BOTANY

PLANT PHYSIOLOGY, GENETICS & ECOLOGY

BLOCK I : PLANT PHYSIOLOGY
BLOCK II : GENETICS
BLOCK III : ECOLOGY

DR.B.R.AMBEDKAR OPEN UNIVERSITY
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PREFACE

This book deals with Plant Physiology, Genetics & Ecology included in the syllabus for the third year of the B.Sc. course offered by the Andhra Pradesh Open University. The topics generally cover the 'core' area of the subject to be studied in the third Year of the Three Year Degree Course in Science. The syllabus, for the sake of convenience, is divided into Blocks, each of which comprises a number of units. Each Block generally covers a specific area of the subject. The units are prepared by the specialists in accordance with a format so designed as to enable the student to read and understand them without much difficulty. Each unit begins with a statement of its objective followed by synopsis. Each Block has at its end assignments intended to test the student's comprehension of the subject matter. Technical terms with which the student may not generally be familiar are also given under the head Glossary.

Recent advances in the fields of Plant Physiology, Genetics and Ecology are also given. The University hopes that this material will help the student to get acquainted with all the principal issues of these fields. Critical suggestions for improving the text are most welcome and they will be incorporated in future edition.

CONTENTS

BLOCK - I
PLANT PHYSIOLOGY

UNIT-1: PLANT CELL - STRUCTURE & FUNCTIONS

Contents

1.1. OBJECTIVES

By the end of the unit, your will be able to:

1. recognise the organisation of cell as unit of structure and functions,
2. differentiate the different parts of the cell, their structure and functions,
3. draw well labelled figures of plasma membrane endoplasmic reticulum, Golgi apparatus, mitochondria, chloroplasts, nucleus,
4. describe the structure and functions of different cell organelles.

1.2. INTRODUCTION

The term "Cell"was first used by Robert Hooke (1665) to describe his investigations in the sections of a bottle cork with the help of crude microscope. In the sections of a bottle cork, he observed a honeycomb like structure consisting of several small compartments bounded by cell walls. He called each compartment a cell. The word cell is derive from the Latin word "Cellula" and this means a small compartment. In his later work, he observed a semi fluid substance within the compartments. He thought that it was a nourishing fluid and paid no attention to it.

Later Grew, Malpighi, Hanstein, Brown and others have observed several plants and brought to light several important features. Hanstein used the term protoplast for the semifluid substance present inside each compartment. Robert Brown (1831) was the first man to recognise the nucleus within the cell.

Presently, the term cell is used to indicate both the cell wall and the protoplast. The protoplasm surrounding the nucleus is being called cytoplasm and that of the nucleus, Karyoplasm.

Several discoveries made about the structure of plant and animal tissues led the botanist Schleiden (1838) and the Zoologist Schwann (1839) to establish the cell theory. This theory states that all living organisms consist of the cell as the fundamental unit. Rudolf Virchow (1855) in his famous aphorism, *Omnis Cellulae a cellula* (i.e., all cells arise from pre-existing cells), stated that the cell division is the central phenomenon in the reproduction of organisms.

The plant cells vary considerably in size, shape, organelle composition and physiological roles. There is no "typical cell" that can serve as an example of all plant cells. So, the diagrammatic representation of the plant cell covering all the important parts are given in the plant cell.

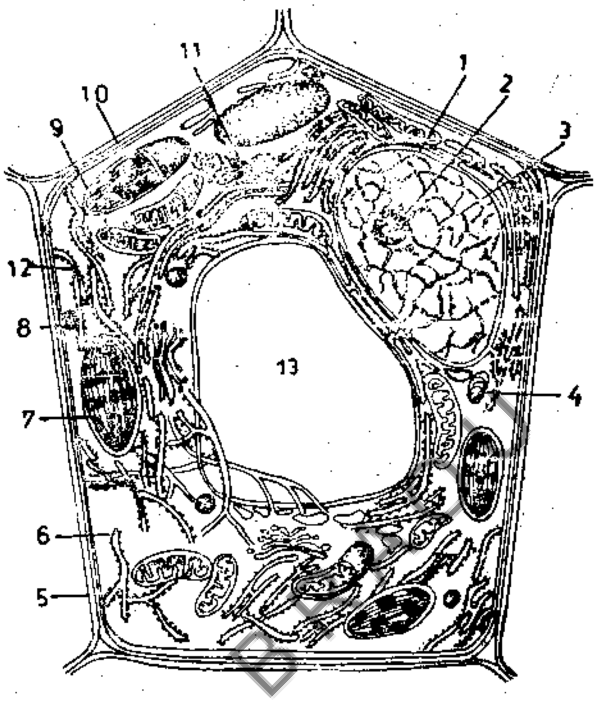

Fig.1.1 Diagrammatic representation of electron photomicrograph of a typical plant cell 1. Nuclear membrane 2. Nucleolus 3.Nucleus. 4.Starch grain. 5.Cell Wall. 6.Endoplasmic reticulum 7.Chloroplast 8.Chromoplast 9.Mitochondrium 10.Middle lamella 11.Dictyosome 12.Ribosome.

1.3. CELL WALL

The cell is covered with a rigid structure called the cell wall. The cell wall has both a supporting and protective function for the plant cell.. The structure, chemical composition and function of the cell wall are dealt in detail in Unit-5 of Course-II of Botony.

1.4. PLASMA MEMBRANE

Inner to the cell wall and surrounding the protoplasm is a thin, delicate, flexible structure called the cell membrane or plasma membrane or plasmalemma. Chemical and physical analysis of cell membrane suggests that it is a lipoprotein.

1.4.1. Structure

After thin sectioning and heavy metal staining Robertson (1959) adapted Danielli and Davson's (1935) model and proposed a unit membrane hypothesis. The hypothesis stated that all biological membranes have almost a uniform structure. It has a bimolecular lipid centre sandwhiched between monomolecular protein layers. Robertson postulated that the total thickness of the membrane is about 75 A^{o} to 100 A^{o} The thickness of each protein layer is about 20 A^{o} to 35 A^{o}. The molecular model of plasma membrane suggested by Robertson is given in Fig. 1.2.

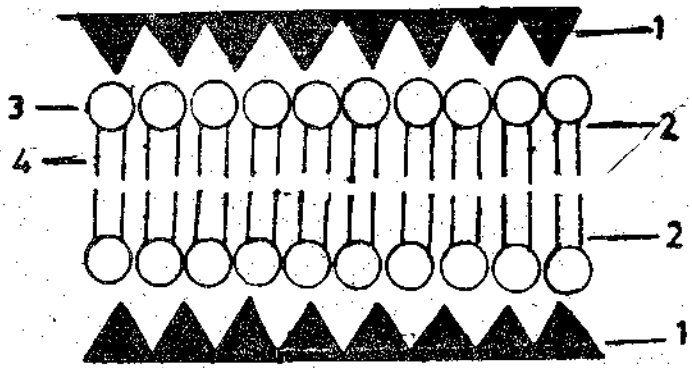

Fig. 1.2 Molecular model of plasma membrane suggested by Robertson. 1. Protein layer. 2. Lipid layer 3. Hydrophilic head. 4. Hydrophobic tail.

Within the last fifteen years so many alternative views have emerged for the structure of plasma membrane. Of them, the fluid mosaic model proposed by Singer and Nicolson (1972) is widely accepted. According to this model the large proteins are dispersed within the bimolecular lipid layer in the form of a mosaic. This is just like the protein icebergs floating in the lipid sea. The treatment of membranes with different salts and different pH's have revealed that the plasma membrane consists of two types of proteins. They are extrinsic or peripheral proteins, present at the surface of the membrane, and they can be easily removed. The intrinsic or intergral proteins are finally incorporated within the lipid bilayer. The proteins are *amphipathic* molecules with their hydrophilic ends protruding into the aqueous phase and their hydrophobic ends embedded in the monopolar regions of the lipids. The model proposed by these scientists is given in Fig 1.3.

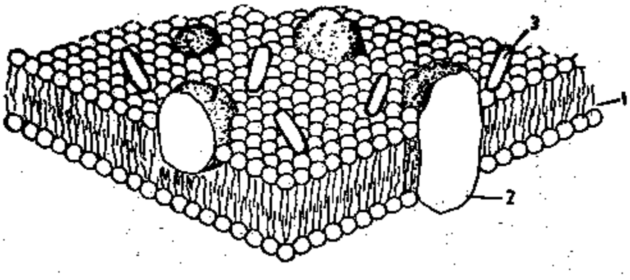

Fig.1.3. A Schematic representation of the three dimentional fluid mosaic model of plasma membrane. 1. Lipid layer. 2. Integral proteins. 3. Peripheral proteins.

Plasma membrane is a differentially permeable membrane and it regulates the movement of substances in both the ways. It permits the entry of some salts and water into the cell from outside and generally prevents substances from moving out of the cell. It also blocks the penetration of most of the toxic compounds into the cytoplasm.

The fluid mosaic model of membranes views a dynamic structure with its permeability barrier (bimolecular layer of lipid) also serving as a solvent for intergal proteins and lipids. Lipids and integral proteins in the bimolecular layer are free to move laterally within their lipid solvent. The carbohydrate moieties of glycolipids and glycoproteins, located on the outside of a membrane, are believed to be important in assuring the proper orientation of proteins in a membrane's structure; they also have a critical role in the phenomena of cell-to-cell recognition and interaction.

1.4.2. Functions

Cell membranes have three main functions: (1) They provide a selectively permeable barrier between two predominantly aqueous compartments; (2) They form a stable and fluid medium for reactions involving lipophilic reactants and provide a matrix for active proteins which catalyze a variety of reactions, including transduction of energy, and (3) They provide a flexible boundary between the cell or organelle and the surrounding medium which in combination with associated protein, has the capacity to transduce information from the exterior environment to the interior of the cell or organelle.

Check Your Progress - 1

What is the difference between the integral proteins and peripheral proteins?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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1.5. CYTOPLASM

The fluid material in between the plasma membrane and the nuclear membrane is called cytoplasm. Under a light microscope the cytoplasm appears as a homogeneous fluid. It is a colloidal complex and exhibits several properties of colloids. The cytoplasm may be more viscous than a liquid. It may approximate itself to a jelly. The cytoplasm can change itself readily from a sol to gel state and *vice versa*, according to Seifriz. Several organelles such as plastids, mitochondria, ribosomes, golgi bodies, lysosomes etc. are present in the cytoplasm.

Electron microscope studies reveal the presence of an extensive network called endoplasmic reticulum.

1.5.1. Endoplasmic Reticulum

Endoplasmic reticulum consists of interconnected flattend sacs called cisternae. Alternatively it may also appear as short tubules or vesicles. The former type usually contains ribosomes attached to the outer surface. This endoplasmic reticulum is known as rough or granular endoplasmic reticulum. The latter type of endoplasmic reticulum is without ribosomes and is known as smooth or agranular endoplasmic reticulum. The endoplasmic reticulum may be connected to the nuclear membrane or plasma membrane.

The endoplasmic reticulum is not only a series of channels which are concerned with transport of materials but also contains a number of enzymes of importance in metabolism. Alteration or additional processing of polypetides can also occur within the cisternae.

1.5.2. Ribosomes

Ribosomes are present virtually in all cells and are distributed freely within the cytoplasm or attached to the endoplasmic reticulum. They are also found within certain organelles such as nucleus, chloroplasts and mitochondria. Ribosomes are small, dense, hemispherical bodies measuring about 17-23 nm in diameter. A small bacterial cell may contain 5-10,000 ribosomes whereas an eukaryotic cell may contain 1,00,000 to several millions. The ribosome of a eukaryote sediments at 18 S and has a molecular weight of about 5 millions. Frequently the ribosomes occur as strings or clumps with as many as 20 ribosomes joined by a thin thread of messenger RNA. These groups of ribosomes are called polyribosomes or polysomes.

Each robosome is composed of two unequal subunits, one sedimenting at 60 S and another at 40 S. The ratio of the velocity to the centrifugal acceleration is called the sedimentation coefficient, S. The sedimentation coefficient has the unit, seconds. Each sub-unit contains RNA and protein in equal amounts. Ribosomes are the sites of protein synthesis.

Check Your Progress - 2

What are polysomes?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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1.5.3. Golgi Apparatus

The structure that we now call as Golgi Apparatus or Golgi complex or Golgi Body was first described in the nerve cells of owl by Camillo Golgi in 1898. There were suspicions till 1950 that the findings were due to artefacts of the fixation and staining methods employed at that time. The use of electron microscope and improved staining techniques in 1950's have established golgi apparatus as a real and universal organelle in living eukaryotic cells. The Golgi Golgi apparatus is divided into a number of smaller, independent units called dictyosomes. The number of dictyosomes ranges from one to several hundreds per cell.

It is suggested that all the dictyosomes are inter-connected by membranous elements to form the golgi apparatus.. Each dictyosome usually consists of a stack of membranous flattened sacs called cistenae (Fig 1.4.). The number of these cisternae in each dictyosome varies from 1-30. In many unicellular algae there is only one dictyosome per cell. The higher plants usually contain 4-7 dictyosomes whereas certain algal and animal cells contain between 20 and 30. The cisternae pinch off vesicles at the edges.

These vesicles contain several substances such as proteins, cellulose, hemicellulose and pectic compounds. The golgi apparatus appears to be involved in the secretion of cellulosic and non-cellulosic wall materials, slimes and mucilages, glycoproteins and various digestive enzymes. They are also useful in the formation of cell vacuoles and cell plate during mitosis. Gogi apparatus is observed in continuation with endoplasmic reticulum in many cases.

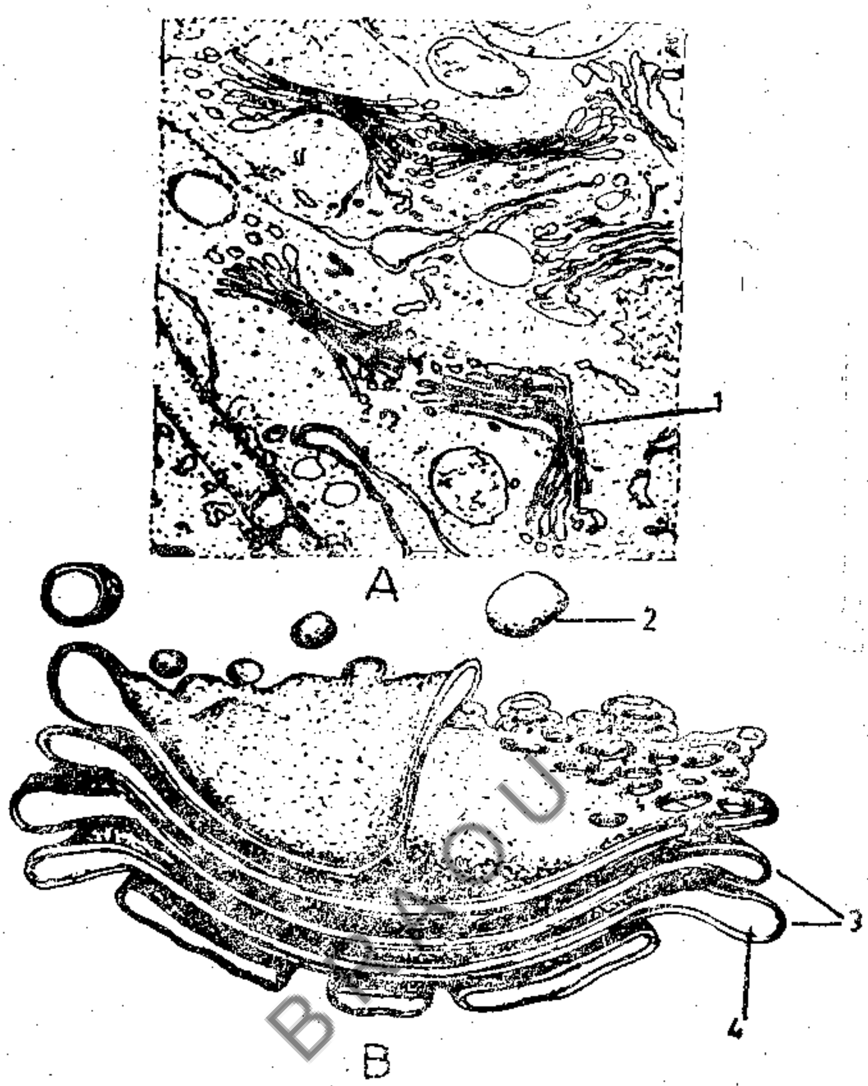

Fig.1.4. Golgi apparatus. A, Few dictyosomes. B. A three dimentional artist's reconstruction of single dyctyosome 1.Single dictyosome. 2. Vesicle. 3. Cisternae. 4. Lumen.

