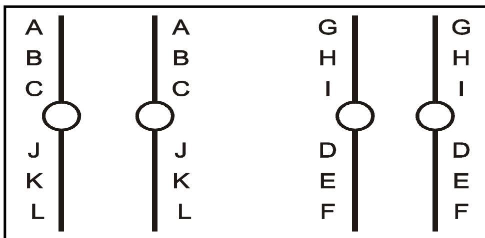
Simple Translocation

(b) Shift translocations: In this, an intercalary segment of one chromosome gets attached to a different portion of a non homologous chromosome.

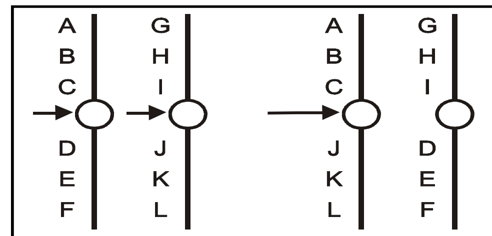


Shift Translocation

(c) Reciprocal translocation: The exchange of chromatid segment between non homologous chromosomes is known as reciprocal translocation. In homozygous condition for reciprocal translocation, there will not be any phenotype changes. In heterozygous condition for reciprocal translocation, one chromosome is normal and another one is a translocated chromosome. In the pachytene stage of meiosis-I, four chromosomes having the gene sequence ABCDEF, ABCJKL, GHIJKL, GHIDEF are arranged in the form of letter X or + cross.

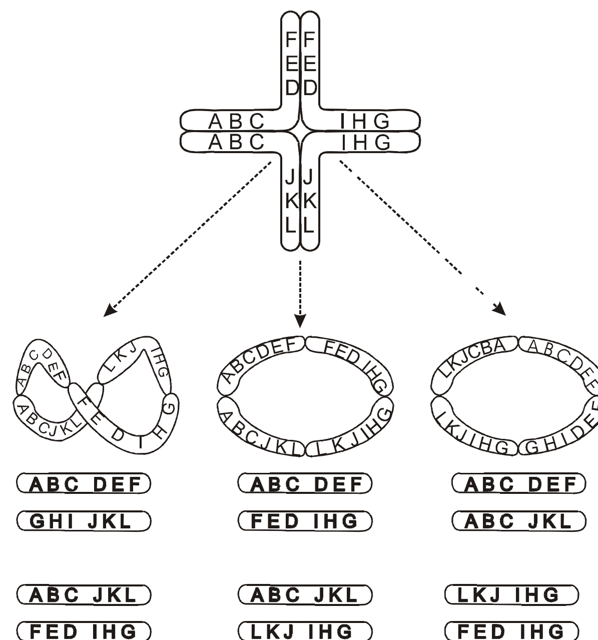


Reciprocal Translocation (Homozygous)



Reciprocal Translocation (Heterozygous)

These are known as quadrivalents. In metaphase-I, these chromosomes show peculiar configurations, namely closed ring configuration (in the form of the number 8) and open ring configuration. If closed ring configuration



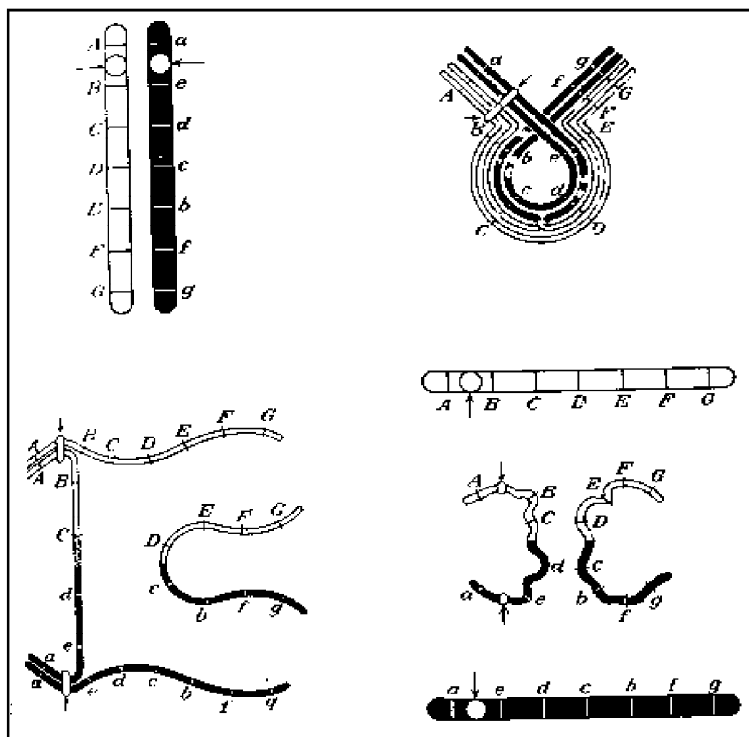
is formed, one gamete gets normal chromosomes and the other gets translocated chromosomes. These chromosomes contain all the genes. So these gametes are viable and functional. If open ring configuration is formed, each gamete shows a normal chromosome and translocated chromosome. These gametes either lack some genes or have additional genes. So these are non functional and non viable.

Crossing over occurs between two homologous chromosomes, but translocation takes place between two non homologous chromosomes. In reciprocal translocation, no loss or addition of chromosome segments takes place, but the chromosome segments become rearranged and new linkage groups are created. They interfere with pairing in homologous chromosomes, and there by reduce crossing-over. Imperfect pairing in synapsis causes partial sterility in pollen or ovules, in plants with heterozygous translocations. This is because, some gametes have deficient chromosomes while others carry duplications.

Inversions: Some times chromosome breaks at two points. The separated segment rotates by 180^0 and realign at the same place. This is known as "inversion". For example, if a chromosome having a gene sequence ABCDEFGH breaks at two points, i.e., at points B and F, and the segment with gene sequence CDE gets separated, rotated by 180° and realigns, the gene sequence in the inverted chromosome will be ABEDCFGH.

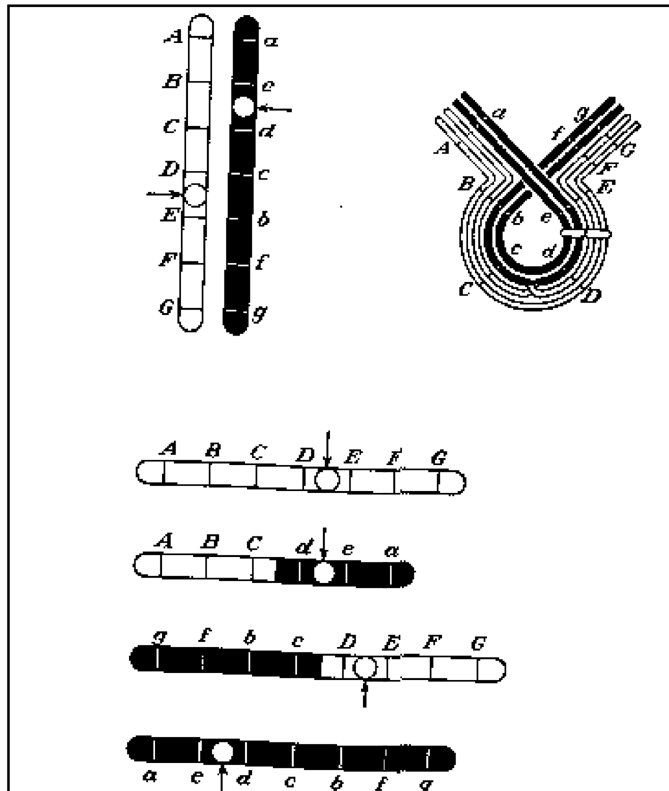
Inversions are of two types

1. Paracentric inversion:- When both the breaks occur on the same side of the centromere, the inversion is known as paracentric. The inverted segment of chromosome is without centromere.



Paracentric Heterozygous Inversion

2. Pericentric inversion: It involves one break on either side of the centromere, and inverted segment contains the centromere.



Pericentric Heterozygous Inversio

Inversion which involves two homologous chromosomes having the same gene sequence, in known as homozygous inversion. These chromosomes form functional gametes. But inversion which involves one of the two homologous chromosomes, is known as heterozygous inversion.

In paracentric heterozygous inversion, a double loop is formed. After crossing over in Diplotene stage, four chromotids with their gene sequences can be seen clearly. Of the four chromotids that are formed, two are normal chromatids, the third chromatid has no centromere and the fourth chromatid has two centromeres. The chromatid segment which connects the two centromeres is known as 'chromatid bridge'. The third and fourth chromatids behave abnormally and do not form gametes. But the normal chromatids form gametes. Of the two normal chromatids, one has a normal gene sequence the otherr one has an inverted gene sequence.

In pericentric heterozygous inversion, of the four chromatids, the first chromatid has a normal gene sequence. The second chomatid shows duplication of gene 'a' and deletion of gene 'f'. The third chromatid shows duplication of gene 'f' and deletion of gene 'a'.The fourth chromatid has an inverted gene sequence . Of these, only the first and third chomatids can form viable gametes and the other two chromatids cannot form viable gametes.

Translocations and inversions involve no loss or gain in the number of genes. They bring about only a rearrangement in the order of genes and change the position of some genes and their neighbours. This rearrangement effects function of genes and causes change in the phenotype. This phenomenon is called 'Position effect'.

17.3 ALTERATIONS IN NUMBER OF CHROMOSOMES

Plants and animals which reproduce sexually are usually diploids. The cells of diploid plants or animals have two sets of chromosomes. One set comes from the female gamete and another set comes from the male gamete. The total complement of genes borne on the haploid set of chromosome is known as genome. Usually the diploid number of chromosomes in a given species is constant. But this is not always true. Some times this number varies in two ways.

1) Alterations in the number of sets of chromosomes

2) Alterations in the number of chromosomes

17.4 ALTERATIONS IN THE NUMBER OF SETS OF CHROMOSOMES (EUPLOIDY) :

In this, multiples of a haploid or basic chromosome set are present in the cells of organisms. These are known as euploids and the phenomenon is known as 'euploidy'. Euploids which have more than two sets are called polyploids and the phenomenon is known as 'polyploidy'. Polyploids may contain 3 sets (3n-triploids) or 4sets (4n-tetraploids) or 5sets (5n-pentaploids) or 6sets (6n-hexaploids) or 7sets (7n-heptaploids) or 8 sets (8n-octaploids) and so on. In polyploidy, the chromosome number is a whole multiple of the basic or haploid number.

Haploids: Euploids which have a single set of chromosomes are known as haploids or monoploids. Haploid plants are formed mostly when diploid organisms loose one set of chromosomes. Eg; Mustard, Cotton, Paddy, Wheat, Maize etc. A monoploid organism has only a single complement of basic chromosomes. Haploids are smaller in size when compared to diploid organisms and they are sterile. The formation of gametes with full genome occurs very rarely, and the fusion of these rare gametes produce homozygous diploids.

Homozygous diploids can be obtained artificially by doubling haploid set of chromosomes present in haploids, by chemical treatment. Similarly haploids can be produced by pollen culture. These two methods are very good tools for geneticists and plant breeders. Haploid animals are very rare in nature. Eg: Male insects – ants, bees and wasps.

Polyploids: Polyploidy has been found to occur in several cultivated and wild plants. Polyploidy in animals is very rare. Organisms with more than two sets of chromosomes are known as polyploids. There are two types of polyploidy. 1. Autopolyploidy 2. Allopolyploidy. In autopolyploidy, the chromosome sets are all of the same kind and are derived from the same species. In allopolyploidy, the chromosome sets are of different kinds and are derived from two or more distinct species.