Check Your Progress - 3

Describe briefly the structure of a dictyosome.

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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1.5.4 Mitochondria

Mitochondria which are also known as chondrisomes were discovered by Kolliker and Altmann in 1880's. The term mitochondria was used by Benda a short time later. The mitochondria usually appear as cylinders (measuring about 3-6 μm long and 0.5 to 1.0μu diameter) or as spheres (about 0.5 to 1.5 m in diameter). The number of mitochondria per cell also shows considerable variation. Some unicellular algae contain only one whereas the cells of higher plants contain several thousands.

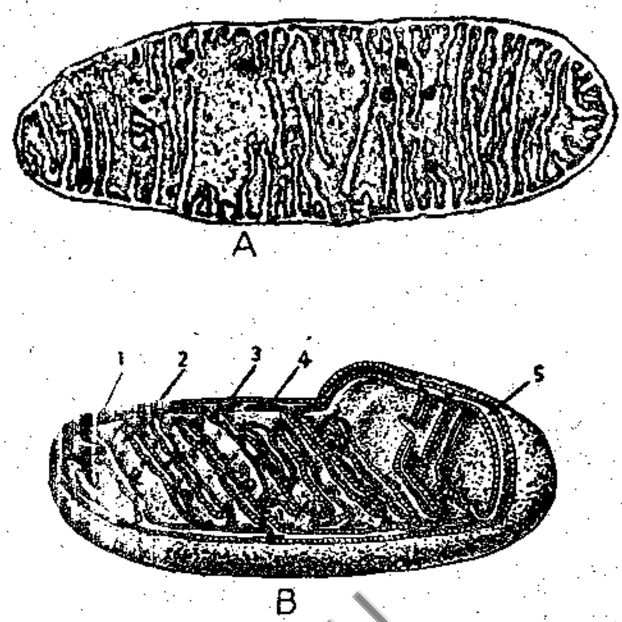

Fig.1.5. Mitochondria. A. L.S.of mitochondrion B.Three dimentional section. 1.Cristae. 2.Matrix 3.Outer membrane. 4.Inner membrane 5.Inter membrane space.

Each mitochondrion is bounded by an envelope consisting of two membranes, each about 5μm wide. The inner membrane is invaginated into the matrix to form projections called cristae. The space between the outer and inner membranes is called perimitochondrial space which is about 8.5 nm wide and the region within the invaginations is known as intercristal space. Regularly spaced stalked particles are found along the matrix side of the inner membrane. These are considered as structural units of mitochondria. Each elementary particle is called oxysome and it consists of a head of about 8-10 nm in diameter borne on a stalk of about 4-6 nm long and 4μm in diameter. There are about 2000 to 4000particles per μm^2 (Fig.1.5).

The space enclosed by the internal membrane is the matrix. The matrix is believed to contain proteins and lipids. A variety of inclusions such as DNA filaments, ribosomes and calcium containing granules are observed in the mitochondrial matrix.

Mitochondria are the sites of chemical reactions of respiration. Pyruvate produced from glycolysis enters the mitochondrion and is oxidised to CO_2 and water via Kerb's cycle or TCA cycle. The stalked particles contain the enzymes responsible for the synthesis of ATP during oxidative phosphorylation. These ATP molecules supply the energy to the cell whenever necessary. Hence the mitochondria are considered as "Power houses of a cell". In addition to this the mitochondria also contain transaminases which are helpful in the production of aminoacids from TCA cycle acids. As the mitochondria contain DNA, ribosomes and related macromolecules, it is presumed that they also carry out the process of transcription and translation during protein synthesis within themselves. Thus these organelles are semiautonomous.

Check Your Progress - 4

What is an oxysome? Describe its structure.

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

1.5.5. Plastids

Immature plant cells contain large mitochondria-like organelles possessing a double membrane envelope with little development of internal membrane system. These are called proplastids. According to the position of these immature cells, the proplastids develop into green chloroplasts (in photosynthetic tissue), colourless amyloplasts or leucoplasts (in root or storage tissue) and variously coloured chromoplasts (in petals and fruits). Plastids were first described by Leeuwenhoek some 300 years ago and the term plastid was used by Schimper in 1880's.

The chloroplasts are usually ellipsoidal or discoidal in shape. They measure about 5-10 μm long with a thickness of 2-3 μm. The number ranges from one in some algal cells to several hundreds in some cells of higher plants. Usually 20-500 chloroplasts are found in each photosynthetic cell of a leaf. Each chloroplast is bounded by a double membrane.

The chloroplast envelope consists of 2 membranes. Each membrane is reported to be about 6-10 nm thick. These two membranes are separated by an electron translucent space of about 1-20 nm in width. The outer membrane generally appears smooth, whereas, the inner membrane is frequently observed with finger like protrusions into the stroma. It is suggested that they are involved in the formation of internal membrane systems even though the chemical composition of the lamellae and envelope are quite different.

The internal structure of chloroplast (Fig.1.6) is a complex of membranes running through the matrix. The matrix which is also called stroma consists of a concentrated solution of proteins. All the enzymes necessary to convert carbon dioxide into sugars are also present in this matrix, and the most important enzyme found in large quantities is RuBP carboxylase. The membranes inside the chloroplast form into a system of thin sacs called lamellae. In some regions several sacs or lamellae are stacked on top of each other to form grana.

The grana are shown to be roughly cylindrical measuring about 0.2 to 0.4um in diameter. Generally 40-60 grana are found in each chloroplast of a photosynthetic cell. Each granum consists of stacks of flattened lamellae bounded sacs known as thylakoids. The intergranal lamellae are called frets. The whole internal membrane system is known as grana-fretwork system. The grana- fretwork system is now regarded as a single membrane continuum with structurally differentiated regions. The simplest granum consists of only two thylakoids, but generally there may be 10- 100 thylakoids in a single stack. The electron translucent region between two adjacent thylakoids is termed partition and the internal translucent regions enclosed by the thylakoids are termed loculi. The ends of the thylakoids in contact with the stroma are called margins and the thylakoids at each end of the stack are termed end granal membranes.

The stroma of chloroplasts also contain ribosomes, circular naked DNA, lipid globules and starch grains. Chloroplasts are the organelles which perform various photosynthetic reactions.

Some of the proteins are also synthesized in chloroplasts. These are also semiantanumous organelles like mitochondria.

Check Your Progress - 5

What is the important difference between the chloroplast, chromoplast and leucoplast?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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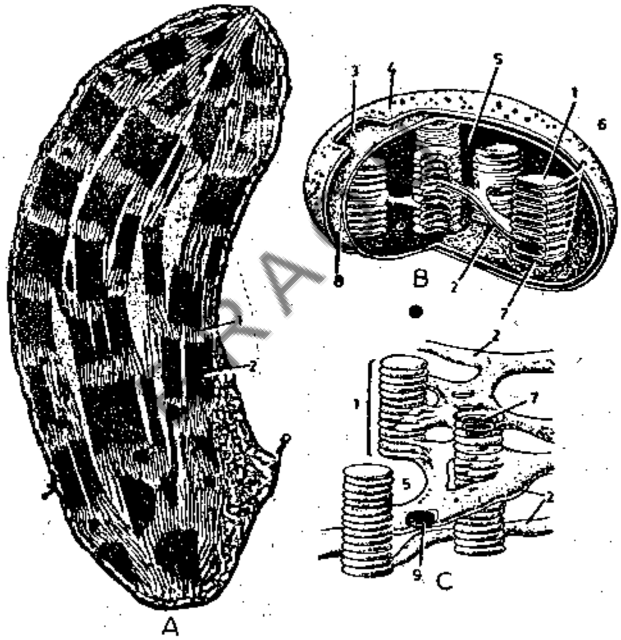

Fig.1.6. Chloroplast A. An election microscopic picture of chloroplast. B. Three dimentional section of a chloroplast. C. The arrangement of thylakoids and stromal lamellae inside chloroplast. 1. Granum. 2. Stromal lamellae. 3. Inner membrane. 4. Outer membrane. 5. Stroma. 6. Thylakoid. 7. Thylakoid compartment. 8. Inter membrane compartment. 9. Channel of stromal lamellae.

1.5.6. Lysosomes

Lysosomes are the membrane-bound vesicles that either bud off from the golgi apparatus or arise directly from the endoplasmic reticulum. Thse are dense bodies of about 400 nm in diameter. The lysosomes contain a variety of digestive enzymes. These enzymes are originally produced by ribosomes. The lysosomes are bound by a single layered membrane. The lysosomes are responsible for the intracellular digestion of materials taken up by endocytosis and also help in self digestion.

1.5.7. Microbodies

Microbodies are also called as peroxisomes or glyoxysomes. These are usually spherical even though some elongated, dumbbell shaped and irregular forms are reported microbodies usually measure about 0.2 to 1.5 μm in diameter.

These are believed to arise from the outgrowths of endoplasmic eticulum. Microbodies consist of a single smooth membrane surrounding a granular matrix. The matrix contain fibrillar, amorphous or crystalline inclusions. The presence of enzymes which catalyse the breakdown of hydrogen peroxide gives the name peroxisomes to these bodies. These are also involved in the conversion of fatty acids to carbohydrates via the glycoxylic acid cycle. So these are called glyoxysomes.

1.5.8. Microtubules and Microfilaments

A series of the fine tubules in the cytoplasm are reported from most of the cells. They measure about 23 cm in diameter, with an indefinite length. These are called microtubules (Fig.1.7). The microtubules are not surrounded by a membrane. Electron- microscopic sections show that the wall of the tubule is composed of repeating elements. In cross section 13 elements can be seen. These elements are suggested to be globular proteins.

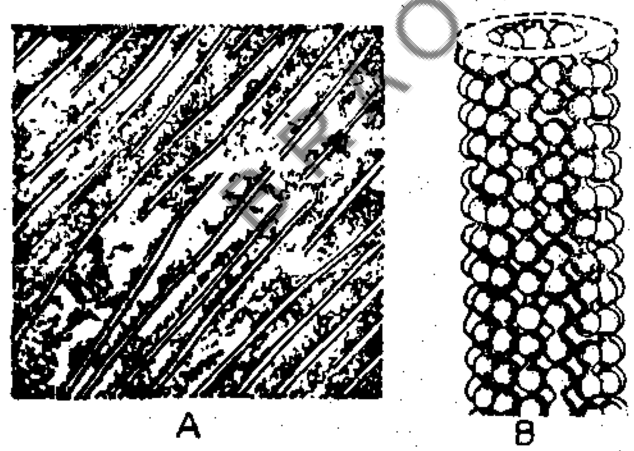

Fig.1.7. Microtubules A.Several microtubules. B. The probable arrangement of the subunits of a microtubule.

Microfilaments are narrower than microtubules. They measure about 5-8 nm in diameter. The microtubuses are responsible for various elastic properties of cells and the movement of cytoplasm and organelles and play an important role in the formation of a cellulose cell wall.

1.6. VACUOLES

The presence of vacuoles is a characteristic feature of all differentiated living plant cells. Vacuoles are usually absent in young meristematic cells. Even if they are present, they are very small. A mature plant cell consists of a single large vacuole occupying about 90% of the total cell volume. The vacuole is surrounded by a semipermeable membrane known as the tonoplast. The contents of the vacuole form the cell sap. The cell sap contains water, mineral ions, sugars, organic acids, amides and amino acids, gums, tannins, lipids, certain pigments and proteins. The proteins include both storage proteins and a variety of enzymes such as acid phosphatase, proteinase and RNase. Vacuoles may also contain crystalline material and inclusion bodies. The inclusion bodies may be simple vesicles or may contain degenerated ribosomes and mitochondria. Vacuoles usually serve as storage organelles for a variety of compounds. The presence of hydrolysing enzymes and degenerated cellular organelles suggests that they are digestive in function.

1.7. NUCLEUS

The nucleus was first described by Robert Brown in 1831. The eukaryotic cells contain a membrane bounded nucleus whereas the prokaryotic cells do not show a definite nucleus with a nuclear membrane. The nucleus is approximately spherical in shape and contains the major part of cellular DNA. The diameter of the nucleus is about 10μm.

1.7.1. Structure

The nucleus is surrounded by an envelop of two unit membranes. Each membrane is about 5-10 nm wide. These two membranes are separated by a gap known as perinuclear space. The width of this space varies between 10 and 60 nm. The envelope is observed with several pores and the diameter of each pore ranges between 10 and 40 nm. It is assumed that the pores are the channels of communication between the interior of the nucleus and cytoplasm. The outer membrane of the nucleus is frequently observed with ribosomes, and may be continuous with endoplasmic reticulum in some places.

Inside the nuclear membrane there is a dense fluid called nucleoplasm or karyolymph and a network of chromatic material. The chromatin material is dispersed throughout the nucleoplasm. During cell division the chromatin material is organised into a definite number of chromosomes. The chromatic material is formed of DNA and histones (proteins). Smaller amounts of acidic proteins and RNA are also reported.

One to four prominent, roughly spherical structures known as nucleoli are usually observed within the nucleus. The nucleoli are not bounded by a membrane. They contain a large proportion of cellular RNA and little of DNA. 70% of nucleolus is composed of proteins. It is now well established that the nucleoli are the sites of ribosomal RNA synthesis. During cell division the nucleolus disappears. Some specific chromosomes known as "Nucleolar chromosomes" are concerned with the reappearance of nucleolus at the end of mitosis.

1.7.2. Functions

The nucleus contains chromosomes which are the carriers of heriditary material, the DNA. Thus nucleus determines the characteristics of the individual organism. The nucleus also regulates the various metabolic processes through synthesis of specific proteins and enzymes. It also regulates the various developmental phases of the individual. Nucleus undergoes division during mitosis and meiosis and thus maintains the continuity of genetic material in all the cells.