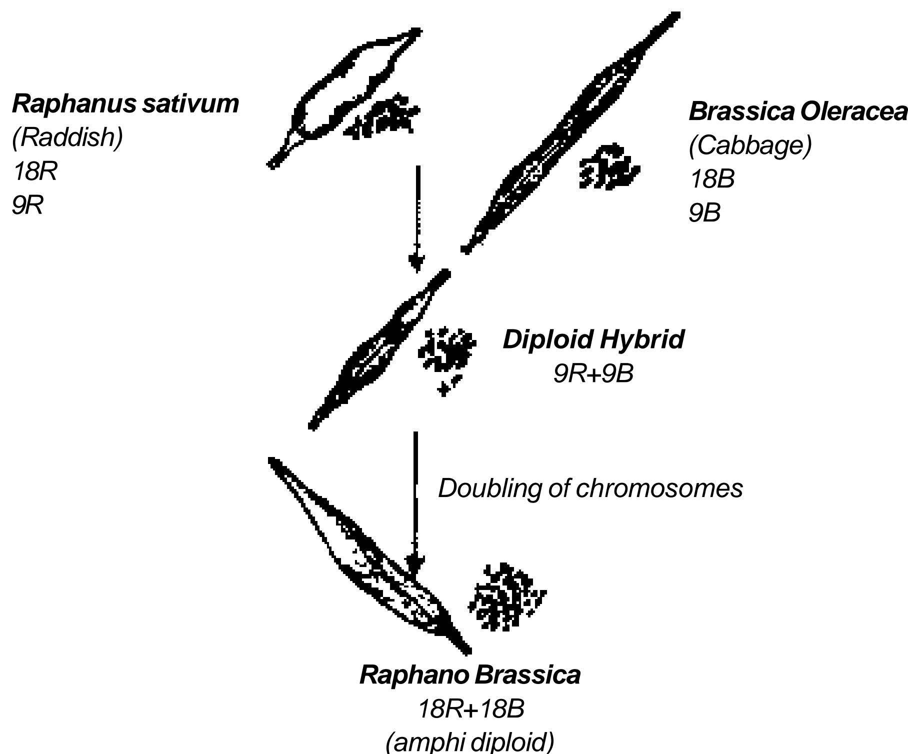
Triploids: Triploids are formed by the union of a diploid gamete (which is formed not due to meiosis) with haploid gamete. Triploids can also be formed by the union of two male gametes with a female gamete. Hence triploids possess three genomes. Abnormal meiosis occurs in triploids and this leads to sterility. So these can be propagated by vegetative methods. Eg: Banana, grapes, pineapple,guava, rose, chrysanthenum.

Tetraploids: These contain four genomes. They are formed either by the union of two diploid gametes, or by the doubling of a diploid genome. Tetraplopids are of two types. Autotetraploids and Allotetraploids. Autotetraploids arise either by the doubling of chromosomes in a diploid zygote or by the union of diploid gametes.

Eg. Groundnut, coffee, potato

Allotetraploids are formed by the doubling of chromosome number in an F_1 hybrid, which is derived from a cross between different species. When two different species or genera are crossed, the F_1 hybrids are sterile, because they receive two different sets of chromosomes. Synapsis does not occur between these chromosomes and gametes are not formed. The doubling of chromosomes in F_1 hybrid will give rise to a tetraploid having two different genomes. Such a polyploid is known as allotetraploid or amphidiploid. Allotetraploids do not occur naturally. Some cytologists have produced allotetraploids in certain plants, by selective breeding methods.

Eg: *Raphanobrassica*



Russian cytologist *G.D Karpechenko* crossed *Raphanus sativus* (2n=18) with *Brassica oleracea* (2n=18). The F_1 hybrids had two sets of 9 chromosomes each. Each set was derived from one of the parents. These F_1 plants are highly sterile due to lack of pairing of chromosomes during synapsis. Among these sterile F_1 hybrids, *Karpechenko* found certain fertile allotetraploids which have 2n=36 chromosomes. Allotetraploids or amphidiploids can also be obtained by doubling of chromosomes in F_1 sterile plants.

Autotetraploidy occurs in *Citrus* species due to polyembryony. *Galax aphylla* is the best example for clear polyploidy. In these, plants are normally larger in size and are more vigorous than diploids. These plants have thicker and stouter stems, larger and more showy flowers, larger fruits and seeds, and often have broader, thicker and darker leaves. In general, polyploids exhibit gigantic characters and, this increases the commercial value, and are useful in agriculture. Many crop plants are allotetraploids. Eg: Wheat, oats, tobacco, cotton. The common tobacco is the result of a cross between *Nicotiana tomentosiformis* and *Nicotiana sylvestris.* Usually allopolyploids are stable. In plants like *Oryza*, one can see both autopolyploidy, and allopolyploidy. Allohexaploids are seen in wheat. In these, three different diplopid genomes are present. High degree polyploidy is seen in crop plants like sweet potato (6n) and sugar cane (8n). But this leads to sterility due to irregularity in meiosis.

17.5 ALTERATIONS IN THE NUMBER OF CHROMOSOMES (ANEUPLOIDY) :

Aneuploidy is a general term used to describe organisms whose chromosome number is not an exact multiple of the basic chromosome number. Thus, if the basic number of chromosomes in a species is 'n' and the diploid number is '2n', organisms having '2n' plus or minus one or more chromosomes are known as aneuploids and the phenomenon is known as aneuploidy. This can be due to an abnormal distribution of the chromosomes (non-disjunction) to the poles during anaphase of meiosis, where, as one daughter cell receives one or more extra chromosomes, and the other daughter cell lacks one or more chromosomes. This addition of one or more extra chromosomes results in hyperploids and the lack of one or more chromosomes results in hypoploids.

Trisomics: Trisomics are those organisms which have an extra chromosome to the normal diploid number (2n+1). This extra chromosome is homologous to one of the chromosomes of a haploid complement. This means that trisomics have a particular chromosome represented in three doses.

Trisomics are formed by the fusion of gametes having n+1 chromosomes and normal gametes (n). These can also be produced artificially by crossing a diploid plant (pollen) with a triploid plant (egg). Scientists could isolate all possible trisomics in Datura, Tomato and maize. In these plants, if the first chromosome is present three times, it is called tripl-1, and if the second chromosome is present three times, it is called triplo-2, and so on (triplo-3, triplo-4, triplo-5 etc). In Datura, chromosomes are termed as ABC...... So, these are known as triplo-A, Triplo-B etc. Triplo-A is also known as rolled leaf trisomic, and triplo-B is known as glossyleaf trisomic because these trisomics express themselves morphologically by rolled leaves and glossy leaves. Humans who are trisomic for the 21st chromosome develop *Down* syndrome, and humans who are trisomic for sex chromosome (x) develop *Klinefelter*'s syndrome.

Tetrasomics: Tetrasomics are those organisms which have two extra chromosomes to the normal diploid number (2n+2). These extra chromosomes are homologous to two of the chromosomes of a haploid complement. This means that tetrasomics have a particular chromosome represented in four doses. Tetrasomics resemble the diploids in many ways. In these, meiosis is more regular than other aneuploids. All the 21 possible tetrasomics are available in wheat.

Monosomics: Monosomics are those organisms which lack one chromosome to the normal diploid number (2n-1). Monosomics are normaly found in polyploids (3n-1, 4n-1 etc.). Monosomic diploids cannot survive because they lack one complete chromosome. But in polyploids, even though one chromosome is lacking in one set, the homologous chromosomes present in the other sets, compensate for it. 21 possible monosomics in wheat, and 24 in tobacco were isolated. Monosomic analysis helps in locating genes on chromosomes. In humans, monosomic condition causes *Turner*'s syndrome.

Nullisomics: Nullisomics are those individuals which lack a single pair of homologous chromosomes
(2n-2). These are also found in polyploids. *Sears* could isolate 21 possible nullisomics in wheat. Nullisomic analysis helps to locate genes on chromosomes.

17.3 SUMMARY :

Alterations in the structure and number of chromosomes bring changes in the phenotype. These are called chromosomal mutations. They are of two types namely 1. Alterations in chromosome structure 2. Alterations in chromosome number. Alterations in chromosome structure are of four types namely, deletions, duplications, translocations, inversions.

Alterations in the number of chromosomes are of two types, namely 1. Alterations in the number of sets of chromosomes 2. Alterations in the number of chromosomes in a set. In the first type, multiples of a haploid or basic chromosome set are present in the cells of organisms. These are called euploids and the phenomenon is known as euploidy. Euploids which have more than two sets are called polyploids and the phenomenon is known as polyploidy. Polyploids contain triploids, tetraploids, pentaploids, hexaploids, heptaploids, octaploids and so on. Poplyploids are of two types namely, 1. Autopolyploids 2. Allopolyploids. Autopolyploids contain similar genomes. Allopolyploids contain dissimilar genomes which are derived from two genetically different species.

Euploids which have a single set of chromosomes are known as haploids or monoploids. Homozygous haploids can be obtained artificially by doubling the haploid set of chromosomes present in haploids. Similarly haploids can be produced by pollen culture. These two methods are very good tools for geneticists and plant breeders.

Aneuploidy occurs in organisms whose chromosome number is not an exact multiple of the basic chromosome number. Thus, if the basic number of chromosomes in a species is 'n' and the diploid number is '2n', organisms having ‘2n’ plus or minus one or more chromosomes are known as aneuploids and the phenomenon is known as aneuploidy. Addition of one or more extra chromosomes results in hyperploids and the lack of one or more chromosomes results in hypoploids. Hyperploids are trisomics (2n+1) and tetrasomics (2n+2). Hypoploids are monosomics (2n-1) and nullisomics (2n-2).

17.4 TECHNICAL TERMS :

Chromosomal mutations, haploids, euploids, polyploids, triploids, tetraploids, autopolyploids, allopolyploids, genomes, aneuploids, trisomics, tetrasomics, monosomics, nullisomics.

17.5 SELF ASSESSMENT QUESTIONS

Essay type questions:

1. Explain various kinds of chromosomal rearrangements.
2. Give an account of polyploidy.

Short answer type of Questions:

1. Euploidy
2. Tetraploids
3. Aneuploidy
4. Trisomics

17.6 REFERENCE BOOKS

1. A.V.S.S. Sambamurthy, 1999 – Genetics – Narosa Publishing House, New Delhi.
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- P.M. VASANTHA KUMARI

CELL BIOLOGY

PLANT PHYSIOLOGY, CELL BIOLOGY & GENETICS MITOTIC DIVISION IN ROOT TIPS OF *ALLIUM CEPA* BY SQUASH TECHNIQUE

Aim : To Observe different stages of mitotic cell cycle in the somatic cells of the given material of *Allium cepa*

Objective : To identify the morphological, chromosomal features characteristic to various stages of mitosis and to describe the process of mitosis.

Materials and Chemicals : Acetic Acid, Alcohol, Aceto Carmine Stain, HCl, Onion root tips of about 5 mm size, glass slides, cover slips, mounting needle, spirit lamp, blotting paper, compound microscope, watch glass.

Preparation of stain : A 2 % solution of Acetocarmine stain is prepared by adding 2gms of carmine powder to 100ml of 45% glacial acetic acid (45 glacial acetic acid + 55ml distilled water). The solution is heated in a reflex condenser for about 24 hours, cooled, filtered and stored.

Procedure : Take two or three root tips of length 1-2 cms or terminal tips of about 5 mm. Place them in a few drops of Acetocaramine and heated in a low flame. Care should be taken that stain should not boil.

Pass them through the following solutions

1. 1:1 Glacial Acetic acid : Alcohol mixture for about 2-5 minutes
2. 6:3:1 Acetic Acid : Chloroform : Alcohol mixture for about 1-3 minutes. This is known as Carnoy's fixative.
3. 90% alcohol. This is the fixative and tips can be placed in this solution for a long period of time.

Place a drop of Acetocarmine stain on a slide. Take the tip portion of the treated root tip and place it in the drop and squash the material by tapping firmly with the flat end of a mounting needle. This facilitates the easy separation of cells from one another. When a uniform suspension of small cell groups are formed, place a cover slip over it. Care should be taken that the stain should not come out, but it should be just sufficient to reach the edge of the cover slip.

The preparation is then heated gently over a low flame of spirit lamp for a few seconds. The slide is then placed between two sheets of blotting paper and firm vertical pressure is applied with thumb over the area of the cover slip. This removes the excess stain and also facilitates flattening of the cells and spreading of chromosomes. The preparation is then ready for the study under a compound microscope.

Observation :

Different stages of mitosis such as interphase, prophase, metaphase, anaphase and telophase were observed. (Fig. 1).

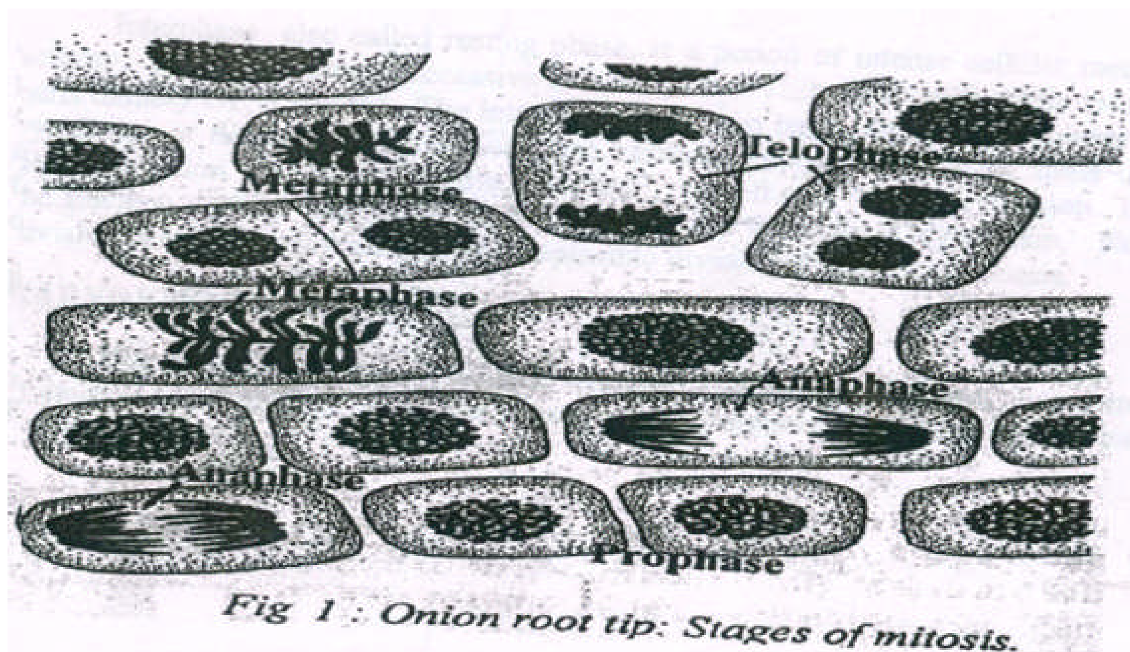


Fig 1 : Onion root tip: Stages of mitosis.

Discussion :

Every organism begins its life as a single cell called **zygote**. This zygote undergoes repeated divisions and multiplies into several thousands of cells. Cell division is essential for the growth and reproduction of organisms. There are two major kinds of cell division. Mitosis and meiosis. Mitosis occurs in somatic cells. Meiosis occurs in reproductive cells. In mitosis the mother cell produces two daughter cells which are identical in size, shape and characters. So it is also called somatic or homeotypic division. In this the two daughter cells produced have equal number of chromosomes. So it is also called equational division. In meiosis, mothre cell produces four daughter cells in which the chromosome number is reduced to half. So it is also called reduction division or heterotypic division. Mitotic cell division was first observed by **Walter Flemming** (1878) in animal cells and Strasburger (1879) in plant cells. It is observed in apical meristems of root and stem tips. Mitosis is a continuous process. For the sake of convenience and easy understanding it has been divided into different stages. They are interphase or resting phase and mitotic phase.

INTERPHASE

Interphase also called resting phase, is a period of intense cellular metabolic activity. It exists between successive cell divisions. This phase is divisible into three parts namely G1, S and G2. The interphase nucleus is large, dark and prominent. The cytoplasm is dense. In the nucleus, the chromatin looks like landed mass due to decondensation. At the end of the interphase, the cell gets ready for division. In this the nucleus divides first and it is followed by the division of cytoplasm. Nuclear division is called karyokinesis and cytoplasmic division is called cytokinesis.

KARYOKINESIS

The dividing nucleus involves a number of sequential changes. For convenience of study it is subdivided into four phases called Prophase, Metaphase, Anaphase and Telophase. (Fig. 2.)

MITOTIC DIVISION

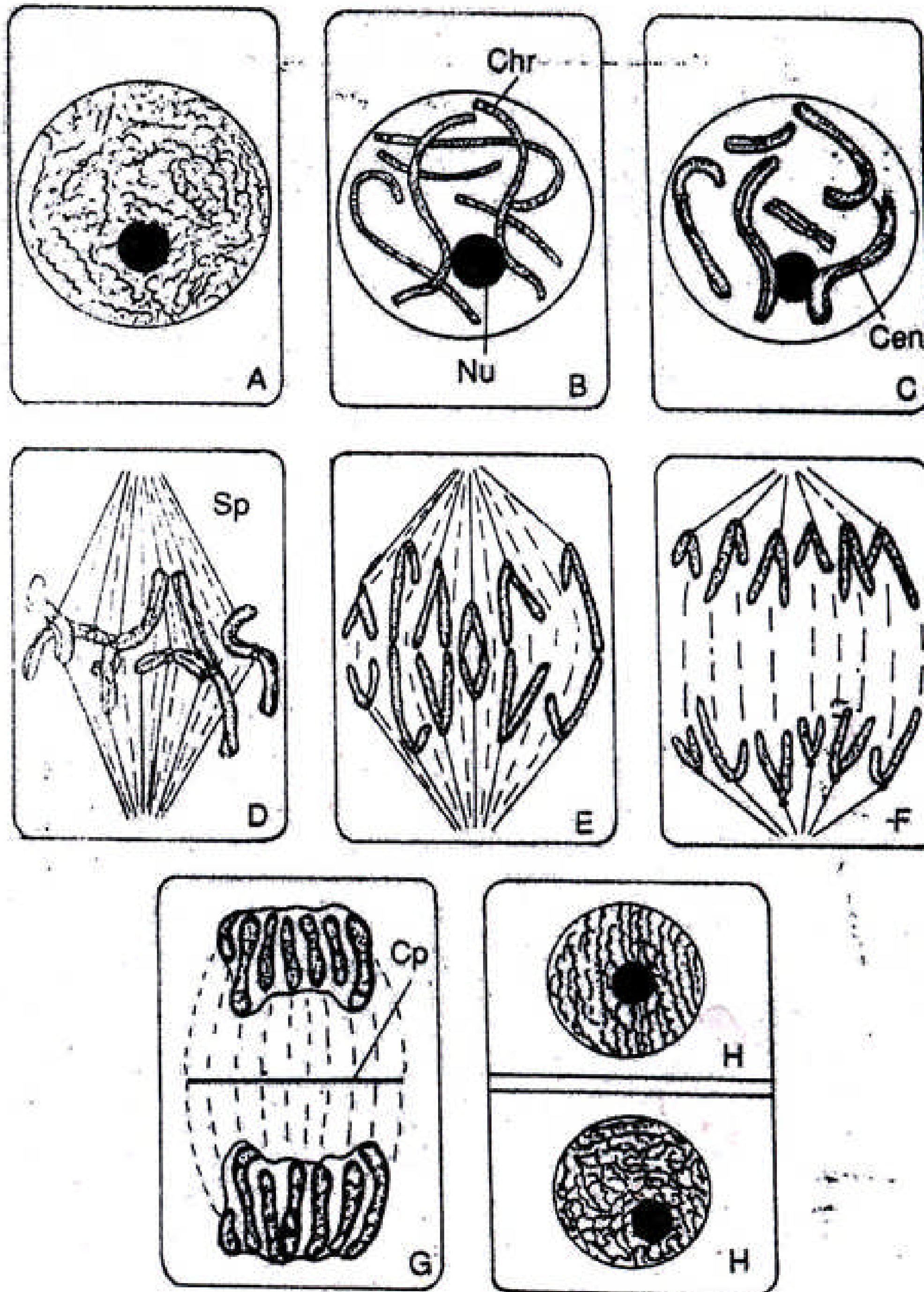


Fig 2 Stages In Mitosis

(A - C Prophase; D - Metaphase; E - Anaphase; F - Late Anaphase ; G - H - Telophase ; Chr - chromosomes; Nu - Nucleus; Cen - centromere; Sp - spindle fibres; Cp - cell plate)

PROPHASE :

In the early stage, the chromatin condenses and appears as slender threads called chromosomes. Each chromosome splits longitudinally into two parts called chromatids, which are united at the centromere. As the prophase progresses, the chromatids become short, thick, and rod shaped structures. Nuclear membrane and nucleoli begin to disintegrate. So, the chromosomes appear to be randomly scattered in the cytoplasm.

METAPHASE :

In this two important changes are present. Formation of spindle fibers and orientation of chromosomes on the equatorial region. A bipolar spindle apparatus is formed by the fusion of microtubules. Bipolar spindle is organised between two poles of the cell. The chromosomes are arranged at the equatorial plate with the help of the spindle fibres. The spindle fibres get attached to the kinetochore part of the chromosomes and bring them to the equator of the cell. Thus all centromeres lie on equatorial plane and their arms hang truly in the cytoplasm.

ANAPHASE

In this stage, the spindle fibers begin to contract causing pressure on the centromeres. So, the centromeres of each of the chromosomes divide and thus the two chromatids are separated. The two sister chromatids with their own centromeres function as daughter chromosomes and migrate to the opposite poles. During the movement of chromosomes to the poles, the centromeres lie ahead followed by arms and the chromosomes, hence the chromosomes appear in the shape of V, L or I.

TELOPHASE

The daughter chromosomes arrived at the poles become deconensed and appear as normal diffuse chromatin. The daughter nuclei are reconstructed by the reformation of nuclear membrane. Nuclei are reorganised. The daughter nuclei then enter the interphase of cell cycle.

CYTOKINESIS

The division of nucleus is generally followed by the division of cytoplasm. Cytokinesis takes place by cell plate formation in the centre of the dividing cell. New cell membrane is formed between two nuclei result in two complete cells.

Dr. T. Srivalli

PLANT PHYSIOLOGY

MAJOR EXPERIMENTS

1.Measurement of osmotic potential by incipient plasmolysis method.

Aim: - To measure the osmotic potential of the plant cell by incipient plasmolysis method

Principle: - Plant cells when placed in an isotonic solution, the protoplast of the cell starts withdrawing from the cell wall. This is called incipient plasmolysis. The concentration of external solution at which the cells show incipient plasmolysis can be regarded as the osmotic potential of the cell sap.

Apparatus: Petriplates, measuring cylinders, beakers, slide, blade, microscope, distilled water, sucrose, compound microscope.

Material: Leaves of Rheo discolor.

Procedure: Prepare stock solution of 1 M by dissolving 34.2 gms of sucrose in 75 ml of distilled water and make up the solution to 100 ml. Prepare different concentrations given in the table.