1.8. SUMMARY

The cells are the basic units of all organisms. Each cell consists of a cell wall, plasma membrane, cytoplasm, endoplasmic reticulum, golgi apparatus, mitochondria, chloroplasts, lysosomes, nucleus etc. The cell wall is a rigid structure and serves both supportive and protective function. The plasma membrane is a differentially permeable membrane and it controls the entry and exit of some salts and water. This is made up of lipids and proteins. The fluid material in between the cell membrane and nuclear membrane is cytoplasm. Several organelles such as plastids, mitochondria, ribosomes, golgi apparatus, lysosomes etc. are preset in the cytoplasm. The interconnected tubular network-like structure is called endoplasmic reticulum. The endoplasmic reticulum with ribosomes on the outer surface is called rough or granular endoplasmic reticulum and without ribosomes is called smooth or agranular endoplasmic reticulum. Ribosomes are 80 S particles present freely in the cytoplasm or on the surfgace of the endoplasmic reticulum. The groups of ribosomes attached together are called polyribosomes or polysomes. Several dictyosomes are interconnected to form the golgi appratus. Each dictyosome consists of 1-30 sac-like cisternae. The golgi apparatus is secretive in function. Mitochondria are sphere-shaped or cylindrical bodies and these are the sites of chemical reactions of respiration. Chloroplasts are ellipsoidal or discoidal particles with an outer envelope and internal lamellar system. These organelles perform various photosynthetic reaction lysosomes are round bodies with a variety of digestic enzymes. The enzymes necessary for the breakdown of hydrogen peroxide and for the conversion of fatty acids to carbohydrates are present in microbodies or glyoxysomes. Microtubules measure about 23 nm in diameter with indefinite length and microfilaments measure about 5-8 nm in diameter. Vacuoles are usually absent in young cells and the mature cells contain a large single vacuole in the centre. The nucleus is about 10 μm in diameter and spherical in shape. The nucleus is bounded by a unit membrane in eukaryotic cells whereas it is absent in prokaryotic cells. It contains the major part of cellular DNA.

1.9. CHECK YOUR PROGRESS: MODEL ANSWERS

1. In the Singer and Nicolson Model of the plasma membrane two types of proteins, *viz.*, peripheral proteins and integral proteins are recognised. Peripheral proteins are present at the surface of the plasma membrane and they can be easily removed whereas the integral proteins are firmly incorporated within the lipid bilayer.

2. As many as 20 ribosomes join together and form clumps with the help of a messenger RNA. These are called polysomes or polyribosomes.

3. The dictyosome consists of a stack of 1-30 membranous flattened sacs. These are called cisternae. The cisternae pinch off several vesicles at the edges and they contain several proteins, cellulose, hemicellulose and pectic compounds.

4. Along the matrix side of the inner membrane of the mitochondria several regularly spaced stalked particles are found. These are called elementary particles or oxysomes. It consists of a head measuring about 8-10 nm in diameter and a stalk measuring about 4-6 nm long and 4 nm in diameter.

5. The main difference between the chloroplast, chromoplast and leucoplast lies in the colour. The chloroplasts are green in colour where as the leucoplasts and chromoplasts are colourless and variously coloured respectively.

1.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. Write an account on the structures and functions of the cell wall and plasma membrane with the help of diagrams.

2. Describe the structure and functions of endoplasmic reticulum with the help of diagram.

3. Describe the structure and functions of chondriosomes and chloroplast with the help of diagrams.
4. Describe the structure and functions of nucleus with the help of diagrams.

II. Answer the following questions in about 10 lines each.

1. Describe the structure and functions of the cell wall.
2. Describe the structure and functions of plasma membrane.
3. Describe the structure and functions of endoplasmic reticulum.
4. Describe the structure and functions of golgi complex.
5. Describe the structure and functions of mitochondria.
6. Describe the structure of chloroplast.
7. Describe the structure and functions of chloroplast.

UNIT - 2: TRANSPIRATION AND STOMATAL MOVEMENTS

Contents

2.1. OBJECTIVES

By the end of this unit you will be able to:

1. define transpiration in plants,
2. describe the different theories of ascent of sap,
3. describe the structure and distribution of stomata,
4. explain the stomatal opening and closing and its significance in transpiration,
5. list out and describe the different internal and external factors that affect transpiration and
6. define and describe the process of guttation.

2.2. INTRODUCTION

Of all the environmental factors that affect plant life, perhaps, the most important one is water. Almost all physiological activities need water. Transoprt of food materials inside the plant body needs water. In photosynthesis, water is one of the raw materials. It is estimated that land plants, on an average, require 500 Kgs of water for each kilogram of dry plant substance. The amount of water in a physiologically active plant ranges from 60% to 95%. Fresh fruits have large water content, e.g., apple 84%, banana 75%, grapes 82% etc. Relatively inactive parts like seeds, however, contain only 10-15% of water.

2.3. TRANSPIRATION

Water enters the plant body from soil through root hairs. Surprisingly, a very large percentage, nearly 98% of the water so absorbed by plants, is not utilised by plants, instead escapes into the atmosphere as water vapour. This process is called transpiration. It is estimated that a single maize plant may transpire about 2 litres of water a day and that an acre of maize may transpire more than 3,00,000 gallons of water in the growing season. This illustration shows how much of water escapes through plants from soil into the air.

So, transpiration may be defined as loss of water from the aerial parts of the plant body into atmosphere in the form of water vapour. Eventhough all aerial parts are involved in transpiration, primarily it is the leaf where the maximum amount of transpiration takes place. The leaves on their surfaces have small openings or pores called stomata through which water vapour escapes into the air. This kind of transpiration is called stomatal transpiration. Besides this, however, some water is lost through the cuticle present on the epidermis of the leaf. This is called cuticular transpiration. This may account for 5 to 10% of the total transpiration. In woody trees, transpiration takes place through the lenticels, present on stems. This is called lenticular transpiration.

Transpiration may resemble evaporation, wherein also water is lost in the form of vapour. The term evaporation is used for the process in which water escapes as water vapour from open water surface areas like rivers, lakes and seas. This is purely a physical process and is controlled by external factors only. Transpiration, on the other hand, occurs from living surfaces and is controlled not only by external factors but by internal factors of the plant body or vital factors.

Structurally the leaf is well suited for transpiration. Between the two epidermises of the leaf, there is the tissue, the mesophyll whose cells are thin walled and there is a lot of intercellular space between these cells. All these spaces together constitute a branched system of air passages which join the larger spaces lying behind the stomata called sub-stomatal chambers. The intercellular spaces make up to 15-40% of the total volume of the leaf.

Water that enters the roots in the soil travelling through the stem reaches the leaf. The upward movement of water from the roots to the tops of trees, is referred to as ascent of sap. Leaving aside small herbs and shrubs, the problem looks very perplexing when we consider trees like *Sequioa* or *Eucalyptus* or a pine which are about 300 feet in height. To transport water vertically to such great heights, a pressure of nearly 10 atmospheres is necessary. The problem then is to consider the forces involved in this remarkable feat.

The pathway for the soil solution inside the plant body is the lumen of xylem vessels. This can easily be demonstrated by removing all the tissues outside the xylem in any branch of a plant and observing whether the leaves lose turgidity. No such loss will be observed. That means the leaves continue to get water and xylem must be the channel for it.

So when the problem of pathway for water movement is settled, the real question is what is the motive force that propels the water to move upwards. There are many theories trying to explain this phenomenon. These are classified as vital theories and physical theories. The supporters of vital theories look upon the pressure of living parenchyma cells as primarry cause for the ascent of sap. On the other hand, those supporting the physical theories deny any such primary role to the living cells and contend that the ascent of sap is purely a physical process in which living cells have no role to play.

2.3.1. Root Pressure

In certain species like grapes when stems are cut at the top specially in spring season, xylem sap exudes from the cut ends. This exudation is attributed to the development of pressure in the sap in the xylem. Activity of living cells is responsible for this exudation and this is called root pressure. Even though root pressure does exist, this may just be sufficient to force a column of water in the vessels to a maximum height of 10 metres only. So this is inadequate to explain water movement in all trees.

Check Your Progress - 1

What is meant by ascent of sap?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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2.3.2. Vital Theories to Explain Ascent of Sap

Westermaier, Godlewski and J.C. Bose are some who vehemently supported vital theories. Westermaier thought that the upward movement of water is effected in the xylem parenchyma while the vessels and tracheids serve as mere reservoirs of water. J.C.Bose was of the opinion that pulsatory activity of the living cells adjoining xylem vessels cause the ascent of sap in plants. The innermost layer of the cortex in dicotyledonous plants shows great deal of pulsatory activity which Bose considered as responsible for ascent of sap.

These views are no more acceptable now, because it is conclusively proved that water moves to the top of plants even when all living cells are killed.

2.3.3. Physical Theories to Explain Ascent of Sap

There are two theories viz., capillary theory and cohesion theory that are proposed to explain the upward movement of water in plants.

Capillary Theory: This hypothesis states that the upward water movement in plants is due to capillary forces. But capillary forces may be sufficient to raise water to a short distance in the stem but cannot account for water rise in trees even of average height. Surprisingly it is now established that vessels of spring wood which are broader than those of autumn wood, conduct more water. As a matter of fact, this evidence refutes the validity of capillary theory.

Cohesion Theory: This theory was first proposed in 1894 and 1895 and subsequently elaborated by Dixon in Great Britain and Renner in Germany early in the 20th Century. Ascent of sap according to this theory, takes place as follows. As transpiration takes place, water is lost from the walls of the mesophyll cells of the leaves. As a result of it, saturation deficit occurs in the cell walls. This makes the cell walls draw water from the cytoplasm of the mesophyll cells. In its turn cytoplasm draws water from the vacuole. In this manner, saturation deficit gets transferred from cell to cell in all the leaves until it finally acts as a cumulative suction pressure on the xylem at the apical part of the stem, which again is transferred to the root system. Thus the movement of water inside the plant is due to the tendency to make good the saturation deficit. Really what causes this quick flow of water from root end to the leaf is the pulling force set in motion by transpiration. Thus transpiration pull is the main force for lifting the water. While moving upwards there is no danger for the continuity of water columns as water molecules within a column are joined to one another by very high forces of mutual attraction, the cohesive forces. Added to this is the adhesion of water molecules to the molecules of the wall lining the xylem which prevents the water filaments from being defected from the vessel walls, thus making their rupture more difficult. Thus the cohesive and adhesive forces of the water molecules amongst themselves and to the wall of the xylem respectively, enable the transpiration pull to lift water as one would pull a cable or wire from the top of a building. Air bubbles may form but do not cause an unsurmountable obstacle, as they dissolve in water in many cases.

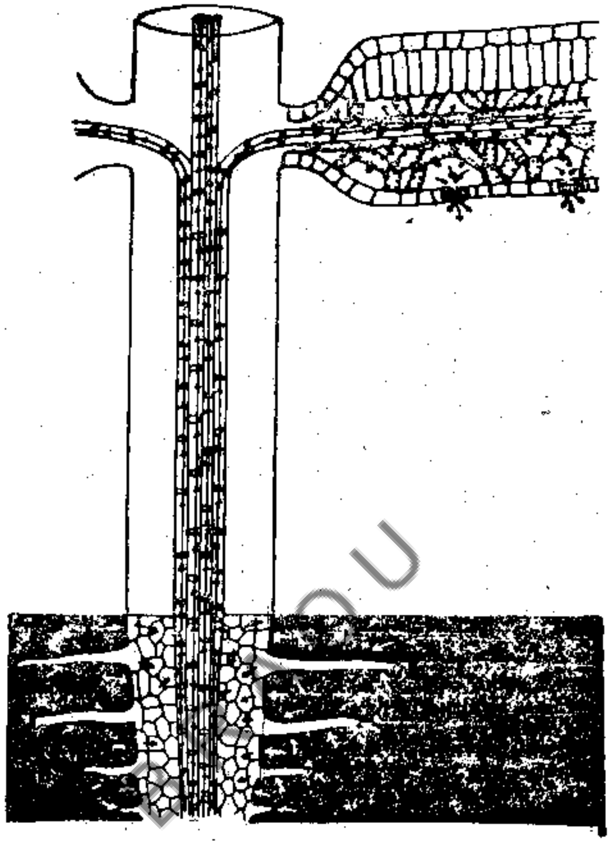

Fig.2.1. Diagram showing the passage of water from the soil to the leaves and into the air. Water enters through the root hairs and other surfaces of the young root (The path of water is shown by arrows.)

Thus the possibility of living cells contributing substantially to the upward movement of water in plant is quite remote. The theory that water ascends in the xylem of plants largely under the influence of force of transpiration from leaf surface, therefore, seems to be probably the correct explanation.

Inside the leaf, travelling through the xylem vessels present in the veins, water ultimately reaches its destination, the mesophyll cells. The cell sap, cytoplasm and the cell walls of the cells of mesophyll get saturated with water. During transpiration, water from the cell walls evaporates into the intercellular spaces of the mesophyll and from them into the stomatal chamber. Finally through the pore the vapour escapes into the atmosphere (Fig.2.1)

2.3.4. Structure of Stomata

These are lens-shaped openings present in the epidermis. These openings lead to intercellular spaces inside the leaf. In reality, this pore is an intercellular space caused by the separation of two specialised epidermal cells called guard cells (Fig.2.2). These specialised epidermal cells differ from the other epidermal cells, in that these contain chloroplasts and in surface view are kidney shaped. These guard cells are so arranged that the concave walls of these kidney shaped cells face each other, due to which the pore is formed. The cell wall of the guard cell is differentially thickened. The wall facing the pore is very much thickened, while the wall away from the pore is normal. It is through this pore that water vapour escapes into the atmosphere. In some plants guard cells are accompanied by epidermal cells which are different from the rest and are called subsidiary cells.

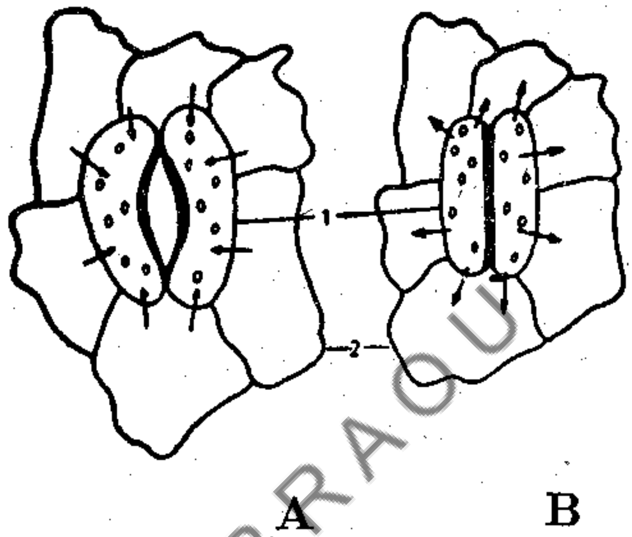

Fig.2.2. Opening (A) and Closing (B) of Stomata. Arrows show the direction of water movement. 1. Guard cells. 2. Epidermis.

Check Your Progress - 2

What are guard cells?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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2.3.5. Distribution of Stomata

Generally the stomata are seen in larger numbers on the lower side of the leaf than on the upperside as in *phaseolus vulgaris* and *Cucurbita pepo*. In some, as in apple, oak and orange, stomata are seen confined only to the lower surface. In plants like maize, they are almost equally distributed on both the surfaces. The number of stomata per square inch of the upper and lower epidermises is given in Table-1.