S.No.	Concentration of sucrose solution	Voluome of stock solution	Volume of distilled water	% of cells showing incipient plasmolysis
1	0.15 M	15 ml	85 ml	
2	0.20 M	20 ml	80 ml	
3	0.25 M	25 ml	75 ml	
4	0.30 M	30 ml	70 ml	
5	0.35 M	35 ml	65 ml	
6	0.40 M	40 ml	60 ml	
7	0.45 M	45 ml	55 ml	
8	0.50 M	50 ml	50 ml	

Prepare the lower epidermal peelings of Rheo discolor. The peelings when observed under microscope should be of one cell in thickenss. The peelings are stored in water. Sucrose solution of different concentrtaions are taken in different petriplates. The peelings are cut into equal bits. Transfer two peellings into each petriplate Peelings should be kept in the petriplates for 30 minutes. Mount the peeling on the slide with the same solution form which it is taken. Observe the peeling under the microscope. Count the number of cells that show incipient plasmolysis Tabulate the results and plot a graph by taking concentration of sucrose solution on x – axis and % of plasmolysed cells on y-axis. Caluculate the osmotic potential by using the formula.

ψ_s = mi RT where m = molarity of the solution

i = ionization constant (For sucrose = 1.0)

R = Gas constant 0.083 litre bars / mole degree
T = absolute temp C^0+273

ψ_s` = - bars (1 bar = 0.1 M pa)

Conclusion: When a cell is placed in hypertonic solution, water moves out of the cell. This results in shrinking of protoplast. This phenomenon is called plasmolysis. The lower epidermal cells contain pink pigment called anthocyanin, Plasmolysed cells can be easily recognised. Incipient plasmolysis is the commencement of plasmolysis when the contents of the protoplast start separating from the cell wall. At incipient plasmolysis the concentration of cell sap is equal to that of sucrose solution. The cell wall does not exert pressure on the cell contents. So water potential is equal to the osmotic potential.

$$\psi_w = \psi_s + \rho$$
$$= \psi_s + 0$$

$$\psi_w = \psi_s$$

2. Differential transpiration by cobalt chloride method.

Aim: To study the differential transpiration by Cobalt chloride method.

Principle: Transpiration occurs through the stomata present on the leaf. Cobalt chloride paper shows change of color form blue to pink depending on dry and wet conditions. Rate of transpiration from the leaf surfaces is compared by color change in the cobalt chloride paper.

Apparatus: Slides, petiplates, dessiccator, clips, stop watch, tray, whatman NO 1 paper.

Chemicals: Cobalt chloride, calcium chloride (anhydrous)

Procedure: Prepare cobalt chloride paper by dipping good grade filter paper in 5 % cobalt chlorde solution. Keep the glass plate flat over the filter paper so that the paper absorbs colour uniformly. Take out the paper from the solution and allow it to dry by hanging it on a rope. The paper is cut into pieces of 2 sqcm and keep them in oven for some time. Dried paper pieces are blue in colour. Transfer them to a petriplate kept in a dessiccator over anhydrous calcium chloride.

Select a dorsiventral leaf. Place one piece of cobalt chloride paper on the upper surface and one on lower surface. Press them with clean glass slide. Make them air tight by clamping with clips. Note the time taken for change of color of the paper from blue to pink. Repeat the experiment with different types of leaves.

S.No	Type of leaf	Time taken for color change in upper surface	Time taken for color change in lower surface
1.	Mesophytic		
2.	Isobilateral		
3.	xerophytic		

Explanation : Anhydrous cobalt chloride paper is blue in color. The color changes to pale pink on moistening. During transpiration the water vapour is released into the atmosphere through the stomata. The change of color of paper depends upon the release of water vapour from the lower and upper surfaces of the leaf.

Conclusion : The rate of transpiration is variable from plant to plant. The rate of transpiration with in the leaf varies in the different surfaces. In mesophytic leaf the rate of transpiration is more on the lower surface. In isobilateral leaf the rate of transpiration is similar with reference to the both surfaces of the leaf. In xerophytic leaves the rate of transpiration depends upon various xerophytic adaptations.

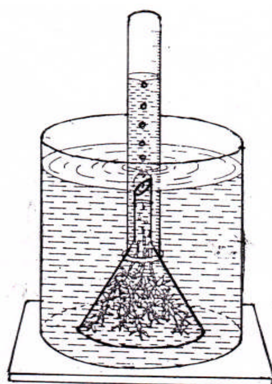
3. Effect of quality of light and concentration of co_2 in the rate of photosynthesis.

Aim: To study the effect of quality of light and co_2 concentration in the rate of photosynthesis.

Principle: Light and co_2 are the major requirements of photosynthesis. Quality of light determines the rate of photosynthesis. Light consists of different wavelengths of light ranging from 400-700 nm. The rate of photosynthesis is dependent on the wavelength of light. Similarly the increase in co_2 concentration increases the rate of photosynthesis upto certain extent because of the principle of limiting factors.

Requirements:- Beaker, test tube, funnel, thread, blade, cellophane paper of different colors, sodium bicanbonate, hydrilla twigs, distilled water.

Procedure: The beaker is filled with water. Add a table spoonful of $NaHCo_3$ and stir the water till the $Na\ HCo_3$ dissolves. Select some fresh plants of Hydrilla having terminal buds. Cut the hydrilla plants under water. Insert the cut ends of the plants into the neck of the funnel. Place the funnel inside the beaker in such a manner so that all plants remain inside the funnel. Invert a test tube filled with water, over the neck of the funnel. This apparatus is used to study the effect of light and Co_2



HYDRILLA EXPERIMENT

Observation: Evolution of bubbles from the cut ends of the stems is observed. The number of bubbles is counted under different conditions Tabulate the results and plot the data on a graph paper

Results:

Effect of quality of light:

		No of bubbles			
S.No	Quality of light	5 mt	10 mt	15 mt	Total
1	Red	-	-	-	
2	Blue	-	-	-	
3	yellow	-	-	-	
4	green	-	-	-	

Effect of Co_2 concentration

		No of bubbles			
S.No	$NaHCo_3$ concentration	5 mt	10 mt	15 mt	Total
1	50 mg/100 ml water	-	-	-	
2	100mg/100 ml water	-	-	-	
3	150mg/100 ml water	-	-	-	
4	200mg/100 ml water	-	-	-	

Inference: Photosynthesis is influenced by external factors such as light and Co_2. The rate of photosynthesis in red light is maximum followed by blue light. Increase in Co_2 concentration increases the rate of P.S. upto a certain extent.

4. Separation of chloroplast pigments by paper chromatography

Aim: To separate the chloroplart pigments by paper chromotography method.

Principle: Paper chromatography separates molecules based on size, shape, mass, charge, solubility and adsorption properties Molecules separate on the basis of their different rates of movement on the paper. The rate of migration depends on the solvent.

Apparatus: Chromatographic chamber, Whatman NO 1. Filter paper, mortar & pestle, measuring cylinders, beaker, capillary tubes, pencil and electric drier

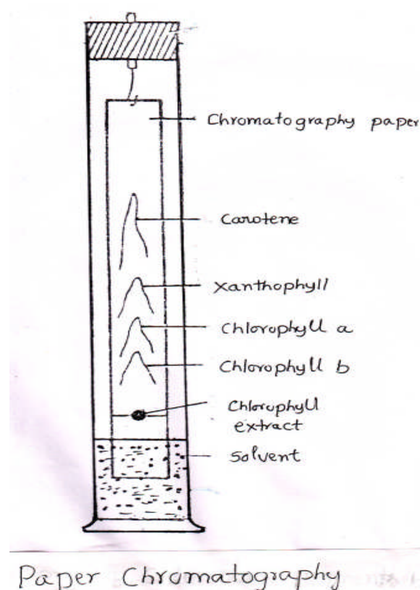
Chemicals: 80 % acetone, petroleum ether, calcium carbonate, quartz sand, muslin cloth,

Materials: Tecoma/palak leaves

Procedure: 10 gms of palak leaves are taken in the mortar. Grind them well whith the pestle by adding 10 ml of 80 % acetone. Add a pinch of calcium carbonate and sand to facititate easy grinding. The paste is squeezed through muslin cloth. The extract is collected in the beaker.

Take chromatographic paper of suitable size that fits into the chromatographic chamber. Draw a line 2 inches above the base. At the centre of the line add a drop of the pigment by means of the capillary tube. Repeat the process of drying and adding some extract until a sufficient quantity of extract is fixed at the centre of the line. After thorough drying the paper is placed in the chrameatographic chamber, containing 9:1 petroleum ether and acetone. The apparatus is kept undisturbed for 30 minutes in a dark chamber.

Observation: The solvent moves in the upward direction separating the pigments at various levels. The pigments separated as light green, bluish green, yellow and orange from bottom to the top.



Paper Chromatography

Conclusion: The pigments present in the leaf are chlorophyll – a, b, xanthophyll and carotene. The respective colors for the pigments are light green chlorophyll-b, bluish green-

chlorophyll-a. Yellow-xanthophyll and orange- carotenes. The solvent while developing on the paper separates the pigments because of the difference in movement.

R.f. Value: Resolution factor expresses the relative movement of the solute and the sovlvent it can be expresssed by the following equation.

$$\text{R.f.} = \frac{\text{Distance travelled by the Solvent}}{\text{Distance travelled by the pigment}}$$

5. Starch- Amylase activity

Aim: Amylase catalyses the hydroysis of starch converting into sugars. The disappearance of blue colour is due to conversion of starch to Glucose.

Apparatus: Test tubes, beakers, measuring cylinder, mortar and pestle. Muslin clothe, stop watch

Chemicals: 1 % starch solution, phosphate buffer of p.H.6.0, iodine solution. Starch solution: Dissolve 1:0 gram of starch in boiling distilled water and made up to 1.00 ml

Iodine solution : Dissolve 10 gms of potassium iodide and make up to 1000 ml with distilled water and to this add 2.5 gms of iodine crystals.

6.0 pH phosphate buffer : 11.86 gms of Di-sodium hydrogen phosphate is dissolved and made upto 1000ml. with distilled water. 9.07 gms of potassium dihydrogen orthophosphate is dissolved in distilled and made upto 1000ml. The above two solutions are mixed in 61:39 ratio respectively.

Enzyme extract : 10 gms of germinating seeds are taken. Grind well in a mortar by adding phosphate buffer.

Procedure: Prepare different dilutions of enzyme extract using the phosphate buffer. Take 2 ml of dilution in separate test tubes. Add 2 ml of starch solution and few drops of iodine to all test tubes. Observe the time taken for disappearance of the blue color. Tablet the results and plot a graph for enzyme dilutions on y – axis and time taken for disappearance of the color on x axis

S.No	Enzyme dilution	Amount of enzyme Extract	Amount of starch	Amount of Iodine	Time taken for disappearance in minutes
1	1:1	2 ml	2 ml	2-3 drops	5
2	1:2	2 ml	2 ml	2-3 drops	9
3	1;3	2 ml	2 ml	2-3 drops	11
4	1:4	2 ml	2 ml	2-3 drops	15
5	1:5	2 ml	2 ml	2-3 drops	20
6	1:6	2 ml	2 ml	2-3 drops	25

Result: The time taken for the disappearance of blue color increases with increase in dilution of enzyme extract.

Inference: Amylase is a hydrolytic enzyme. It catalyses the hydrolysis of starch converting it into disaccharide and finally to glucose – a monosaccharide. To this starch when iodine drops are added, the disappearance of blue color indicates that the enzyme converts polysaccharide into monosaccaharide.

6. Estimation of Protein content present in the given sample by Biuret method

Aim: To estimate the amount of protein present in the given sample byBiuret method.

Principle: This is a general test for proteins. The –CO-NH-groups of protein form a purple complex with copper ions in an alkaline medium. Since all proteins contain the peptide bond, the method is fairly specific and there is little interference from other compounds.

Chemicals: Copper sulphate, sodium hydroxide, sodium potassium tartarate, potassium iodide, standardised Bovine Serum albumin, unknown protein.