Table 1 : Distribution of stomata per square inche (S I Unit) of leaf surface.

Plant	Upper epidermis	Lower epidermis
Apple (*Pyrus malus*)	none	2,50,000
Bean (*Phaseolus vulgaris*)	26,000	1,60,000
Maize (*Zea mays*)	39,000	64,000
Oak (*Quercus suber*)	none	3,75,000
Orange (*Citrus sinensis*)	none	2,90,000
Pumpkin (*Cucurbita pepo*)	18,000	1,75,000
Sunflower (*Helianthus annus*)	55,000	1,00,000

When fully opened, the maximum width of the pore is 3 to 12 μm and the length is 10 to 40 μm. The number of stomata per unit area of the leaf is called stomatal frequency. Stomatal index is defined as the percentage number of stomata as compared to all the epidermal cells including stomata in a unit area

$$I = \frac{S}{S+E} X\ 100$$

Here, I=stomatal index, S=Number of stomata, E=number of epidermal cells per unit area. Eventhough it may appear from the above table that there are so many stomata in a leaf, the total pore area, when all the stomata are fully open is 1 to 2 percent of the total leaf area.

2.3.6. Methods of Measuring Transpiration

There are several methods to measure the rate of transpiration. Three methods are described below.

1. Weighing method: This is a gravimetric method. In this method, a potted intact plant is selected and it is watered thoroughly. Then the pot and root system are covered by metal containers or metal sheet or a sheet of polythene to ensure that no evaporation takes place from them and that whatever water is lost is only from the aerial parts of the potted plant. Now the pot is weighed at regular intervals. The differences in weight is due to transpiration only. The rate of transpiration is usually expressed as grams per unit area per hour. In arriving at the total leaf area, care must be taken to see whether the stomata are confined to only one side or present on both sides. Any change in weight due to photosynthesis and respiration will be negligible.

2. Volumetric Method: This is also called potometer method. This particular potometer is known as Ganong's potometer (Fig.2.3). This apparatus is made up of a glass tube bent upwards at one end and downwards at the other end. A portion of the potometer is a narrow capillary tube, with known diameter and a scale on it. It is bent down with a small hole at its bulbous end. The other arm which is bent upwards has a stopper at the base of a water reservoir. A twig of a mesophyte is cut under water to avoid entry of air bubbles into the stem and is inserted into another arm (potometer well) of the potometer with the help of a rubber stopper. The entire potometer is filled with water and is made air tight. The bulbous end with a hole is kept in a beaker with coloured water. After a while an air bubble is introduced through the hole in the bulbous end of the capillary tube. As transpiration proceeds, the air bubble moves forward in the graduated horizontal tube. By noting the time taken for the air bubble to travel the capillary tube, whose diameter is known, the amount of water transpired can be calculated. In this experiment, it is presumed, that the amount of water transpired is equal to the amount absorbed.

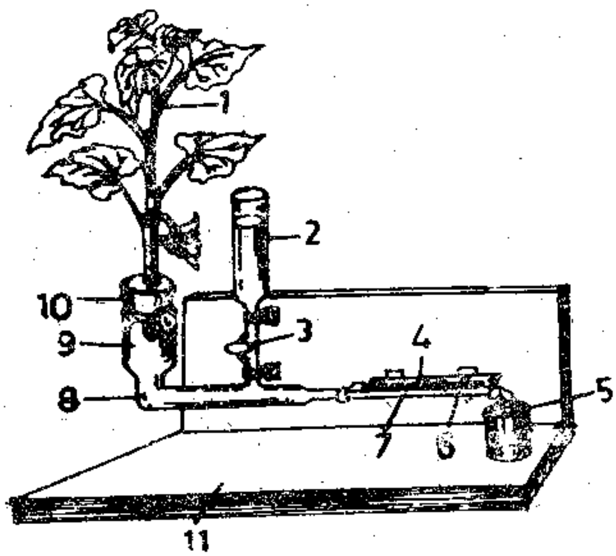

Fig. 2.3. A potometer used for measuring transpiration 1. Transpiring platn. 2. Water reservoir. 3. Stopcock. 4. Millimeter ruler. 5. Water level during readings. 6. Capillary. 7. Bubble. 8. Water filled system. 9. Potometer well. 10. Rubber stopper. 11. Wooden potometer support and rack.

3. Cobalt Chloride Method: Filter paper is dipped in 3 percent cobalt chloride solution. This is then dried in hot air oven. This filter paper will be blue in dry condition and pink when moist. Bits of dried cobalt paper are placed on the leaf surfaces and to avoid entry of atmospheric moisture, glass plates or mica sheets are used. X-ray films are placed upon the filter paper bits and held securely.

The blue colour changes into pink. In some plants this change takes place quickly while in some slowly. So this method is useful in comparing the transpiration rates of different plants.

2.4. STOMATAL MOVEMENTS

Generally in many plants stomata open during day time i.e., in the light and close in the night (darkness). However, in certain plants having CAM (Crassulacean acid metabolism) type of photosynthesis, stomata are open during night and closed during day. The movement of the stomata is due to entry and exit of water in and out of the guard cells.

Guard cells have all the organelles like mitochondria, nucleus, peroxisomes, ribosomes, spherosomes, endosplasmic reticulum and chloroplasts. These chloroplasts have prominent grana, and accumulate starch. The other epidermal cells surrounding the guard cells have less number of organelles and no chloroplasts at all. The amount of starch in the guard cells increases slowly in the night and decreases in the day. Protoplasmic threads, the plasmodesmata, connect the guard cells with the surrounding epidermal cells, but no such connections exist between cells of mesophyll and guard cells.

During day time, water enters the guard cells from the surrounding epidermal cells and reaches the vacuoles of the guard cells. This results in the increase in the turgidity of the guard cells. This brings about changes in the volume and shape of the guard cells. The wall of the guard cell, away from the pore, as a result of increased turgidity bulges out, thus pulling away the thickened walls surrounding the pore. As the two guard cells thus separate out, the pore increases in size. Thus the stomata open. However, when water gets out of the vacuole of guard cells, turgidity falls, the outer bulged wall moves forward and so thickened cell walls also come close to each other. Thus stomata get closed. This happens in the night. In this context, it should be noted that when compared to other epidermal cells guard cells are small in size.

and metabolically more active and have a special ability to undergo the rapid changes due to turgidity.

Many plant physiologists tried to explain the principles underlying the stomatal movements. The fundamental fact accepted by all is that stomatal movements are caused due to changes in the turgor pressure of the cells. The forces at work which bring about these changes are interpreted differently.

2.4.1. Starch-Sugar Hypothesis

Three scientists are associated with this hypothesis. They are Lloyd, Loftfied and Sayre. During day time, CO_2 concentration in guard cells is low as CO_2 is consumed in photosynthesis and this results in the increase of the pH value of the cells. This high pH value favours hydrolysis of starch into sugars, which is osmotically active and thus there is an increase in the turgidity of the cells. Thus guard cells move apart and so stomata opens. In the darkness, the CO_2 concentration increases as a result of respiration in the guard cells. This results in a fall of pH value in the guard cells, thus lowering the turgor pressure, and so the guard cells get nearer closing the stomata. The enzyme phosphorylase present in the chloroplast is said to catalyse the conversion of starch into sugar in the presence of inorganic phosphate.

$$\text{Starch+Inorganic phosphate} \underset{\text{pH 5}}{\overset{\text{Phosphorylase}}{\rightleftharpoons}} \text{Glucose-1-phosphate}$$

This hypothesis is not acceptable now, because firstly there are some plants in which no starch is present at all in guard cells e.g.,*Allium;* secondly there are plants with variegated leaves and etiolated leaves, where guard cells do not contain chloroplasts and thirdly the conversion of starch into sugars is very slow and thus cannot explain the quick stomatal movements.

2.4.2. Modern View

This is called theory of ion fluxes. This was first proposed by Fujino in 1967. The guard cells, in opened condition has higher concentration of potassium (K^+) ions. The epidermal cells and subsidiary cells, when present are the store houses of K^+ ions. The entry of K^+ ions in large numbers into the guard cell, increases its osmotic potential. This results in the rise of turgor pressure of the cell and thus stomata open. There is a large experimental evidence for this migration of K^+ into guard cells from the epidermal cells. It is also demonstrated that during day time, guard cells synthesise organic acids, specially malic acid. In the cytoplasm of the guard cells, malic acid dissociates into hydrogen ions (H^+) and malate ions. The hydrogen ions move out of the guard cells into the surrounding epidermal cells while K^+ move into the guard cells, thus maintaining electroneutrality. The malate ions inside the guard cells balance the excess K^+ ions. The organic acids could be produced in many ways,i.e. starch hydrolysis, carboxylation of phosphoenol pyruvate or through conversion of glycolate. Thus the influx of K^+ follows the synthesis of organic acids.

The potassium malate so formed in the guard cells moves into the vacuoles of guard cells. This results in increasing the osmotic potential of the guard cells. So water moves into the guard cells from the epidermal cells. This results in the increase of turgor pressure of the guard cells and the stomata open.

The sequence of events as light falls on leaf is given below

Light → Malic acid production → Dissociation of malic acid into hydrogen and malate ions → Inflow of K+ and outflow of H+ through plasma membranes
↓
Stomata open ← Turgor pressure increases ← Osmosis of water from surrounding epidermal cells ← Transport of potassium malate into vacuoles of guard cells

Another important factor in the modern concept is that the transport of potassium ions into the guard cells is an active mechanism and that ATP is supposed to provide the necessary energy for this transport. Considerable evidence is there to support this. The studies of Ramadas and Raghavendra (1976) have demonstrated that when guard cells receive light photophosphorylation takes places in the chloroplasts of the guard cells as a result of which ATP is produced. The necessity of light, for stomatal opening perhaps is more for ATP synthesis rather than for carbon assimilation.

Thus, according to modern view the opening of guard cells is due to the entry of potassium ions into the guard cells, finally reaching the cell sap in the form of potassium malate. This increases the osmotic potential of the guard cells and turgor pressure and leads to stomatal opening. The entry of K^+ ions is an active mechanism and needs energy, which is supplied in the form of ATP, which is produced in the phtophosphorylation that takes place in the guard cells.

Now, with the onset of darkness all these changes in guard cells take place in the opposite direction. K^+ ions are now transported out of guard cells into the surrounding epidermal cells by hydrogen-potassium ion exchange pump and the organic acids are consumed in the metabolic process. As a consequence water gets out of the guard cells whose turgor pressure comes down and the stomata get closed.

2.5. FACTORS AFFECTING TRANSPIRATION

The rate of transpiration is influenced by internal factors as well as external factors. Root-stem ratio, leaf area and structure are examples of internal factors while light, temperature, wind and availability of water are the external factors.

2.5.1. Internal Factors

Root-Shoot ratio: Rate of transpiration increases with an increase in the ratio of root and shoot provided the other conditions are favourable. *Sorghum's* root system is more extensive than that of maize. So the rate of transpiration per unit area of leaf is more in sorghum than in maize.

Leaf area: The greater the leaf area the higher is the loss of water, eventhough there is no proportionality between them. A big plant may lose more water but the rate of transpiration by a smaller plant may be greater per unit area. When leaves of any plant are removed, the total water lost may be less, but rate of transpiration per unit area will be more.

Leaf Structure: Plants growing in dry area show many adaptations like thick cuticle, sunken stomata, well developed palisade and hairs on the epidermis. All these adaptations are to reduce the rate of transpiration. Under dry conditions mostly cuticular transpiration takes part.

2.5.2. External Factors

Light: as already stated light makes stomata open, thus increasing transpiration. Due to light the temperature of the leaf rises. This accelerates evaporation of water from the saturated cell walls of the mesophyll cells into the intercellular spaces of the leaf and finally into the stmosphere. Thus light increases rate of transpiration.

Temperature: We have already studied that at extremes of temperature of O^oC and $30\text{-}35^oC$ and above, stomata close and so reduce transpiration. A rise in temperature of the atmosphere upto $25\text{-}30^oC$ increases the rate of transpiration.

Wind: As moist air above the leaf is removed and replaced by drier air, wind increases the rate of transpiration. When plants are exposed to sudden wind currents, there will be sudden increase in transpiration rate, but it is followed by a sudden fall. This is due to the cooling effect brought on to the evaporating surfaces. Also, it should be noted that guard cells get closed in strong wind currents.

Availability of Water: If adequate supply of water is not available, there will be fall in transpiration rate and if this situation continues, it will result in wilting.

Atmospheric Pressure: Transpiration and atmospheric pressure are inversely proportional. If there is a reduction in atmospheric pressure, say from 1 atm to 1/2 atm, the rate of transpiration gets doubled. This is the reason why transpiration rate is increased at mountain tops as the atmospheric pressure there is less.

Antitranspirants: For the last few years, research has been going on chemical substances, which when applied to plants, reduce the rate of trnspiration without being injurious to the plant in any other way. The term 'antitranspirant, is used for those chemicals which when applied, retard transpiration.

Colourless plastics, silicone oils and low-viscosity waxes are one kind of antitranspirants used. When these are sprayed they form thin films on the leaf surface, which is permeable to oxygen and carbon dioxide but not to water.

Another group of antitranspirants are those which when applied bring out stomatal closure. Phenyl mercuric acetate, a fungicide and salicylic acid are examples of this type. When applied as a foliar spray at a concentration of 10^{-4}M, phenyle mercuric acetate has been found to be very effective. But there are reports that this substance has certain toxic effects on fruits, vegetables and also it interferes with photophosphorylation. A plant growth regulator, abscisic acid is also used to induce stomatal closure.

By using antitranspirants, it is possible to save the crops under drought conditions without the yield being decreased and to keep fruits and cut flowers maintain their freshness at least temporarily.

2.6. GUTTATION

In transpiration, water is lost from the plants as water vapour. But sometimes, water is also lost from plants in liquid state. This process is called guttation. In early summer, it is a common sight to observe early in the morning, water drops at the tips on the grass blades on the margins of leaves of some herbaceous plants like potato, tomato, nasturtium etc. However, this should not be confused with dew. Dew may be present as thin layer on the leaves, whereas in guttation these drops are seen on tips or on the margins of leaves. It is reported that one single leaf of *Colocasia*, oozed half a cup of water through guttation in one single night.

Guttation is commonly seen when absorption by roots is more rapid while the conditions for rapid transpiration are not favourable. This is the result of active absorption of water by roots and development of root pressure.

At the margins and tips of leaves in plants where guttation is seen, there are special stomata present. These are called hydathodes or water stomata (Fig.2.4). The hydathode is surrounded by a group of thin walled parenchyma cells. These are guard cells but they do not show any movement. So hydathode remains open always. Below the pore there are loosely held parenchyma cells which are colourless, called *epithem*. The xylem endlings of veins are seen below the epithem. During nights when there is more absorption of water and less transpiration, xylem sap is forced out of the hydathodes.

Thus drops of water are formed at the tips and margins of leaf. These drops contain organic and inorganic solutes. These drops may get evaporated or may be absorbed. Guttation can be demonstrated by covering a well watered potted plant. e.g., seedlings of maize or barley with bell jar.

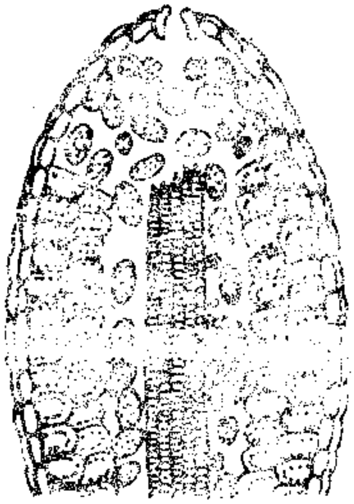

Fig. 2.4 V.S. of leaf margin showing hydathode (Note apical pore with guard cell.)

Check Your Progress - 3

Define guttation

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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2.7. SIGNIFICANCE OF TRANSPIRATION

As already stated plants transpire large quantities of water absorbed from the soil, utilising hardly 2 percent of the water for the metabolism. The question is whether transpiration is beneficial to plants, or not.

According to some, transpiration is necessary for water movement in plants. But there is enough evidence that even in the absence of transpiration, upward movement of water does take place. The flow of water through the plant induced by transpiration provides a transport system for minerals from the soil. The ions are taken up by plants both actively and passively. Active transport of ion is brought about by utilising energy. On the other hand passive transport does not involve energy. The rate at which ions are absorbed by plant roots and translocated to the shoots is altered by the rate of transpiration. When once the ions reach the xylem, transpiration speeds up the ion transport. A number of investigators have found that transpiration increases the quantity of ions reaching the plant shoots, irrespective of the ion uptake being active or passive. Although the role of transpiration in increasing the absorption is controversial, it is well established that larger amounts of potassium and phosphate ions get into the plants as a result of transpiration.

One of the most important effects of transpiration is the inevitable decrease of water in the tissues of the plant particularly when absorption is not rapid. Even in the well-watered soils, continuous transpiration depletes the soil water and causes desiccation. This is serious in young seedlings in which roots do not grow deeper.

One inevitable fact is, gaseous exchange must take place throughout the life of the plant and for this stomata must remain open. So long stomata are not closed, transpiration continues to take place even though the air is saturated with water vapour. So transpiration is an inevitable process in plants, which, in excess, is definitely dangerous to plants. Some consider this as inevitable evil.

2.8. SUMMARY

Water absorbed by roots is transported through the xylem of roots and stem to the leaves. This is called ascent of sap. Of the several theories, cohesion theory explains this process better. Although a large amount of water is absorbed by the plant only a fraction of it is utilised by them. The bulk of water is lost from the plant in the form of water vapour through small pores on the leaves called stomata. This is called transpiration. The process of transpiration regulated by stomatal opening is different from evaporation of water which is purely a physical process. Stomata are small pores, a few microns in length and about 1-2M (Microns) in width, found in the epidermis of the leaves. These pores surrounded by guard cells are different from other epiderrmal cells in structure and function. Stomata are distributed mostly on the lower epidermis than on the upper epidermis. In certain plants the stomata may be equally distributed on both surfaces. In submerged water plants, there are no stomata. The amount of transpiration can be determined by observing the loss of weight in a potted plant, during a period of transpiration. The rate of transpiration is measured more accurately by a volumetric method involving the use of a simple apparatus called potometer. The rates of transpiration on either side of the leaves, or in different plants can be measured by a rough method called cobalt-chloride method. Mechanisms of stomatal opening and closing include Sayre's starch-sugar conversion hypothesis. This is an old one and it is no longer accepted. The recent theories include ion fluxes mediated by energy. The potassium ions enter the guard cells at the expense of ATP and increase turgidity of cells, causing the stomata open. During light, photophosphorylation in the chloroplasts of guard cells generates ATP. Both internal and external factors affect transpiration, the internal factors include (a) root/shoot ratio (b) leaf area and (c) the number of stomata present in the leaves and the cuticle. External factors include (a) light (b) temperature (c) wind velocity (d) water availability (e) atmospheric pressure. Guttation is the loss of water in liquid form through specialised 'hydathodes'. Significance of transpiration is that it is a necessary evil as gas exchange is necessary for the operation of photosynthesis and respiration also takes place through stomata. Certain ions are taken up by the plants in larger quantities due to transpiration.

2.9. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The upward movement of water from the roots to the top of trees is called ascent of sap.
2. The specialised chlorophyll containing kidney shaped cells that surrounded the lens-shaped openings in the epidermis of leaves is called guard cells.
3. Usually plants loose water in the form of water wapour in transpiration. But some times the water is lost in a liquid state through hydathodes. This process of losing water in the form of liquid is called guttation.

2.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. How are the stomatal movements explained?

2. What are the factors affecting transpiration? Explain each one of them.

3. Describe the behaviour of stomata in succulent plants.

II. Answer the following questions in about 10 lines each.

1. Define transpiration. How does it differ from evaporation?

2. Describe the structure of stomata and add a note on their distribution.

3. What is the significance of transpiration?

UNIT- 3: MACRO AND MICRO-NUTRIENTS

Contents

3.1. OBJECTIVES

By the end of this unit you will be able to:

1. explain the historical account of macro and micro-elements,
2. list out the various elements that are present in the plant body,
3. describe the criteria for the essentiality of the elements,
4. describe the role of various macro- and micro-elements in plant nutrition and also the deficiency symptoms of them.
5. list out and describe various methods of studying plant nutrition, and
6. list out the indicator plants which will indicate the presence of certain minerals in the soil.

3.2. INTRODUCTION

Plants, with a few exceptions like bacteria and fungi, are autotrophic, while all animals without exception are heterotrophic. The plants are autotrophic because they have chlorophyll in their cells and with the help of chlorophyll they manufacture complex organic substances they require, making use of simple inorganic compounds and radiant energy from the sunlight. With the exception of carbon, oxygen and hydrogen, plants get all the required inorganic substances from the soil. Hence this inorganic nutrition is also called mineral nutrition.

But the actual mineral requirements of plants became known only after the results of water culture experiments were made known. In these experiments plants are grown in containers filled with nutrient solution medium. About this we learn a little later in detail. Around the year 1860 Sachs, a great German Botanist, made a solution of different mineral salts required by plants in different proportions and cultivated plants in that nutrient medium. Similar experriments were carried out by scientists like Knop and Pfeffer using mineral salts in different proportions. They cultivated different plants in these nutrient solution media. In these media three salts i.e., calcium nitrate, potassium dihydrogen phosphate, magnesium sulphate are available in higher proportion, while iron salts are available in small proportion. While some used sodium nitrate and others substituted this by ammonium nitrate. Thus by the end of 19th century it was established that there are ten elements which are necessary for the growth of higher plants. These are carbon, hydrogen, oxygen, phosphorus, potassium, calcium, magnesium, nitrogen, sulphur and iron. Besides these elements, some more elements, are required by plants for their normal healthy growth. This fact came to light due to the extensive researches of some scientists like Warrington and Brenchley of U.K. and Hoagland, Stout and Arnon of the U.S.A. However these elements are needed by plants only in very small amounts. These elements are boron, zinc, manganese, copper, molybdenum and chlorine. Thus a total of 16 elements mentioned above are found to be necessary for the healthy growth of higher plants. Besides these elements, there are instances where certain species are found to require other elements also. For example, turnips and beet roots are seen to grow well in the presence of sodium. Likewise silicon, an element abundant in monocots, and *Equisetum*, seems to stimulate the growth of several plants. The mere fact that an element is present in a particular plant need not necessarily mean that it is essential for the plant.

3.3. CHEMICAL ANALYSIS OF PLANTS

The fact that there are several elements in the plant body can also be demonstrated by chemical analysis. In this method, the plant body is heated to approximately $100^{o}C$ for several hours in an oven, when the water in the plant is driven away. The remainder is called dry matter. Generally the amount of water in plants varies from 10% to 95%. This value varies not only between different species but also between different parts of the same plant body. Thus seeds contain minimum amount of water while some succulent plants contain as high as 95%. The dry matter contains both organic and inorganic compounds. A large part of dry matter, nearly 90% is made up of organic compounds such as carbohydrates, proteins, aminoacids etc. When the dry matter is heated upto 600^{o}-$700^{o}C$ then the organic matter decomposes and comes out as carbondioxide, sulphur, water and phosphorous. The nonvolatile residue is the one that is made up of inorganic compounds. An analysis of this residue, known as ash, shows the presence of potasssium, magnesium, calcium, aluminium and iron. This composition varies with species, age and environment.

Table-1: Elements in a plant body, their ranges and their form.

Sl. No.	Element	Range in the plant	Form in which it is available to plants
1.	Carbon	45 percent	CO_2, HCO_3
2.	Oxygen	43 percent	O_2, H_2O
3.	Hydrogen	6 percent	H_2O
4.	Nitrogen	1-3 percent	NO_3^-, NH_4^+
5.	Potassium	0.3-6 percent	K^+
6.	Calcium	0.1-3.5 percent	Ca^{++}
7.	Sulphur	0.05-1.5 percent	SO_4^{+}
8.	Phosphorous	0.05-1 percent	H_2PO_4, HPO_4
9.	Magnesium	0.05-0.7 percent	Mg^{++}
10.	Chlorine	100-300 ppm	Cl^-
11.	Iron	10-1500 ppm	Fe^{+++}, Fe^{++}
12.	Manganese	5-1500 ppm	Mn^{++}
13.	Zinc	3-150 ppm	Zn^{++}
14.	Copper	2-75 ppm	Cu^+, Cu^{++}
15.	Boron	2-75 ppm	BO_3^{--}, $B_4O_7^{--}$
16.	Molybdenum	trace	MoO_4

3.4. CRITERIA FOR ESSENTIALITY OF ELEMENTS

In 1939 two American Scientists, Daniel Arnon and Perry Stout, made important contributions to the studies on mineral nutrition. They conducted several experiments on tomato and its nutritional needs. They introduced three criteria to establish the essentiality of an element. They are:

1. The element must be essential for the normal growth and reproduction of the plant and in its absence growth and reproduction should be affected.
2. The requirement must be specific and that no other element can act as a substitute for it.
3. The action of the element in the plant must be direct but not simply causing some other element to be more readily available or simply antagonising a toxic effect of another element.

These essential elements required by plants are arbitrarily divided into macro-nutrients and micro-nutrients. The former are required by plants in relatively large amounts and the latter in smaller amounts. The elements carbon, hydrogen, oxygen, nitrogen, phosphorous, calcium, sulphur, potassium and magnesium are regarded as macro-nutrients. Chlorine, iron, manganese, boron, zinc, copper, molybdenum are called micro-nutrients.

Out of the 9 macro-nutrients, carbon, hydrogen, and oxygen are the 3 elements which are taken in by plants in very large amounts. These three constitute nearly 90-94 percent of all the elements in a plant body. These enter the plant as water and as carbon dioxide. The former enters from the soil into the plant body. Carbon dioxide is supplied by air. Eventhough water is not considered as a nutrient, it is indispensable for many biological activities. As a universal solvent it is the medium of transport inside the plant. It is a constituent of many large organic molecules in the plant. Most important of all is the fact that the hydrogen required in photosynthesis comes from water. Carbon enters as carbon dioxide. Without this element there will be no biological molecules. Without these three elements there is no life.

Check Your Progress - 1

What are the three important elements that are taken in large quantities by the plant?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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3.5. ROLE OF ELEMENTS IN PLANT NUTRITION

When a certain element is not available in the soil, plants develop certain deficiency symptoms characteristic to it, from which it should be possible to determine the role of the element in plant nutrition. Sometimes these symptoms may be due to absence of more than one element. This kind of situation occurs in alkaline soils. In such cases, it would be rather difficult to determine the element concerned. We are now dealing with deficiency symptoms found on aerial parts only. The deficiency symptoms may be present in root system also. But as it is underground, it would be difficult to observe.

3.5.1. Macro-Nutrients

Nitrogen: This is available in the form of nitrates and ammonium salts in the soils. Of all the deficiencies, nitrogen deficiency is very common in many soils. The parent material from which the soils are formed do not contain nitrogen. Whatever nitrates or ammonium salts are present in the soil are easily lost, both through leaching by rain water in the soil or by conversion of it into nitrogen gas by micro-organisms. Thus the soils become poor in nitrogen.

Nitrogen is a constituent of a number of organic compounds like amino acids, proteins, co-enzymes etc. Chlorophyll unique to the plant kingdom has in its molecule 4 nitrogen atoms. In many metabolic processes nitrogen has a role.

When nitrogen is present in very small amounts in the soil, the plant growth is arrested. When the nitrogen is insufficient the leaves of such a plant turn to yellow colour. This to called chlorosis. This is due to lack of chlorophyll. This condition is seen specially in older leaves. In extreme cases, such leaves fall off. In such plants young leaves remain green, because soluble nitrogen would be transported to them from the older leaves below. In plants like tomato, maize, nitrogen deficiency results in purplish colouration on the stems, petioles and lower leaf surfaces. This is due to accumulation of anthocyanin pigments.

When nitrogen is available in abundance in the soil, plants show excessive vegetative growth and the plants appear dark green. But the root system is poorly developed. Potato plants grown in such conditions show the same features and tubers formed are small. In several crop plants excessive nitrogen retards flowering and seed formation. However, there are certain plants where abundance of nitrogen favours flowering.

Phosphorous: This element enters the plant as phosphate ion. This is then converted into organic form. Molecules containing phosphorous are many like sugar phosphates, nucleotides, nucleic acids, and coenzymes like NADP and energy-rich compounds like ATP and ADP. Thus phosphorous plays an important role in photosynthesis, respiration, and protein synthesis. Also as a constituent of DNA and RNA, phosphorous has a role in heredity.

Deficiency of phosphorous results in stunted growth but such plants are green in colour in contrast to nitrogen deficiency. Sometimes this may result in the accumulation of anthocyanin pigments. Maturity of the plant may be delayed. In maize and tomato phosphorous deficiency leads to purpling of leaves on the lower sides near the veins.

Phospates are easily transported inside the plant body. It is easily lost from old leaves to young leaves and growing parts. As a result the symptoms of deficiency are first seen in the old leaves.

Potassium: Eventhough this element is present in large quantities, in the soil most of it is in a form not suitable for plant intake. Eventhough plants take this element, in large amounts this does not form a stable part of any compound as is the case with nitrogen and phosphorous. Many life processes like respiration, protein synthesis, Photosynthesis and transpiration are dependent upon potassium. Protein synthesis is known to require large amounts of potassium. Even so, the exact role of the element in that process is not known. It is well known that plants deficient in potassium are low in protein content and high in aminoacids, which are building blocks of proteins. A respiratory enzyme pyruvate kinase is activated by potassium. Here potassium gets strongly bound into the enzyme molecule and causes it to act as a catalyst in breaking down starch molecule. So when potassium supply is limited, it results in accumulation of starch as respiration gets retarded.

It is widely accepted now that potassium plays a key role in the opening and closing of the stomata.

This element also is redistributed from mature parts to young parts. So the deficiency symptoms are first noticeable in the mature leaves of the lower regions of the plant. In some legumes, this deficiency results in white patches on the leaves. Stems of such plants are weak. The cells in the pith region tend to get broken. The root system of these plants is susceptible for attack by root rotting organisms.

Check Your Progress - 2

What are the life processes that are dependent on potassium?