Biuret reagent: 3 gms of copper sulphate and 9 gms of sodium potassium tartarate are dissolved in 500 ml of 0.2 N sodium hydroxide solution. Stir the solution. To this add 5 gms of potassium iodide and make upto 1000 ml with 0.2 N NaOH.

Standard Protein solution: Dissolve 5 mg of serum albumin in 100 ml of distilled water.

Procedure: Different concentrations of standardised protein solution – 0.2, 0.4, 0.6, 0.8 and 1.0 are pipetted into the test tubes. Add distilled water to make upto 4 ml in each test tube. Blank is prepared by adding of 4 ml of distilled water. Add 6 ml of Biuret reagent to each test tube. Keep the test tubes at 37°C for 10 minues during which purple color will develop. The O.D. of each test tube is measured at 520 nm using the reagent blank. Repeat the experiment with unknown protein solution. Estimate the quantity of unknown sample with the help of standard graph.

S.No.	Standardised protein	Distilled Water	Bluret reagent	O.D. value
1	0.00 ml	4 ml	6 ml	
2	0.2 ml	3.8 ml	6 ml	
3	0.4 ml	3.6 ml	6 ml	
4	0.6 ml	3.4 ml	6 ml	
5	0.8 ml	3.2 ml	6 ml	
6	1.0 ml	3.0 ml	6 ml	

7. TITRABLE ACIDITY ESTIMATION IN LEMON OR TAMARIND LEAVES

Aim: To estimate total and titrable acidity of the given leaf.

Materials and apparatus: Lemon or tamarind or Bryophyllum leaves, conical flasks, Burettes, Pippets, centrifuge, 0.1 N HCl, 0.1 N NaOH, Bromothymol blue indicator and phenapthalene indicator.

Chemical reagents:

(a) 0.1 HCl – Take 8.6 ml of concentrated HCl. To this add distilled water and make up the volume upto 1000 ml.

(b) 0.1 N NaOH – Take 4 gms of NaOH, to this add distilled water and bring the volume upto 1000 ml.

Procedure: Take 5 gms of given leaf material, grind it well with the help of pestle and mortar, by adding 20-30 ml of distilled water. Filter the grinded material through 3 layers of muslin cloth. Collect the extract and subject to centrifuge at 3000 rpm for 5-10 minutes. Collect the supernatant and add distilled water to bring the volume to 100 ml in a standard flask.

Pippet out 10 ml of the extract from the standard flask into a conical flask and add 2 drops of Bromothymol blue indicator. Then liquid turns yellow colour, and titrate against 0.1 N NaOH, taken in the burette. Appearance of green colour stands for end point, stop the titration and record the volume of NaOH used. To the same conical flask add 2 drops of phenapthalene indicator and titrate against 0.1 N HCl. Reappearance of yellow colour indicates the end point. Then record the volume of HCl used. Repeat the titrations atleast 4 times to get accurate results.

Calculate the titrable acidity in the leaf using the following formula:

$N_1 V_1 = N_2 V_2$ and $N_3 V_3 = N_4 V_4$

Titrable acidity: $N_1 V_1 = N_2 V_2$ where N_1 = Normality of NaOH (0.1 N)
V_1 = Vol. of NaOH (from the burette reading)
N_2 = Normality of the extract unknown
V_2 = Volume of the extract (10 ml)

$$\therefore N_2 = \frac{N_1 V_1}{V_2}$$

Titrable acidity = - - - - - - - - - N

For calculating Total acidity: $N_3 V_3 = N_4 V_4$

where N_3 = Normality of HCl (0.1 N)

V_3 = Volume of HCl (from the Burette reading)

N_4 = Normality of the extract (unknown)

V_4 = Vol. of the extract (10 ml + vol. of NaOH used)

$$N4 = \frac{N_3V_3}{V_4}$$

Total and titrate acidity of given leaf = $N_2 + N_4$ - - - - - - - - N

Inference: Plants contain some organic acids, among them only few are titrable. Tritrable acidity is the acidity which is contributed to weak or strong acids, present in the free state or formed during metabolism. These are easily tritrable against weak alkali (NaOH). Total acidity is the acidity which is contributed to H^+ ions, fatty acids, amino acids, certain proteins and some aromatic acids. This can be known by the titration against weak acid (HCl) and strong indicator such as phenapthaline.

8. EFFECT OF TEMPERATURE ON MEMBRANE INTEGRITY – BEET ROOT SLICES

Aim: To study the effect of temperature on membrane integrity using beet root slices.

Material and apparatus: Beet root, Beaker, thread, stand, scalpel, distilled water, burner, tripod stand, thermometer.

Procedure: Cut a block of beet root and wash away the excess pigments by distilled water thoroughly. Tie the block with a thread and suspend it in a beaker (500 ml) containing distilled water. The beaker is placed on a tripad stand. Note the initial temperature of water and heat the beaker on a burner. Record the change in temperature of water inside the beaker and gently stir it by a glass rod. Carefully note the temperature at which leaching of pigment from the block starts. This can be observed more accurately if the beaker is placed against a piece of white paper.

Observation: Record the water temperature at which leaching of pigment starts.

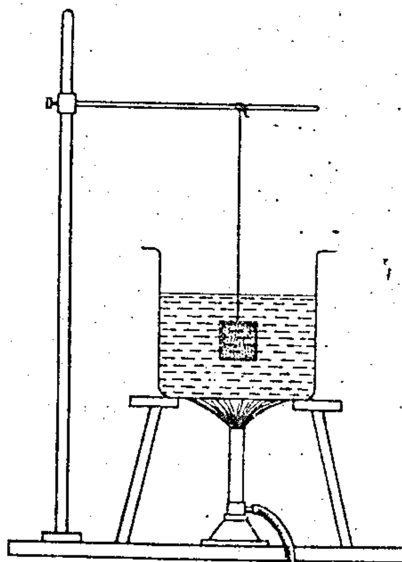


Fig. Effect of temperature on membrane integrity

Inference: The leaching of pigment is due to the loss of permeability of cell membrane due to high temperature. Repeat the experiments atleast 3 times to get a more accurate result. The effect of temperature on any biological system is classified into 3 stages. (a) low temperature, (b) moderate temperature, (c) maximum temperature. When temperature is raised, the activity of particular system can also be enhanced. But at maximum levels, the system loses its integrity and cannot perform concerned activity. The pigment released from beet root block is anthocyanine.

9. MEASUREMENT OF PLASTID MOVEMENT IN HYDRILLA

Aim: Measurement of plastid movement in Hydrilla plant.

Apparatus: A portion of a freshly collected young leaf of Hydrilla, slide, microscope, water, hot water and cool water.

Principle: Cell is a structural and functional unit of an organism. The living nature of cell lies with protoplasm. It is a jelly like colloidal substance undergoing series of chemical reactions and regulates the entire cellular activity. The major characteristic features of living system is expressed by protoplasm and its components such as movement, permeability and metabolic activities etc.

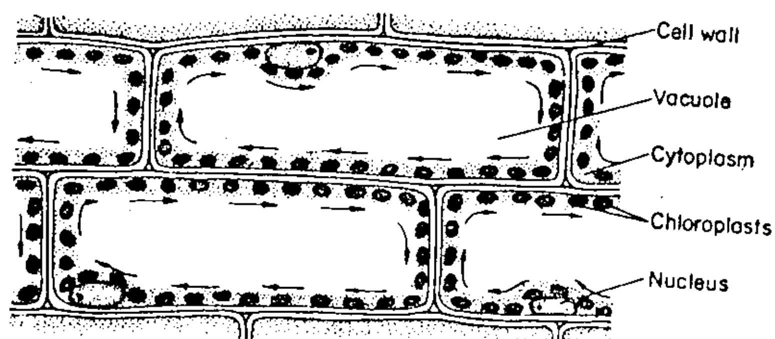
Procedure:

(A) Take a freshly collected young leaf of hydrilla, mount it on a slide in water and then examine under microscope.
(B) Add a few drops of hot water and put on the mounted strip and reexamine after 3-5 minutes.
(C) Finally add a few drops of ice-cold water on the mounted leaf strip and examine after 3-5 minutes, remember to observe the movement of plastids for every change you made.

Observation:

(A) The rotatary movement of the chloroplasts along the boundary wall of the cell is observed.
(B) The rate of movement of chloroplasts in the protoplasm is increased.
(C) The chloroplast movement has almost ceased.

Fig. Streaming movements of chloroplasts



Inference:

(A) The protoplasmic streaming movement indicates its living nature.
(B) The exogenous supply of energy enhances the rate of movement.
(C) The stress conditions stop the movement of chloroplastids.

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10. DETERMINATION OF RATE OF TRANSPIRATION BY STOMATAL FREQUENCY

Aim: To determine the rate of transpiration by using stomatal frequency.

Apparatus: microscope
Stage micrometer
Glass slides
Cover slips etc.

Material: Leaves of Rhoeodiscolor, Bougainvillea, Nicotiana.

Procedure: A stage micrometer is taken and it is kept under microscope with 10x eye piece and 40x objective and the diameter of the microscope field area is calculated by adapting the formula Πr^2. Boil the leaves in chloral hydrate solution (or) chlorinated soda. Peel and upper and lower epidermis of all leaves using foreceps, mount them separately using glycerine. Examine the slides under microscope. Notedown the number of stomata and epidermal cells. The stomatal frequency to calculated for all the above mentioned plants for 1 sq.mm. Tabulate the results.

Table

S.No.	Name of the plant	Number of epidermal cells	Number of stomata	Stomatal frequency
1	Rhoeodiscolor			
2	Bougainvillea			
3	Nicotiana			

Results: The number of stomata within the area of field of visionis determined and from it the number of stomata per square cm is calculated. This gives the stomatal frequency of the leaf.

Explanation: Loss of water in the form of water vapous through stomata is called 'Transpiration'. As stomata are involved in the transpiration, estimation of stomata number is prerequisite for estimating the rate of transpiration. Generally xerophytes show sunken stomata so they can reduce the water loss mesophytes show more stomata. So they show transpiration at normal rate. Mesophytes are expected to be with hypostomatic condition. So they contain more number of stomata on the lower surface than upper surface. The number of stomata per unit area leaf surface may be quiet different on leaves of two plants of the same species.

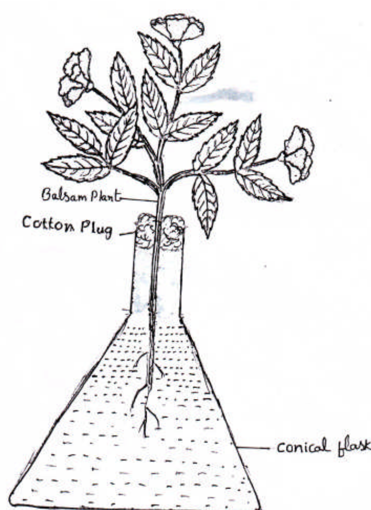
MINOR EXPERIMENTS

1. Demonstration of transport of water through Xylem

Aim: To demonstrate the movement of water through xylem.

Materials: Balsam plant, conical flask, water, cotton plug, Safranin, blade, slide, glycerine, coversleep and microscope.

Procedure: Take a Conical flask. Fill the Conical flask with water and add a few drops of safranin. Keep the plant with the roots in the conical flask. Fix a cotton plug at the mouth of the flask. Keep the apparatus for a few hours. Take a thin section of the stem. Mount the section in glycerine.



Demonstration of Transport of water through Xylem

Observation: It is observed that the stem, petioles and petals become red in color. The t.s of the stem shows staining of xylem as red colored tissue. This indicates that the solution moved through xylem.