Note:(a) Write your answer in the space given below

(b) Compare your answer with the one given at the end.

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Sulphur: Sulphur is absorbed as sulphate ions. Roots metabolise the required amount of this element and the rest is transported into the shoot system. In the shoot system it is incorporated into two aminoacids-cysteine and methionine, which are then incorporated into proteins. Respiratory compound coenzyme A, vitamins like thiamine and biotin are some of the important sulphur containing compounds.

Generally, soils are not deficient in this element. When this deficiency is present, general yellowing of the affected leaves is seen. Sulphur is not easily redistributed from the older regions. So the deficiency symptoms are first noticeable in the younger leaves. In tea plant, sulphur deficiency results in the falling off of the leaves and drying up of the shoot apex. In tomato, for the same reason shoots and roots become weakened and the stem gets hardened.

Magnesium: The chlorophyll molecule has one atom of magnesium. This shows how essential this element is for the most important metabolic activity of the plant world, namely, the photosynthesis. Roots and micro organisms, which lack chlorophyll, also require magnesium but its role here is to activate numerous essential enzymes. For example many enzymes which utilise energy in ATP are activated by magnesium. Cells where lipid synthesis takes place contain magnesium in large quantities. *Vaucheria*, growing in magnesium deficient soils do not possess oil bodies. Proper amounts of magnesium are essential to maintain the binding of ribosomal subunits which are the sites of protein synthesis.

Unlike potassium, this element is required in small amounts. As this is easily redistributed, any deficiency is first seen in the old leaves. This deficiency leads to interveinal chlorisis. As chlorophyll is absent, other pigments become more pronounced. In some cases leaves become dried up and necrotic spots are formed. These are seen in between veins. The margins and tips of the leaf may be bent outward. Magnesium deficiency is seen in some sandy soils.

Calcium: This element is found in the plant cells inside the vacuole as crystals of calcium oxalate in some and as calcium carbonate in others. In addition, this element is present in the middle lamella, between two cell walls as calcium pectate. Some scientists are of the opinion that this middle lamella acts as cement binding the neighbouring cells. Instances are there, however, where middle lamella is devoid of calcium. Calcium is utilised by plants in making the plasma membranes. The property of semipermeability of the cell membranes is also due to calcium.

The root requirement of calcium is 100 times to that of shoot. Calcium helps legumes in the formation of root nodules. Groundnut needs calcium for the growth of gynophore which pushes the fruit into the soil.

In some plants, calcium deficiency leads to reduction of mitochondria in the cells. In cotton, calcium deficiency leads to accumulation of starch in leaves and depletion of the same in stems and roots. Calcium may also help in the transport of soluble form of starch. Nitrate distribution also is aided by calcium.

When compared to other elements the redistribution of calcium from older to younger parts is meagre hence the deficiency is visible in the terminal buds and root tips. The root tips become black in colour and gelatinous. In tobacco, tips and margins of young leaves bend down and the leaves get curled.

Calcium deficiency results in disorganisation of nuclear envelope, plasma membrane, tonoplast, mitochondria, golgi bodies and plastids. Thus calcium is concerned with membrane integrity and aids in permeability of various ions and metabolites.

Check Your Progress - 3

What is the important symptom of calcium deficiency in roots?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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3.5.2 Micro-Nutrients

Iron: This is considered by some as a macro-nutrient, but many now regard this as micro-nutrient.

Plants absorb iron in ferric (Fe^{+++}) state but inside the plant body it participates in all activities in ferrous (Fe^{++}) state. Iron deficiency leads to the inhibition of chlorophyll synthesis. Iron is an essential activator for enzymes catalysing reactions leading to chlorophyll synthesis.

Electron carriers like cytochromes, ferredoxins contain iron. Cytochromes have a role to play both in respiration and phtosynthesis. Iron deficiency leads to reduction in the manufacture of energy rich ATP, and metabolic activities dependent on its synthesis.

Iron is very immobile inside the plant. So iron deficiency symptoms are seen in young leaves which show interveinal chlorosis. In some cases leaves may be completely bleached. Leaves show necrosis.

Manganese: This is absorbed in divalent (Mn^{++}) state. This element plays important role in respiration, photosynthesis, and in activiating enzymes.

The deficiency of this element leads to retardation of chlorophyll synthesis and improper formation of the grana of chloroplasts. In the absence of this element, the chlorophyll breaks down in sunlight.

Manganese is known to activate several enzymes. For example, it activates one or more enzymes involved in fatty acid synthesis, and those responsible for DNA, RNA synthesis. In Krebs cycle also this is known to activate a respiratory enzyme. In photosynthesis this takes part in splitting water and releasing oxygen.

Manganese deficiency leads to chlorisis in young leaves and sometimes necrosis. Stems appear yellowish green, often hard and woody. This deficiency leads to a disease called 'grey speck of oats' in oats and 'Marsh spot of peas' in peas.

Molybdenum: This element forms a component of the enzyme nitrate reductase which reduces nitrate to nitrite. This is also necessary for nitrogen fixation by bacteria like *Azotobacter, Clostridium, Rhizobium* and by algae such as *Anabaena* and *Nostoc*.This element is required in the conversion of inorganic nitrogen (nitrate, nitrite, ammonia) into organic nitrogen (amino acids, amides, proteins). In the absence of this element glycolysis is affected and thus synthesis of amino acids also gets affected.

For many plants of all elements this one is required in very small amounts. Deficiency of this disease in cauliflower results in a disease called "whip tail" in which the leaves of the apical bud are not formed properly but grow into long whip-like structures. In citrus, molybdenum deficiency leads to yellow spots on the leaves in between veins.

Check Your Progress - 4

What is the reason of whip tail disease in cauliflower?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Zinc: Even by 1926 it was known that zinc is essential for plant growth. This is required to produce the hormone, indoleacetic acid (IAA) which is needed for normal enlargement of the cells. When there was deficiency of this element, it was observed that there was less of the hormone in the plant. When zinc was added to such a plant, there was an increase in the hormone in the plant. Skoog (1940) who studied the relationship between zinc and IAA was of the opinion that this element keeps IAA in active state and in its absence IAA gets oxidised. Zinc is also an essential part of some enzymes like alcohol dehydrogenase and certain peptidases like carboxypeptidase. Zinc deficiency results in decrease in protein synthesis.

Zinc deficiency in many plants such as tobacco, tomato, mustard, sunflower, cotton leads to a disease called 'little leaf in which leaves become small in size and narrow. In some plants leaves at the top show mottled chlorosis and curved down. Still in others a disease called 'rosette' appears in which the internodes get shortened. In others the leaves are shed early. In older leaves whitish chlorosis streaks appear in between veins. Zinc deficiency causes a disease in bean, peas, citrus, tomato, peach etc. When zinc is sprayed on such plants, deficiency symptoms disappear.

Copper: This element is absorbed both in cuprous (Cu^{+}) and cupric (Cu^{++}) states. But inside the plant it primarily exists in cupric form. It is necessary component in some enzymes like polyphenol oxidase. It may also play a catalytic role in nitrogen fixation.

Copper deficiency leads young leaves to become dark green in colour, get twisted and otherwise misshaped. In extreme cases the leaves are shed and the plant looks dried up. In citrus gardens, copper deficiency leads to a disease called 'die back' in which young leaves die. In trees like apple, pear, citrus, this deficiency results in the formation of blisters on the bark, from which gum exudes. This disease is called 'exanthema'. In tomato, copper deficiency leads to deposition of calcium oxalate crystals in large amounts in the spongy parenchyma of the leaves.

Check Your Progress - 5

Describe the symptoms of exanthema.

Note: (a) Write your answer in the space given below

(b) Compare your answer with the one given at the end.

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Boron: This is largely absorbed from the soils as soluble borate ion. Even though the necessity of this element is known for many years, as to how exactly it works inside the plant has become clear only in recent years. Boron is involved in the transport of carbohydrate inside the plant body. Borate ions combine with sugars to form borate complex and it is in this form that sugar is transported through the phloem. It is observed in plants growing with boron deficiency, that apical regions show retardation in growth and there is slowing down of activities like cell division, cell differentiation and respiration. All these show lack of proper supply of sugars. Normalcy is restored when once boron is supplied. This element is understood to help in increasing RNA synthesis. This is also found in chloroplasts. It is said that boron is necessary for lignification of xylem and sclerenchyma.

Boron deficiency first is seen in the death of stem tips. This results in the development of lateral branches whose tips also die. The leaves have thick coppery texture, sometimes they curl and become brittle. This is called 'bronzing'. This deficiency leads to the stunted growth of roots and the flowring getting arrested. In storage organs like beets, their deficiency results in the disintegration of internal tissues. This disease is called heart rot of beets.

Chlorine: This element enters the plant as chloride ion and remains in this form without forming a part of any organic molecule. It is found that chloride is necessary for proper growth of beet roots. Chlorine acts as an enzyme activator for one or more reactions in photosynthesis in which water is split and oxygen is liberated.

Chlorine deficiency in tomato leads to the wilting of the tips of leaflets and the leaves become chlorotic and nectrotic and the end leaves become bronze in colour. Roots become stunted as tips become enlarged. Tomato plants do not fruit in the absence of this element and wilting is common and by adding 20 lbs of chlorine per acre this can be neutralised.

Most of the higher plants require these sixteen elements. However there are more required by some plants. A few of such elements are mentioned below.

Sodium: *Atriplex vesicaria*, grows on arid soils where salts are present in plenty. Vegetables like turnips, beets and the blue green algae require this element for healthy growth.

Silicon: This is present in all plants, but is abundent in monocots. *Equisetum* and diatoms also have this element. In rice, silicon when present makes the straw stiffer and thus helps the plant to stand erect.

Aluminium: This is found in most plants. In *Hydrangea macrophylla* the blue colour of the flower is due to this elment. It is found necessary for the growth of stems and roots of citrus cuttings. But definete demonstration that it is essential for any plant has not yet been made.

Cobalt: This is necessary for the growth of blue green algae. In leguminous plants there is an increase in the formation of nitrogen fixation and nodule formation, in the presence of this element. For bacteria cobalt is necessary because it is a parrt of vitamin B_{12}. The necessity of this element for other plants has not been conclusively established.

Selenium: Some species of *Astragalus, Stanleya* absorb this element. Other species growing in the same area do not absorb this element. This is found to stimulate growth of some species of the above mentioned genera. Selenium is found to replace sulphur in some amino acids, in the plants where it is found and such plants are toxic.

Strontium: It is found that in the leaves of elm, this element is present. Some plants absorb radioactive strontium. Some plants are known to take strontium in place of calcium. But how exactly this element is useful to plants is not known.

Vanadium: A number of leguminous plants and green algae grow better in the presence of Vanadium. It is known that vanadium can replace molybdenum requirement in some microorganisms. Nitrogenase the enzyme involved in biological nitrogen fixation, contains vanadium as a cofactor, in addition to molybdenum.

3.6. METHODS OF STUDYING PLANT NUTRITION

Already mention was made of Sachs and Knop, the German plant physiologists, who a hundred years ago made nutritional experiments with plants using water culture medium instead of soil.

3.6.1. Hydroponics

The technique of cultivating plants in water culture (nutrient solutions) is called hydroponics and also soiless culture. A nutritional solution is made by using the salts containing all the required elements in a particular ratio. It was later observed that many plants grow much better if oxygen is also supplied to the roots in the form of air bubbles into the solution. In order to find out the role of an element, that particular element is eliminated from the solution and when the plants are grown, all the changes in the plant are noted and they usually indicate the role of the element in the plant nutrition. The plant is grown in such a way that the root system is present in the container having the nutrient medium, while the shoot system is outside and is given support at the mouth of the container so that the plant can stand erect. In this way vegetables like tomatoes, radish, and lettuce can be cultivated.

However, there are some disadvantages in this method. As roots are submerged in water, air has to be constantly supplied by forcing air bubbles through the solution so that oxygen reaches the roots. Secondly, there is a need to change the solution once in a few days or sometimes even daily as the composition of the solution changes because plants absorb some ions more than other ions. This results in a change of pH of the medium. For instance, as a results of absorption of nitrate ions more rapidly, hydroxyl (OH^-) ions or bicarbonate (HCO_3^- ions are released into the solutions. Both these increase the pH of the solution and so it becomes alkaline. This results in the reduction in the availability of iron, manganese and zinc ions. Hydroponic solutions must be kept in darkness as algae are likely to grow when exposed to light.

3.6.2. Sand Culture

To avoid some of these disadvantages of water culture, some used a method called sand culture. In this method cleaned quartz sand is used as a medium. One advantage in this is that the plants need not be given additional support to stand erect. Also, the roots get proper aeration and are in darkness naturally. Depending upon the plant cultivated and its age, the nutrient solution must be added at suitable intervals and in required amounts. Some times a substance called perlite is substituted for quartz.

Some of the common nutrient solutions and their chemical composition, are as follows:

a. Knop's Solution

Salt	Molarity
$Ca(NO_3)_2 \, 4H_2O$	0.003
KNO_3	0.002
KH_2PO_4	0.0015
$MgSO_4 . 7H_2O$	0.0008
$FePO_4$	trace

b. Arnon. Hoagland Solution (1940)

Salt	gram litre
KNO_3	1.02
$Ca(NO_3)_2$	0.492
$NH_4H_2PO_4$	0.23
$MgSO_4 . 7H_2O$	0.49
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$CuSO_4.5H_2O$	0.08
$ZnSO_4 . 7H_2O$	0.22
$H_2MO_3 . H_2O$	0.09
$FeSO_4.7H_2O$(0.5%)	0.6 ml/L
Tartaric acid 0.4%	Thrice a week

c.Hewitt Solution

Salt	g/l	Salt	g/l
KNO_3	0.505	$CuSO_4 5H_2O$	0.000245
$Ca(NO_3)_2$	0.820	$ZnSO_4.7H_2O$	0.000296
$NaH_2PO_4 2H_2O$	0.208	H_3BO_3	0.0001860
Ferric citrate	0.0240	$(NH_4)_6Mo_7O_{22} . 4H_2O$	0.000035
$MnSO_4$	0.002230	$CoSO_4 7H_2O$	0.000028
		NaCl	0.00580

There are many solution media with different formulae for plant nutrition, thre[illegible]f which are detailed above. No particular solution is suitable for all plants. The suitability of [illegible]dium for a plant is determined by temperature and light. For instance plants tend to abso[illegible]itrates on sunny days than on cloudy days. Also depending upon the stage of devel[illegible]re is a need to change the constituents of the solution. Phosphorous, for instance, is [illegible]ore in the early stages of plant growth than during adult stages.

3.6.3. Special Techniques for Studying Micro Nutrie[illegible]

The early plant physiologists like Sachs and Knop did not know mi[illegible]and their role. This is mainly due to the fact that these elements reached the plants as contaminants of salts used, as these are impure. Subsequently with better techniques pure salts came into the market. This made the microelements unavailable to the plants under experimentation.