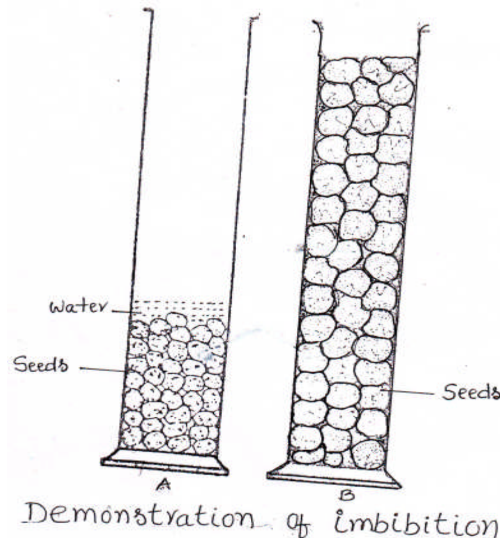
Inference: Absorption of water takes place through the root hairs. Water moves in the upward direction through xylem vesseles. This upward movement of water against gravity is called ascent of sap. The ascent of sap is due to transpiration occurring in the leaves.

2. Demonstration of imbibition

Aim: To demonstrate the phenomenon of imbibition

Requirements: Seeds of Bengal gram , wheat, groundnut, water, beakers, blotting paper

Procedure: Take 5 gram of the seeds. Place them in a beaker containg water. After 2 hours remove the seeds. Blotting paper is used to drain the excess water. Weigh the soaked seeds. The gain in weight of different kinds of seeds is compared.



Demonstration of imbibition

Observation: The imbibed seeds are swollen.

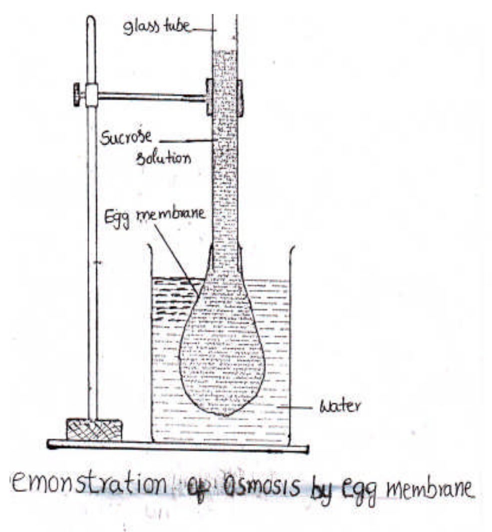
Inference : The increase in volume of the seeds is due to a phenomenon called imbibition. It is a special type of diffusion. The dry seeds are having low water potential. When placed in water, water moves into the seeds because of water potential gradient. The seed consis of colloidal material. The proteins and carbohydrates show maximum imbibition. The rate of imbibition depends upon the seed coat and the type of food material in the seed.

3 . Demonstration of Osmosis by Egg membrane

Aim: To demonstrate the osmosis by the egg membrane

Requirements: Egg membrane, dilute Hcl, large beaker, graduated tube , sugar solution, stand

Procedure: Prepare an egg membrane by disolving the shell of the egg in dilute Hcl. Make a hole at one end and remove the contents of the egg. Fill the egg membrane with sucrose solution through the hole and fit a graduated tube in the hole. Tie a thread tightly to tube at the inserted end. And note the level of the sugar solution in the graduated tube. Keep the apparatus undisturbed for a few hours in a beaker containing water.



emonstration of Osmosis by egg membrane

Observation: Level of the sugar solution increases in the tube

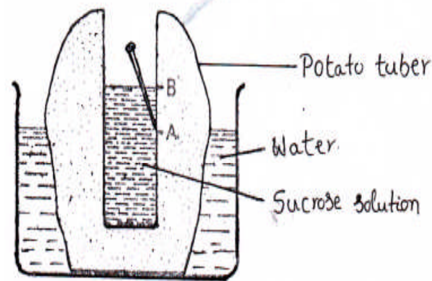
Inference: The rise of solution level inside the egg osmoscope is due to endosmosis of water through the egg membrane.

4. Potato osmoscope

Aim: To demonstrate osmosis with the help of potato osmoscope

Requirements: Potato tuber, petridish, sucrose solution, scalepel, pins

Procedure: A large potato is selected. The skin of the potato is peeled. The edge portions are sliced off to give it the shape of a rectangle. Scoop out carefully tissues from the central part of the potato to create a deep cup like cavity. Fill the cavity with sucrose solution. Place the osmoscope in petridish containing water and keep it for 1 hour.



Potato Osmoscope

Observation : The rise in the level of the solution in the cavity of the osmoscope is observed.

Inference : The rise of level of solution is due to the movement of water from the petridish into the osmoscope. It is the cell to cell osmosis water from a region of higher concentration of water to a region of lower concentration.

5 FERMENTATION

Aim: To demonstrate the process of fermentation.

Apparatus: Kuhne's fermentation vessel, beaker etc.

Chemicals: Glucose, water, yeast etc.

Procedure: Take 10% of glucose in the beaker, change it into solution. Add a little quantity of yeast to it as a starter culture. Transfer this mixture to the Kuhne's fermentation vessel. The complete upright tube and half of the bent (side)bulb is filled. The open end of the bulb is plugged with cotton. The entire apparatus is allowed to stand for few hours.

Observation: As gas is forming and being collected in the upright tube the solution level in the vessel is slowly decreased. The gas collected in the tube is CO_2. It can be proved by introducing caustic potassium into the vessel. The alcohol smell is noticed from the solution.

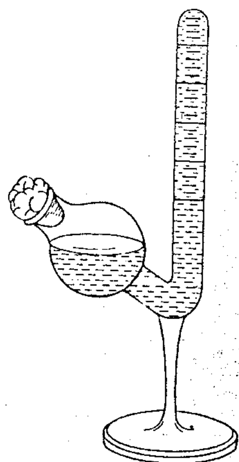


Fig. Fermentation vessel (Kuhne vessel)

Explanation: Yeast ferments sugar solution present in the Kuhne's fermentation vessel and converts it into alcohol and CO_2 under anaerobic conditions.

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2C_2H_5OH + 2CO_2$$

The fermentation of sugar stops when the concentration of C_2H_5OH reaches 10-15%, the condition where yeast cells are killed.

6 PHOTOTROPISM

Aim: To prove the phototrophic movements of the plant (or) to prove phototropism.

Apparatus: Seedlings growing in a pot, Heliotrophic chamber – it is a wooden chamber having holes of different diameters and they can be opened and closed as desired.

Procedure: Keep the pot with seedlings inside the heliotrophic chamber, allow the plant to grow in darkness for 2-3 days by closing all the holes. Later open a hole and let diffuse light into the chamber. A potted plant of same species growing in dark room should be taken as control.

Observation: Plant, which is subjected to light treatment is bent towards the light coming direction and the plant present in the dark room is straight.

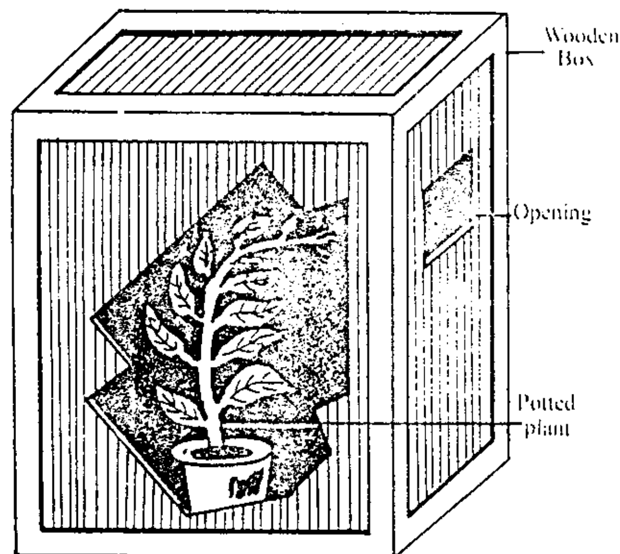


Fig. Phototropic chamber

Explanation: If a growing plant is exposed to unilateral light, it causes unequal distribution of hormones (auxin) in the stem tip. The darker side of the tip receives more hormone than the light side, because hormones are light sensitive. So much hormone on dark side causes more growth on that side so that plant tip bends towards the light. This is called phototropism.

7 ARC AUXANOMETER

Aim: Measurement of vertical growth of the plant, using arc auxanometer.

Apparatus: Arc auxanometer, potted plants and thread with weight

Arc auxanometer is a metallic instrument fitted to a vertical stand. It consists of an axle and a pulley. The axle holds a pointer, which can move up and down on the scale and shows reading. The movement of scale depends on pulley rotation.

Procedure: Take a thread and place it on the pulley. Tie one end of the thread to the growing tip of the potted plant and tie another end of the thread to a weight. Note down the initial reading of the point and allow the plant to grow for a few days and must not forget to take readings at regular intervals of growth period i.e. one day.

Observation: As plant grows, the one end of the thread tied to the plant goes up and the other end, tied to the weight goes down with the help of pulley. With the rotation of axle, the pointer moves down.

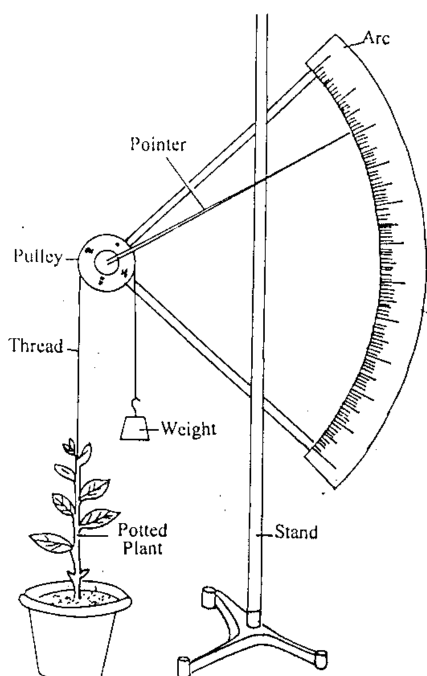


Fig. Arc Auxanometer

Explanation: Growth is an important process of any organism. It is the result of many metabolic processes. Generally in multicellular organisms, growth is confined to apical parts such as stem tip and root tip where apical and subapical meristems are present. The cells in those regions undergo continuous elongation and divisions cause growth. In plants growth is a continuous process. Rate of the growth can be measured by dividing the actual growth with time in hours.

$$\text{Rate of the growth} = \frac{\text{Actual growth}}{\text{Time in hours}}$$

If the actual growth is 3 mm in 12 hours then the rate of growth

$$\frac{3}{12} = 0.25 \text{ mm / hour}$$

References:

1. O.P.Sharma, **United Practical Botany-III**, Pragati Prakashan.
2. S.S. Santra, T.P. Chatterjee, A.P. Das, **College Botany Practical, Vol. I**, New Central Book Agency (P) Ltd.
3. J. Jayaraman, **Laboratory Manual in Biochemistry**, Wiley Eastern Ltd.

Genetics Practicals

PREPARATION OF KARYOTYPES FROM DIVIDING ROOT TIP CELLS

AIM : To observe karyotype from root tip cells of the given material.

OBJECTIVE : To identify different types of chromosomes based on karyotype analysis.

PRINCIPLE : Karyotype is the phenotypic appearance of the entire chromosome complement of the species. It represents all the chromosome types based on their morphology in the total complement. The diagrammatic representation of the karyotype is the idiogram. Katyogram is the actual representation of the karyotype performed from the microphotograph, as well as from the drawing.

At metaphase of mitosis, the chromosomes assume the most condensed structure and their length, shape and size-differentiation assume cytotaxonomic importance. Individual chromosomes can be reliably identified at mid and late metaphase and for this we need to arrest the movement of chromosomes. This is achieved by colchicine treatment.

MATERIALS AND CHEMICALS : Onion roots or Aloe vera roots, aceto carmine stain, acetic acid, alcohol, HCl, chloroform, glass slides, cover slips, mounting needle, spirit lamp, blotting paper, compound microscope and watch glass.

PROCEDURE : For karyotype analysis, pretreatment of the material is needed prior to fixation. The karyotype analysis involves the following steps.

PRETREATMENT : Fresh young root tips of about 1 cm long are taken and transferred to corked glass tube containing a suitable pretreatment agent such as colchicine. The tube is kept at 10^0C – 12^0C for a specific period of time i.e., for about 1 hour.

FIXATION : After keeping the root tips for a specific period of time in colchicine solution, the root tips are taken out and thoroughly washed under running tap water. Now the pretreated root tips are placed in a suitable fixative such as 1:3 acetic acid: ethanol for 24 hours. After 24 hours, the root tips are removed from the fixative and changed into 70% ethanol solution. In this solution, the root tips can be stored for longer duration at 4^0C.

STAINING : Fixed root tips are stained as in mitosis by squash technique.

SQUASH TECHNIQUE : Two or three root tips of length 1-2 cms or terminal tips of about 3mm, are taken and placed in a few drops of acetocaramine and heated in a low flame. Care should be taken that stain should not boil. They are passed through the following solutions:

1) **1:1 Glacial acetic acid : Alcohol mixture** for about 2-5 minutes.
2) **6:3:1 Acetic acid: Chloroform : Alcohol mixture** for about 1-3 minutes. This is known as Carnoy's fixative.
3) **90% Alcohol :** This is a fixative and tips can be placed in this solution for a long period of time.

A drop of acetocaramine stain is taken on a slide. The tip portion of the treated root tip is taken and placed in the drop and the material is squashed by tapping firmly with the flat end of a mounting needle. This facilitates the easy separation of cells from one another. When a uniform suspension of small cell groups are formed, a cover slip is placed over it. Care should be taken that the stain should not come out, but it should just be sufficient to reach the edge of the cover slip.

The preparation is then heated gently over a low flame of spirit lamp for a few seconds. The slide is then placed between two sheets of blotting paper and a firm vertical pressure is applied with thumb over the area of the cover slip. This removes the excess stain and also facilitates flattening of cells and spreading of chromosomes. The preparation is then ready for study under a compound microscope.

OBSERVATION : Well scattered metaphase plate with distinct constriction of chromosomes is found under the oil immersion objective and can be drawn with camera lucida or drawing prism. Depending upon the position of the centromere, the chromosomes should be arranged, and arm ratio should be calculated. First of all, chromosomes should be arranged according to their total length, the biggest pair as first and the smallest pair as the last. In the second attempt, chromosomes should be separately arranged as per position of centromere.

PROBLEMS ON MENDELISM

Solve the following problems whcih are based on Mendelism Monohybrid Cross, Dihybrid Cross, Back Cross and Test Cross.

Problem 1: Monohybrid Cross :

Mendel crossed pea plants that produced round seeds with those of wrinkled seeds. From a total of 7324 F2 seeds, 5474 were round and 1850 were wrinkled. Using the symbols W and w for genes,

a. Symbolise the original P cross

b. Symbolise the gametes of parental generation

c. Symbolise the F_1 progeny

d. Represent a cross between two F_1 plant

e. Symbolise the gametes of F_1 plants

f. Summarise the expected F_2 results under phenotypes, genotypes, phenotypic ratio and genotypic ratio.

Solution:

Round seeds: 5,474

Wrinkled seeds: 1,850

Round seeds and wrinkled seeds are in the ratio of 3:1.This suggests that the cross is a monohybrid cross.

P Generation: WW (Homozygous Round seeds) X ww (Homozygous Wrinkled seeds)

Gametes: (W) (w)

F_1 Progeny: W w — Heterozygous Round

Cross between two F_1 plants: W w X W w

Gametes: (W) (w)

F_2 results

	W	w
W	WW round	Ww round
w	Ww round	ww wrinkled

Phenotypic ratio - 3 round : 1 wrinkled
Genotypic ratio - 1 WW : 2 Ww : 1 ww

Problem 2: Monohybrid Cross :

a. Several black guinea pigs of same genotype were mated and they produced 29 black and 9 white offspring. What would you predict on the genotypes of the parents?

b. Heterozygous black (Bb) guinea pigs were mated to homozygous recessive (bb) whites. Predict the genotype and phenotype ratios expected from back crossing the black F1 progeny to (i) the black parent and (ii) the white parent.

Solution:

a.) Black : 29

White : 9

These are in the ratio of 3:1 which suggests a monohybrid ratio. Monohybrid cross takes place between two F1 organisms having the same genotype.

Genotypes of parents: Bb X Bb

b. i)

Back cross with black parent: Bb (Black F1 offspring) X BB (Black parent)

Gametes: B b | B

	B	b
B	BB Black	Bb Black

Phenotype : All are black

Genotype : BB Homozygous Black : Bb Heterozygous Black

1 : 1

b. ii)

Back cross with white parent: Bb (Heterozygous Black) X bb (Homozygous white)

Gametes: B b | b

	B	b
b	Bb Black	bb White

Phenotypic ratio: Black : White

1 : 1

Genotypic ratio : Bb : bb

1 : 1

Problem 3 : DIHYBRID CROSS :

In summer squash, white fruit colour is governed by a dominant allele (W) and yellow fruit colour by a recessive allele (w). A dominant allele (S) at another locus produces disc shaped fruit, and its recessive allele (s) yields spherical shaped fruit. If a homozygous white disc variety of the genotype WWSS is crossed with a homozygous yellow, spherical variety of the genotype wwss, all the F_1 plants produced white coloured and disc shaped fruit of the genotype WwSs. If the F_2 is allowed to cross at random, what would be the phenotypic ratio in the F_2 generation?

White colour allele : W
Yellow colour allele : w
Disc shape allele : S
Spherical shape allele: s

Cross WWSS wwss
Homozygous White Disc Homozygous Yellow Spherical

Gametes (WS) X (ws)

F_1 WsSs

Heterozygous White Disc

Gametes (WS) (Ws) (wS) (ws)

F_2 Generation

	WS	Ws	wS	ws
WS	WWSS White Disc	WWSs White Disc	WwSS White Disc	WwSs White Disc
Ws	WWSc White Disc	Wwss White Spherical	WwSs White Disc	Wwss White Spherical
wS	WwSS White Disc	WwSs White Disc	wwSS Yellow Disc	wwSs Yellow Disc
ws	WwSs White Disc	Wwss White Spherical	wwSs Yellow Disc	wwss Yellow Spherical

Phenotypic : Ratio White Disc 9 : White Spherical 3 : Yellow Spherical 3 : Yellow Spherical 1

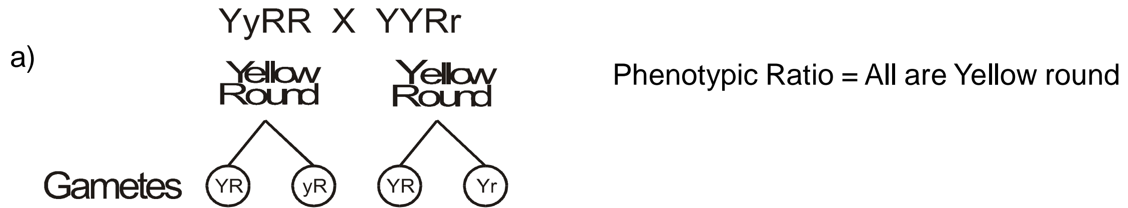
Problem 4 : DIHYBRID CROSS :

In sweet pea, yellow seed coat (Y) is dominant over green (y), and round shape (R) is dominant over wrinkled (r). What phenotypic ratios could be obtained in F1 progeny of the following crosses?

Yy RR x yy Rr

Yy Rr x Yy Rr
Yy rr x yy rr
Yy Rr x yy rr

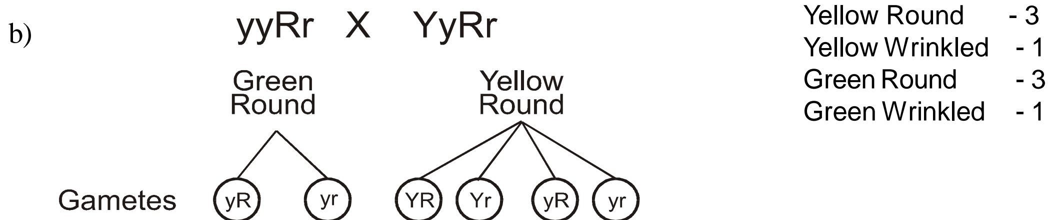
a)



Phenotypic Ratio = All are Yellow round

	YR	Yr
YR	YYRR Yellow Round	YYRr Yellow Round
yR	YyRR Yellow Round	YyRr Yellow Round

b)

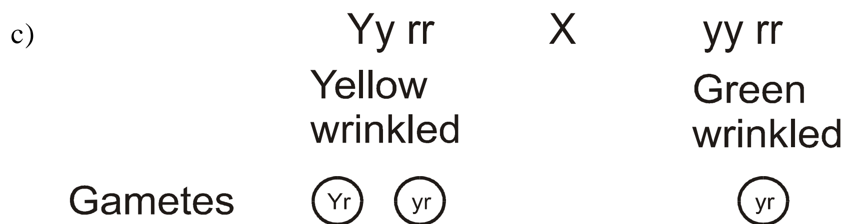


Yellow Round - 3
Yellow Wrinkled - 1
Green Round - 3
Green Wrinkled - 1

	YR	Yr	yR	yr
yR	Yy RR Yellow Round	YyRr Yellow Round	yyRR Green Round	yyRr Green Round
yr	YyRr Yellow Round	Yyrr Yellow Wrinkled	yyRr Green Round	yyrr Green Wrinkled

Phenotypic Ratio = 3:1:3:1

c)



	Yr	yr
yr	Yy rr Yellow wrinkled	yy rr Green wrinkled

Yellow Wrinkled - 1
Green Wrinkled -1

Phenotypic Ratio = 1:1

d)

YyRr X yy rr
Yellow round   Green wrinkled

(YR) (Yr) (yR) (yr)   (yr)

	YR	Yr	yR	yr
yr	Yy Rr Yellow Round	Yy rr Yellow Wrinkled	yy Rr Green Round	yy rr Green Wrinkled

Yellow round - 1
Yellow wrinkled - 1
Green round - 1
Green wrinkled - 1
Phenotypic ratio = 1:1:1:1

Problem 5 : DIHYBRID TEST CROSS :

A cross between a yellow round and green wrinkled seeded pea plants gave the following four types of progeny in equal proportions.

Yellow round
Yellow wrinkled
Green round
Green wrinkled

What are the genotypes of the parents?

Equal proportions of progeny suggests the dihybrid test cross ratio 1:1:1:1. Dihybrid test cross involves F1 plant and double recessive parent i.e.,

Genotype   Yy rr   X   yy rr

F1 Plant   Double Recessive Parent

Problem 6

As Mendel discovered, gray seed colour in peas is dominant to white. In the following experiments, parents with known phenotypes but unknown genotypes produced the listed progeny:

Parents		***Progeny***	
		Grey	*White*
a. Gray x	*White*	*82*	*78*
b. Grey x	*Grey*	*118*	*39*
c. White x	*White*	*0*	*50*
d. Grey x	*White*	*74*	*0*
e. Grey x	*Grey*	*90*	*0*

a) Parents - Grey X White
given progeny 82 78

Phenotypic Ratio of above progeny 1 : 1

The above ratio suggests that the cross is monohybrid test cross. It involves F_1 and recessive parent.

Genotype of parents - Gg X gg

b) Parents - Grey X Grey

Given Progeny 118 39

Phenotypic Ratio of above progeny 3 : 1

The above ratio suggests that the cross is monohybrid cross which involves 2 F_1 plants.

Genotype of parents - Gg X Gg

c) Parents - White X White

Given Progeny 0 50

Phenotypic Ratio of above progeny All are white

The above progeny suggests that the cross involves 2 recessive parents.