These elements are required by plants in very small concentrations and this is usually indicated as ppm (parts per million). The following are the ranges of requirement of these elements: iron (0.5 to 5.0), manganese (0.1 to 0.5), boron (0.1 to 1.0), Zinc (0.02 to 0.2), copper (0.01 to 0.05), molybdenum (0.01 to 0.05), chlorine (upto 5.0). Higher concentrations of these elements are harmful for plants, it is difficult to remove these elements from macroelements. But there are methods by which they can be removed to a large extent. The micronutrient cations present in a solution can be absorbed by insoluble calcium carbonate and aluminium. These cations can be made to combine with diphenyl thiocarbazone and the compounds so formed by solvents like carbon tetra chloride or chloroform.

Next the water to be used for making nutrient solution must be purified. Generally there will be some impurities in the tap water. So the water has to be distilled and a glass still without exposed metal parts, is necessary to prevent zinc and copper contamination. In recent times, ion- exchang resins are used. As the water first passes through the resin that asborbs cations, hydrogen ions are exchanged for cations. Next while passing through resins that absorbs anions, contaminant anions like those of molybdate, borate, chlorate are absorbed by the resin and hydroxyl ions are exchanged. Thus pure water is obtained.

3.6.4. Contaminations by Containers and Atmosphere.

Generally these experiments can be conducted in containers made up of borosilicate. But, the element boron will be available to plants grown therein. So when studies are made on boron, it is advisable to use containers made up of polythene.

Atmospheric dust falling on the solution or the plants themselves may supply some micronutrients to the plants. This should be prevented by taking suitable precautions.

Plants get miroelements like copper and zinc from seeds. It is better to use the seeds from the plants which are not additionally supplied with micronutrients.

3.7. INDICATOR PLANTS

In mineral exploration, now-a-days scientists are using plants to indicate the presence of minerals in the soils. Such plants are called indicator plants. Some plants absorb some metals, while other plants growing at the same place do not take that metal. Already mention was made about how *Astragalus* and *Stanleya* absorb the element Selenium, while other plants at the same place do not absorb. Thus the presence of those plants indicate the presence of selenium in the soil. Likewise *Gypsophila patrini* (Caryophyllaceae), *Becium hombeli* (Labiatae),*Meruga latifolia* (a moss) and blue green algae *Cirosiphon geniculatus* are known to grow in places rich in copp[illegible]r.*Polycarpea synandra* grows in places rich in lead. A fern called *Asplenium adulterium* [illegible]nown to grow in places rich in nickel.

3.8. [illegible]ARY

Scie[illegible]dge on plant nutrition began with the classical experiment of Jan Baptist von Helmon[illegible] Century. But more difinitive contributions were made in 19th century mainly by Germ[illegible]d British scientists who have proved the need and roles of macronutrients, N.P.K. L[illegible] Sachs, Knop and Pfeffer grew the plants in nutrient media and studied their growth and specified the physiological roles of almost all the macronutrients. Later, Warrington, Brenchley, Hoagland, Arnon and Stout proved the need for micro-nutrients. Chemical analysis of the plants reveal that about 60 elements are found in them; but the presence of the element in plant ash does not necessarily mean that the element is needed for the nutrition of the plant. The chemical analysis of the plant indicates the relative concentrations and the requirement of the plant in a general way. By conducting elegant experiments Arnon and Stout proposed 3 criteria for determining the essentiality of elements in plant nutrition. The physiological role of various elements in plants has been established by conducting experiments in deficient media and determining the difficiency symptous and correcting the symptoms by the addition of deficient elements. Their symptoms not only reveal the role of the nutrients, but also applied value in correcting the deficiency symptoms of crop plants grown on mineral dificient soils. Methods of studying plant nutrition and the technique concerning the purification of media for micronutrients have been developed as a result of the continuous efforts of Hoagland, Shive, Stout and Arnon in the U.S.A. and Hewitt in Britain. As a result, a number of media for growing different kinds of plants in soilless nutrient media and also in certain cases in sand culture have become known. These studies not only re established the essentiality of the 16 macro and micronutrients, but also had the understanding of physiological roles and mechanisms of ion absorption and antagonism. Certain plants grow only in soils where a large quantity of any heavy metal is present. Such plants in fact indicate the presence of an important mineral. They are known as indicator plants.

3.9. CHECK YOUR PROGRESS: MODEL ANSWERS

1. The three important elements that are taken in large quantities by the plant are carbon, oxygen and hydrogen.
2. The life processes that are dependent on potassium are respiration, protein synthesis, photosynthesis and transpiration.
3. Due to the defieiency of calcium, the root tips become black in colour and gelatinous.
4. The deficiency of molybdenum causes white tail disease in cauliflower.
5. The symptoms of exanthema disease are the blisters on the bark resulting of the exudation of gum in trees like apple, pear and citrus.

3.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. Enumerate micro-nutrient elements in plant nutrition. Describe how they are utilised by plants?
2. What are the three elements required by plants in very large amount. Give a brief account of their role?
3. What is soilless culture? Describe how this is performed?

II. Answer the following questions in about 10 lines each.

1. What are the criteria for determining the essentiality of the elements for plant growth?
2. Besides 16 macro- and micro-elements, mention any three elements which are absorbed by plants and describe their role.
3. What is sand culture? What are its advantages over water culture?
4. Give an account of boron in plant nutrition.
5. What is the role of nitrogen in plant nutrition?

UNIT - 4: ION UPTAKE

Contents

4.1. OBJECTIVES

By the end of this unit you will be able to:

1. list out the methods of uptake of ions in plants, and
2. describe the various methods of uptake of ions in plants.

4.2. INTRODUCTION

Plants absorb inorganic salts in the form of cations and anions. Early workers believed that inorganic salts were passively carried into the plant through diffusion along with absorption of water. It was assumed that the translocation of absorbed salts to different parts of the plant was dependent on transpiration pull. But these assumptions could not explain adequately the higher concentration of salts in the cell sap of plant tissues than in the surrounding medium. For example, *Nitella* an alga, accumulates K^+ ions almost 100 times more in the cell sap than in the surrounding medium.

It was observed that osmotically active substances diffused along concentration gradients from the soil solution into the plant. The osmotic concentration inside the cell was maintained at a low level due to the utilization of absorbed salts for metabolic activities. This osmotic theory sufficiently explained the absorption of small quantities of salts along concentration gradient but did not account for absorption of larger quantities of salts along concentration gradient and rapid translocation of the absorbed salts.

The phenomenon of salt accumulation is mostly found in meristematic cells and decreases as cells mature. Moreover the rate of respiration is also more in meristematic cells than in maturing cells. This indicates the involvement of energy in ion uptake by cells against concentratian gradient which is an active process.

4.3. METHODS OF UPTAKE OF IONS IN PLANTS

There are several methods of uptake of ions in plants. They are given below.

4.3.1. Passive Absorption

Salt absorption by root system takes place with intimate contact with soil solution, passive or non-metabolic absorption of ions has been demonstrated. It was found that when a plant tissue was kept in a medium of relatively high concentration, there was an initial rapid uptake of ions until an equilibrium was reached. Consequently, the ion concentration in this part of tissue would be approximately equal to that of the external solution. If the above tissue is returned to the low salt medium, some of the ions taken in, will diffuse out into the external medium. In other words a part of a cell or tissue is apparently open to free diffusion of ions. It is called apparent free space or outer space.

It is possible to calculate the volume of "outer space" when a tissue is immersed in a solution of known concentration of non-metabolite like mannitol (a sugar - alcohol) and allowed to attain equilibrium. The amount of salt taken up is determined.

Hope and Stevens found that Been root tips, when immersed in a KCl solution, reached equilibrium in 20 minutes in the absence of metabolic energy by using metabolic inhibitor. Further the volume of KCl in external medium when increased, the volume of tissue allowing free diffusion also increases. Since active transport was inhibited, it is concluded that passive accumulation of ions against a concentration gradient must have taken place. The term "pparent free space" was introduced to describe the apparent volume allowing for free diffusion of ions.

This brings up the question how the ions accumulated against a concentration gradient without participation of metabolic energy. This is explained through ion exchange mechanisms, through the establishment of Donnan equilibria and Mass flow hypothesis.

4.3.2. Ion Exchange

Ions are obsorbed through the surfaces of the cell walls or membranes of cell by exchanging with ions of equivalent charge from external solution. For example the cation K^+ of the external solution, exchanged with a hydrogen ion H^+ absorbed to the surface of the cell membrane. In ion exchange neutrality is maintained. Firstly, if uptake of anions exceeds cation uptake, hydroxyl (OH^-) and bicarbonate (HCO_3^-) ions are transported outwardly from inside from the cells. Similarly if, the cation uptake is more rapid than anions, some hydrogen (H^+) ions are exchanged by cells. The ion exchange process is explained as follows:

4.3.3. Carbonic Acid Exchange Theory

CO_2 from respiration is continuously released at the root tips. It combines with the water to form carbonic acid (H_2CO_3) and the latter dissociates into H^+ and HCO_3^- ions. A zone of carbonic acid is developed in the root tip because of high respiratory activity. The H^+ ions exchange with cations absorbed on the clay micelle, will enter the soil solution and diffuse to the root surface. In the same manner the H^+ ions from the root surface may exchange with cations in the soil solution that are released from the clay micelle.

Recently Jenson and his co-workers revealed from their experiments that in tissues, definite ion exchange places in the cell wall are located in the form of pectic carboxyl compounds ($RCOO^-$) which are negatively charged. That enables exchange of K^+, Ca^{++} and Mg^{++} cations from soil solution with H^+ ion.

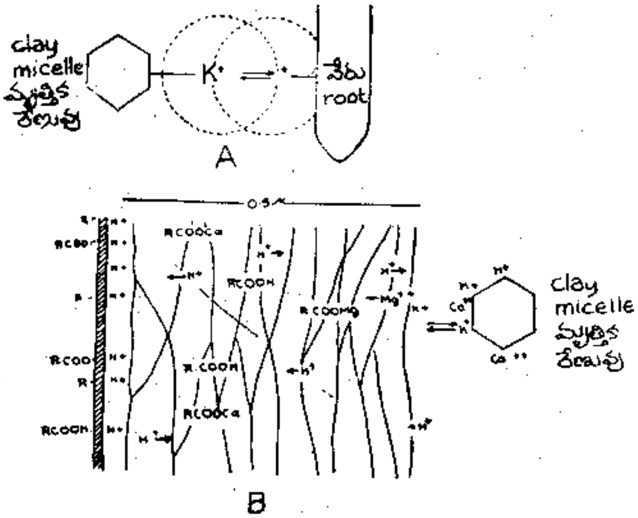

Fig.4.1 A. Cations (H^+) adsorbed to the root wall, get exchanged with K^+ cations from the clay micelle. B. The cations (K^+, Ca^{++}, Mg^{++}) from soil water getting exchanged with H^+ cation from the negatively charged pectic carboxyl compound ($RCOO^-$) sites in the cell wall.

4.3.4. Donnan Equilibrium

This theory takes into account the effect of fixed or indiffusible ions. A differentially permeable membrane separates cell from the external solution, that is, the membrane is permeable to some ions and not to others. Suppose the cell has an internal concentration of anions to which the membrane is impermeable, such ions are known as fixed anions. For example, if you obnserve the number of anions on both sides of the membrane shown in the figure 4.2, the membrane is

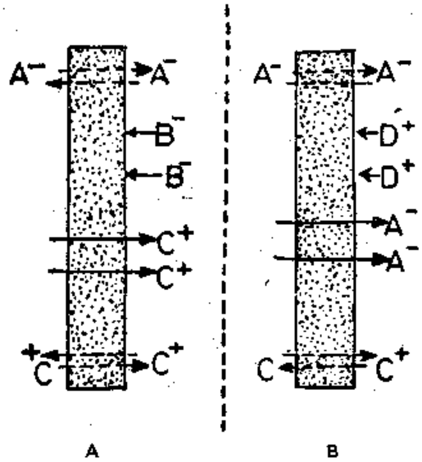

Fig.4.2 A.Membrane is impermeable to anion, B^-, causing additional cations, C^+, to diffuse across from the outside (accumulation of cations). B.Membrane is impermeable to the cations, D^+, causing additional anions, A^-, to diffuse across from the outside (accumulation of anions).

permeable to anion A^- and cation C^+ but impermeable to the anion B^-. The ions A^- and C^+ can move freely through this semi-permeable membrane until an equilibrium is reached between those ions. But as the anions are arrested inside the membrane, the membrane is negatively charged. To balance this, some of the cations C^+ will enter the membrane resulting in the greater increase of cations inside the cell than inside its surrounding medium. The same thing happens when cations are accumulated inside the membrane due to its impermeability for those cations. Thus, the ions enter the cell against concentration gradient without metabolic energy. In some algae the K^+ ion concentration is 30 times more than the surrounding medium that can be explained by Donnan's equilibrium. (Fig. 4.2).

Check Your Progress - 1

What are fixed ions?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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4.3.5. Mass Flow Hypothesis

Many workers have proposed that ions move through roots along with the mass flow of water under the influence of transpiration. When there is a high rate of transpiration, a high rate of absorption of soil water is also observed by the root system.

The loss of water from xylem vessels through transpiration creates negative tension in the xylem vessels, ultimately the roots absorb more water. Along with this water, more number of ions enter the plant against concentration gradient with involvement of energy.

From this discussion, it is clear that at least a part of the total salt uptake by a plant may result from passive absorption as follows:

(i) Free diffusion of ions along concentration gradient into the apparent free space of a tissue (ii) accumulation of ions against concentration gradient due to ion exchange or Donnan equilibrium and (iii) lastly, mass flow of ions through roots due to 'transpiration pull'. In all these processes, no metabolic energy is involved.

4.3.6. Active Absorption

The analysis of the vacuolar sap of plants immersed in solutions of known salt concentration have demonstrated that both anions and cations are accumulated by plants against concentration gradients. The extent of accumulation is such that the passive processes like ion exchange and donnan equilibria cannot account for the accumulation that occurs. In many plants, it is found that light enhances the absorption. Young plants with actively growing roots absorb more salts.

Analysis of the ion accumulation in the sap of *Nitella* and *Valonia*, reveals, that potassium and certain other ions are present in higher concentrations inside plant cells than in the environments.

Since ion accumulation is inhibited when the metabolic activity of the plant is inhibited by low temperature, low oxygen tension and metabolic inhibitors etc., we can assume that ion accumulation as it occurs in plants requires metabolic energy. The transport of ions with the aid of metabolic energy has been termed active transport. Various hypothesis have been suggested to explain active transport.

4.3.7. Lundegardh's Cytochrome Hypothesis

Lundegardh observed that there was a quantitative relationship between anion absorption and salt respiration. When a respiring tissue was transferred from the water into a salt solution, the respiration rate increased. This increased respiration other than ground respiration is known as "salt respiration". It was also noted that salt respiration and anion absorption were inhibited by cyanide or carbon monoxide. The carbon monoxide specifically inhibits the cyto-chrome oxidase which is an electron acceptor. Based on these observations Lundegardh advanced a theory with the following assumptions.

Absorption of anions is independent of cations; only anions are actively transported through the cytochrome system which acts as an anion carrier. Cations move passively to balance the electrical differece caused by the accumulation of anions on the inner surface.