Genotype of parents - gg X gg

d) Parents - Grey X White

Given Progeny 74 0

Phenotypic Ratio of above progeny All are grey

The above progeny suggests that the cross involves 1 dominant parent and 1 recessive parent.

Genotype of parents - GG X gg

e) Parents - Grey X Grey

Given Progeny 90 0

Phenotypic Ratio of above progeny All are grey

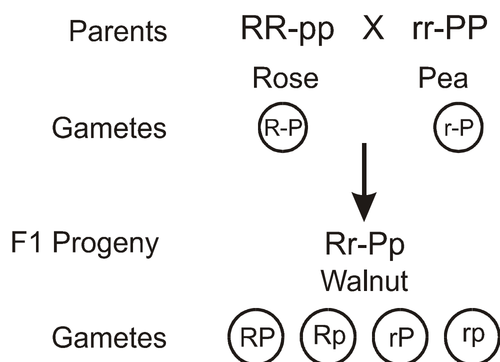
The above progeny suggests that the cross is back cross which involves F_1 plant and dominant parent.

Genotype of parents - Gg X GG

PROBLEMS ON GENE INTERACTION :

Problem 7 :

Two non allelic gene pairs govern the comb shapes in fowls. The genotype R-P produces a walnut comb, R-pp produces a rose comb, and rr-P produces a pea comb. rr-pp produces a single comb. If a pure rose combed variety (RR-pp) is crossed with a pure pea combed variety (rr-PP), give the appearance of the F1 and F2 progeny.



	RP	Rp	rP	rp
RP	RRPP Walnut	RRPp Walnut	RrPP Walnut	RrPp Walnut
Rp	RRPp Walnut	RRpp Rose	RrPp Walnut	Rrpp Rose
rP	RrPP Walnut	RrPp Walnut	rrPP Pea	rrPp Pea
rp	RrPp Walnut	Rrpp Rose	rrPp Pea	rrpp Single

F2 Progeny – Walnut 9: Rose 3 : Pea 3 : Single 1

Problem 8 : COMPLEMENTARY GENES :

In sweet peas, when genes C and R are present separately, produce white flowers. But when both are present, the flower colour is purple. Give the flower colour of the offspring of the following crosses.

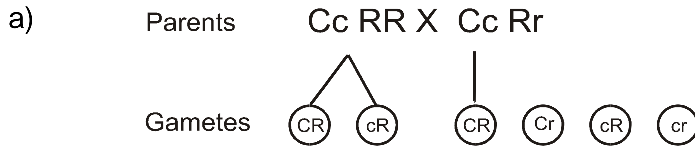
a) CcRR x CcRr

b) ccRr x CcRr

c) CCrr x ccRR

Solution:

a)

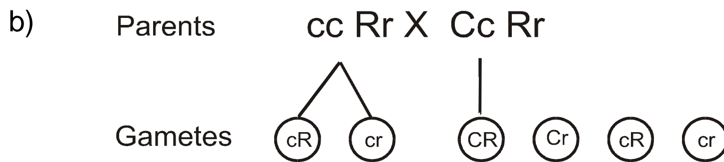


Progeny

	CR	Cr	cR	cr
CR	CCRR Purple	CCRr Purple	CcRR Purple	CcRr Purple
cR	CcRR Purple	CcRr Purple	ccRR White	ccRr White

Purple Flowers : 6
White Flowers : 2

b)

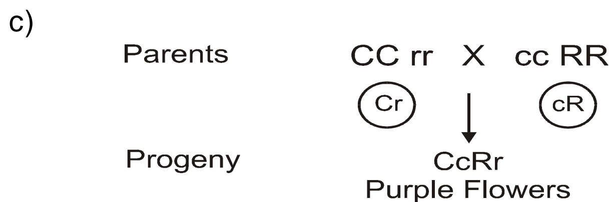


Progeny

	CR	Cr	cR	cr
cR	CcRR Purple	CcRr Purple	ccRR White	ccRr White
cr	CcRr Purple	Ccrr White	ccRr White	ccrr White

Purple Flowers : 3
White Flowers : 5

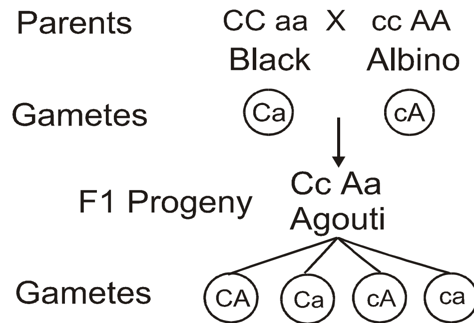
c)



Problem 9 : SUPPLEMENTARY GENES :

When a true breeding black cat (CCaa) is crossed with an albino (ccAA), all the F1 offspring are of agouti type (CcAa). The F2 generation consisted of 45 agouti, 15 black and 21 albino. Explain the F2 results with the help of a checker board.

Solution:



F_2 Progeny

	CA	Ca	cA	ca
CA	CC AA Agouti	CC Aa Agouti	Cc AA Agouti	Cc Aa Agouti
Ca	CC Aa Agouti	CC aa Black	Cc Aa Agouti	Cc aa Black
cA	Cc AA Agouti	Cc Aa Agouti	cc AA Albino	cc Aa Albino
ca	Cc Aa Agouti	Cc aa Black	cc Aa Albino	cc aa Albino

Agouti	9
Black	3
Albino	4

9:3:4 ratio suggests the interaction of supplementary genes. Here there are two dominant genes C & A. One dominant gene (C) will produce its effect whether the other dominant gene is present or not. But the second dominant gene (A) can produce its effect only in the presence of the first dominant gene.

Problem 10 : EPISTATIS :

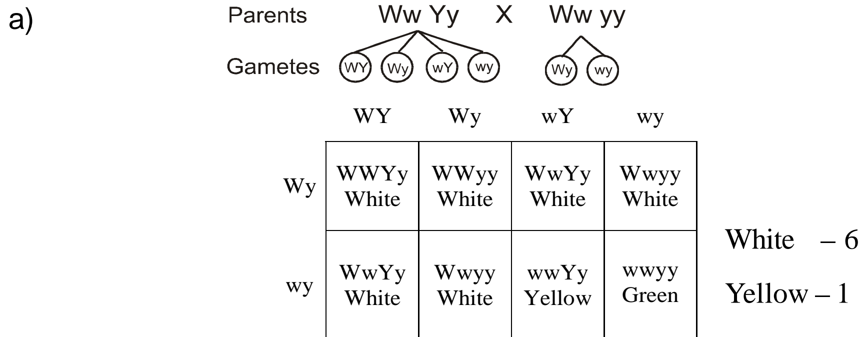
In summer squash, the gene for white fruit colour (W) is epistatic to the gene for yellow fruit colour (Y). Yellow (Y) is dominant over green (y). Determine the fruit colour of the offspring of the following crosses.

a) WwYy x *Wwyy*

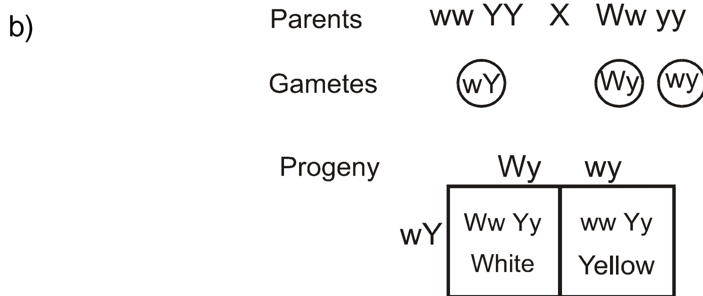
b) wwYY x *Wwyy*

c) Wwyy x *wwYy*

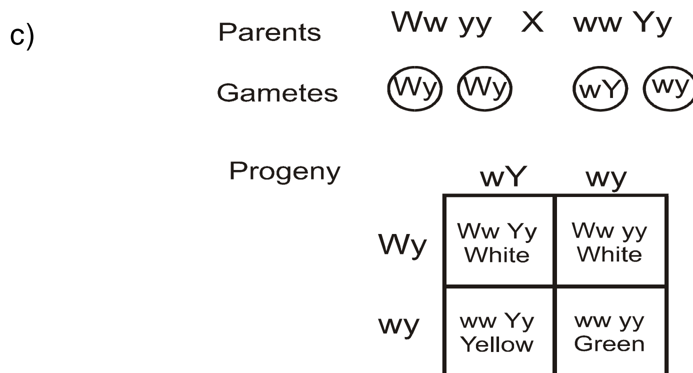
a)



b)



c)



The results of the above process are due to the gene W (white) which is epistatic to the gene Y (yellow). So if gene W is present it suppresses yellow colour.

PROBLEMS ON LINKAGE

Problem 11 :

In maize, there are 10 pairs of homologous chromosomes. The recessive gene (bm) for brown midrib is located on chromosome V. Another gene brevis (bv) causes short internodes.

a. How many linkage groups are there in maize?

b. What is the probability that a second gene brevis (bv) will be linked with the gene for brown midrib?

c. What would be the offspring phenotypic ratios resulting from self pollination of a plant which is heterozygous for these alleles (+bm +bv), if the alleles lie on different chromosomes?

d. What would be the offspring phenotypic ratios of the dihybrid cross if bm and bv are linked with no crossingover?

Solution:

a. The number of linkage groups = The number of homologous pairs

So, there are 10 linkage groups in maize.

b. The probability that a second gene brevis (bv) will be linked with the gene for brown midrib (bm) is 1/10.

c. If the alleles lie on different chromosomes, they assort independently and the F_2 phenotypic ratio will be 9:3:3:1 which is a typical dihybrid ratio.

The phenotypic ratio will be 9 ++ : 3 bm+ : 3 +bv : 1 bmbv

d. If bm and bv were linked (present on the same chromosome), the F_2 ratio will be 3 ++ : 1 bm which resembles a monohybrid ratio.

Phenotype ratio will be 3 ++ : 1 bmbv

Problem 12

Find out the linkage relationship (coupling or repulsion) between the two genes in a dihybrid individual (Aa, Bb) which when test crossed, gives the following data.

Parents	*Aa Bb* *Dihybrid Parent*	*X*	*aa bb* *Double Recessive Parent* *(Test Cross Parent)*

Aa Bb	:	*40%*
aa bb	:	*40%*
Aa bb	:	*10%*
aa Bb	:	*10%*

Solution:

The test cross parent contributes genes a and b. Since AB and ab are the combinations which appeared in greater frequency, genes A and B must be on one chromosome, and a and b on the other homologous chromosome of the dihybrid parent, i.e., in the coupling phase AB/ab. The genotype *Aabb* and *aaBb* represent the two recombinant types (Ab and aB).

A B

a b

Coupling Phase

Problem 13

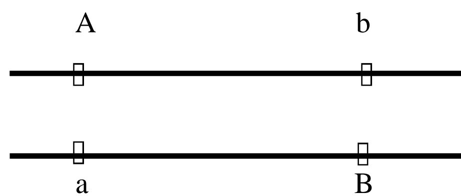
Find out the linkage relationship between the two genes, when a dihybrid parent (AaBb) was test crossed and gave the following progeny.

Parents	*Aa Bb*	*X*	*aa bb*
	Dihybrid Parent		*Double Recessive Parent (Test Cross Parent)*

Aa bb	*:*	*40%*	Coupling phase
aa Bb	*:*	*40%*	
Aa Bb	*:*	*10%*	
aa bb	*:*	*10%*	

Solution:

The genes A and b must be on one chromosome and genes a and B on the other chromosome (repulsion). Test cross parent contributes genes a and b. The remaining gene combinations Ab and aB are more frequent than the recombinations AB and ab.



Repulsion Phase

- P.M. VASANTHA KUMARI