An oxygen concentration exists from the outer to the inner face of a membrane and this favours oxidation at the outer face and reduction at the inner face respectively. This can be observed from Fig.4.3. Dehydrogenase reactions on the inner surface produce protons (H^+) and electrons (e^-). The electrons move over via a cytochrome chain Fe^{+++} acting as electron carrier getting it reduced to F^{++} while anions move inward. At the outer face the reduced cytochrome liberates the electron and picks up an anion. The liberated electron unites with a portion of (H^+) and oxygen to form water. At the inner face the oxidised cytochrome ion becomes reduced by the electron released in a dehydrogenase reaction, thus releasing the anions inside.

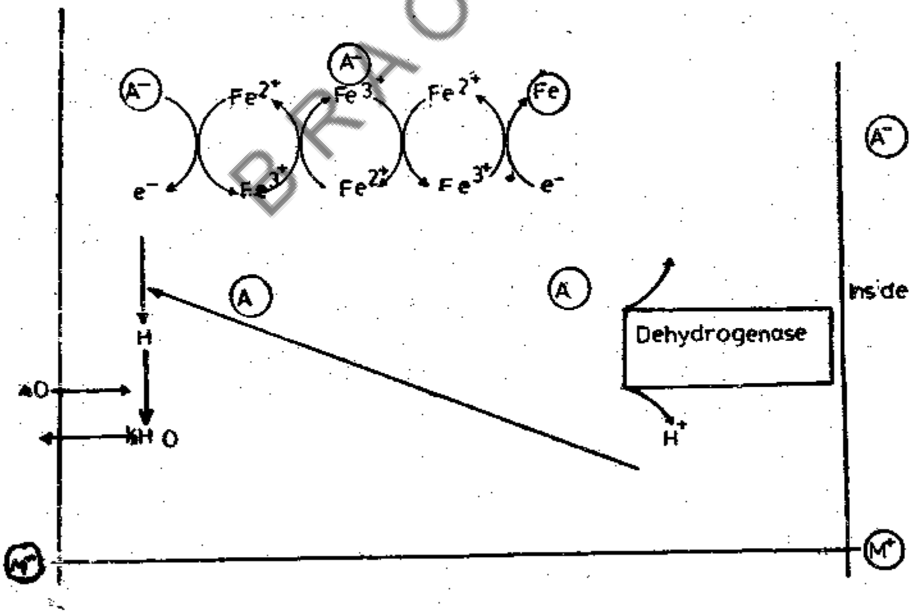

Fig.4.3 A diagrammatic representation of Lundegardh's cytochrome theory on salt absorption. The anions (A^-) are actively absorbed via "cytochrome pump". The cations (M^+) are absorbed passively.

Eventhough the cytochrome hypothesis explained the involvement of metabolic energy in ion uptake, it has not been universally accepted and has been criticized by a number of investigators. This theory has been criticized on the following grounds:

Robertson (1951) found that D N P (2,4- Dinitrophenol), an inhibitor of oxidative phosphorylation increased respiration but decreased salt respiration indicating the involvement of phosphorylation in salt accumulation.

According to cytochrome hypothesis only anions are capable of stimulating respiration. However, Overstreet and Handley (1955) observed that both sodium and potassium cations stimulated respiration.

If cytochrome is the only carrier for all anions, there should be an antagonism between anions like chloride, bromide, nitrate, sulphate etc., for binding sites. But antagonism is not exhibited even when chloride and nitrate ions are there in the solution simultaneously.

In this hypothesis the ratio of the monovalent anions, and the oxygen molecule should be 4, as one oxygen molecule combines with 4 electrons. But investigators found that this ratio exceeded 4.

Check Your Progress - 2

What is salt respiration?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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4.3.8. Hypothesis Involving ATP

The D N P (2,4- dinitrophenol) the inhibitor of oxidative phosphorylation, preventing salt absorption presents a strong evidence for the involvement of ATP in active salt absorption.

Bennet - Clark has proposed a mechanism for active absorption that utilizes ATP. He has suggested that phospholipids may be important in the transport of ions across membranes otherwise impermeable. In this transport lecithin, a phospholipid, is synthesized and hydrolyzed in a cyclic manner picking up ions on the outer surface and releasing them on inner space. The synthesis of at least one of the components of this phospholipid cycle requires ATP. The diagram showing "The phospholipid cycle" and how it might proceed in ion transport is given in Fig.4.4.

4.3.9. Sodium and Potassium Pumps

Mac Rabbie has done some experiments on *Nitella* and proposed that poitassium cations are accumulating in *Nitella* cell actively against concentration gradient. According to her experiments, it is revealed that plasmalemma is more permeable than tonoplast and there are potassium pumps on the plasmalemma that are responsible for accumulation of potassium in the cell sap against concentration gradient. Along with potassium pump, sodium pump is functioning but the sodium pump expels the cations thus preventing the entry of sodium into the cell. It was revealed that for the functioning of potassium and sodium pumps, energy is required, and that energy is released when the ATP is hydrolysed by adenosine triphosophatase enzyme. It was assumed by many workers that for anion pumps that allow the phosphate and chloride ions into the cell, the energy is available from photosystem II of the "Light reaction" of photosynthesis but not from the hydrolysis of ATP by the enzyme.

Most of this work is carried on animal and bacterial systems and it is assumed that similar pumps exist in plant cells also.

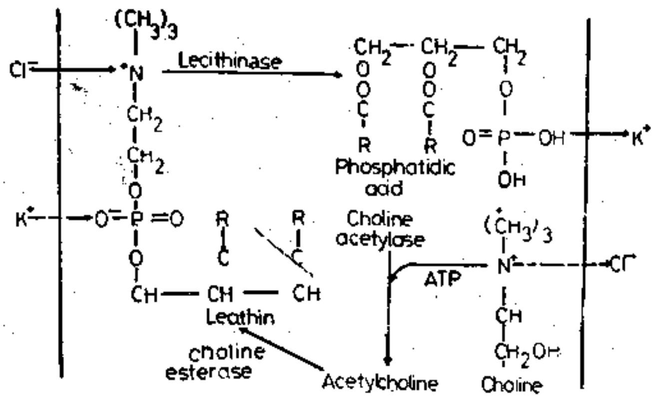


Fig.4.4. Diagrammatic representation of the phosphatide cycle. At left, ions from outer space are picked up by the lecithin. Hydrolysis of the lecithin-ion complex releases ion to inner space. Lecithin is then resynthesized.

4.3.10. Carrier Concept

The space in a tissue or a cell to which ions penetrate through the aid of metabolic energy is termed as "inner space". The "inner space" comprises the vacuoles and protoplasts of all cells of a tissue that are interconnected by plasmodesmata. The area between the outer and inner space is impermeable to free ions. Passage across this area is thought to require some specific carriers. that combine with ions in outer space and release them in inner space. This impermeable barrier is referred to as a membrane and assumingly carriers exist with it.

The most important feature of the carrier theory is the formation of an intermediate carrier - ion complex, which is capable of moving across the impermeable membrane. Ions released in the inner space cannot move out and are thus accumulated. During the formation of ion-carrier complex metabolic energy is utilized. Carrier hypothesis is most widely accepted. A model giving a simplified description of the carrier concept is shown in the figure.4.5.

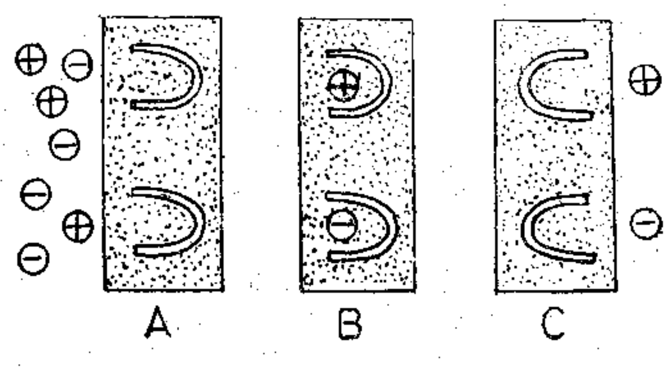


Fig.4.5 Model explaining the carrier concept. A.Membrane is impermeable to ions B. Carrier-ion complex is formed. C.Ions are released into inner space.

4.3.11. Nature of Carriers

Eventhough it is evident that ions are transported by carriers through the impermeable membrane, the carriers are not isolated so far. Epstein and other workers assumed that the carriers may be of protein in nature. Based on experiments on ion uptake with the use of protein synthesis inhibitors, Steward and Sutcliffe proposed that carriers could be proteins. When the inhibitors of protein synthesis, are used the ion absorption is also reduced. Ample evidence is not there to prove that proteins alone are the carriers of ions, but we must agree that there exists relationship between ion uptake and proteins.

Pressmen and other workers have revealed from their experiments, that an antibiotic which is a polypeptide acted as a specific carrier for potassium and aided for its accumulation. They have proved that, the antibiotic acted as an ionophore or ion carrier. Recently it was demonstrated that the unhydrated potassium ion is bound to the polypeptide molecule of valinomycin potassium complex, thus adding to the concept that some of the polypeptides are capable of forming complexes with ions thus behaving as ion carriers.

4.4. SUMMARY

The earlier workers assumed that inorganic salts were passively carried into the plant along with the absorption of water. "Osmotic theory" sufficiently explained the diffusion of salts along concentration gradients from soil solution into the plant but did not account for absorption of salts against concentration gradient. The concept of involvement of metabolic energy in the ion uptake was put forth by Pfeffer in 1900. The uptake of ions even against concentration gradient without participation of metabolic energy is known as passive absorption. Passive absorption is explained through "ion exchange" mechanism, "Donnan's equilibrium" and "Mass flow" hypothesis. The transport of ions with the aid of metabolic energy across the impermeable membrane has been termed as "active transport". The active transport is explained by various hypotheses: (i) Lundegardh's cytochrome hypothesis (ii) hypothesis involving A T P, (iii) sodium and potassium pumps and (iv) carrier concept. Steward, Epstein and other workers assumed that the carriers of protein in nature are located in the cell membrane. From the works of Pressman and other workers, it is revealed that the ion carrier is a polypeptide that combines with ions forming intermediate ion carrier complex and transport the ion into cytoplasm or cell vacuole resulting in the accumulation of ions. The ion uptake by the plants is partly by passive absorption. Accumulation of salts against concentration gradient is generally by active transport.

4.5. CHECK YOUR PROGRESS: MODEL ANSWERS

1. Suppose the cell has an internal concentration of anions to which the plasma membrane is impermeable, such ions are called anions.
2. The respiratory rate increases when a respiratory tissue is transferred from water to salt solution. This increased respiration other than ground respiration is called salt respiration.

4.6. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. What are the methods of ion uptake in plants? Define active and passive absorption.
2. Explain the rate of Donnan equilibrium in ion uptake.

3. "Accumulation of ions against concentration gradient is facilitated to some extent by ion-exchange" - Discuss.

4. Explain the concept of carrier involved in active absorption of ions in plants.

5. Discuss Lundegardh's cytochrome hypothesis.

II. Answer the following questions in about 10 lines each.

1. What are sodium and potassium pumps?

2. What is the nature of carriers?

3. Explain the phenomenon of mass flow ?

UNIT-5: TRANSLOCATION OF SOLUTES

Contents

5.1. OBJECTIVES

By the end of this unit you will be able to

1. describe the structure of the tissue responsible for the translocation of the solutes from source to sink,
2. describe and differentiate the apoplast from symplast,
3. describe various methods that are adopted to measure the rate of translocation,
4. list out the different methods of translocations of solutes and describe them separately, and
5. explain the electro-osmosis theory and lateral transport of ions.

5.2. INTRODUCTION

Green plants synthesise sugars by a process known as photosynthesis in the presence of sunlight. However, the distances separating the photosynthetic cells of the leaves from the living cells of other parts are relatively long. The need for a rapid and efficient translocation system, to translocate the organic solutes from the source to sink (site of utilization) becomes apparent. It was found that specialised cells of phloem tissue called sieve tube elements are involved in the translocation of solutes.

5.3. GIRDLING OR RINGING EXPERIMENT

Hertig demonstrated in 1837 by the girdling or ringing experiments that nutrients will collect above a stem "girdle" causing the stem tissues to bulge. The girdling technique comprises the complete removel of a ring of bark from a stem or branch, leaving the wood (xylem) intact. If the girdle is made midway on the stem with the leaves, substances translocated from the leaves will accumulate above the girdle. This proves that the phloem and not the xylem is concerned with the movement of materials from leaves.

From the "girdling" experiment we can arrive at the following conclusions:

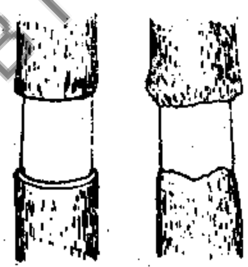
Water along with dissolved salts is translocated against gravitational force through xylem vessels.

The photosynthetic products (sugars) are translocated from leaves to growing parts (stem tips, flowers, fruits and roots etc.) through the phloem tissue.

The translocation of solutes is independent of the gravitational pull and the direction of translocation changes according to the source of the solutes and sites of requirement of those solutes (sinks). This is evident, when a ring of bark is removed between matured leaves and stem tips, with the matured leaves below the girdle. The substance accumulated below the girdle, indicating the direction of translocation from matured leaves (source) to the growing shoot apex (sink) (Fig.5.1).

5.4. ANATOMY OF PHLOEM TISSUE

The phloem tissue is primarily composed of sieve tube elements, phloem parenchyma and companion cells. The phloem parenchyma cells often contain chloroplasts and the companion cells are living cells. The state of sieve elements is not so clear. The mature sieve elements appear to contain living protoplasm although they do not have a nucleus. The main components of the sieve tubes are longitudinally arranged rows of individual cells called sieve elements. These sieve elements are separated by perforated cross walls called sieve plates. The protoplasm may be present in filaments through the cells and appears to pass through the sieve plates so that a sieve tube may contain a more or less continuous protoplast. Sieve elements can be plasmolyzed, indicating the presence of differentially permeable membrane, but they apparently lack any well defined tonoplast. The process of fixation usually destroys or alters the internal structure of sieve tubes and commonly leads to the formation of substantial plugs of slimy substance across the sieve plates called slime bodies. The difficulty of getting an exact picture of internal structure of living phloem cells has added greatly to the problem of understanding the process of phloem translocation. No satisfactory theory has been putforth.



A B

Fig.5.1 A trunk of a tree (A) immediately after a ring of bark has been removed, and (B) after a longer period of time. Note that materials being translocated from the leaves have accumulated in the region above the girdle causing it to bulge.

5.5. APOPLAST AND SYMPLAST

In 1932 the German Physiologist E.Munch developed the concept of the apoplast and symplast in the roots, illustrated in the Figs.5.3 & 5.4. The apoplast consists of all the space in the roots that roughly equates with free space, that is, cell walls and intercellular spaces plus the tissue in the stele which gives free access to water mainly the xylem vessels. The important point to note is the apoplast is discontinuous and is separated into two regions. One consists of the cortex of root and the tissues external to the endodermis. The other is the tissue of the stele, including the xylem located inside the endodermis. The endodermis acts as barrier because of the casparian strip, a heavily suberized thickening of the cell walls that prevents the passage of water from outside to inside or back. The water enters the stele only when it passes through the membranes and cytoplasm of the endodermis. The root may thus be thought of an osmometer, the endodermis being the osmotically active membrane. Dissolved substances or solutions may diffuse or flow unrestricted via the apoplast through the cortex to the endodermis, but to enter the stele they must pass through the differentially permeable membranes of endodermis.

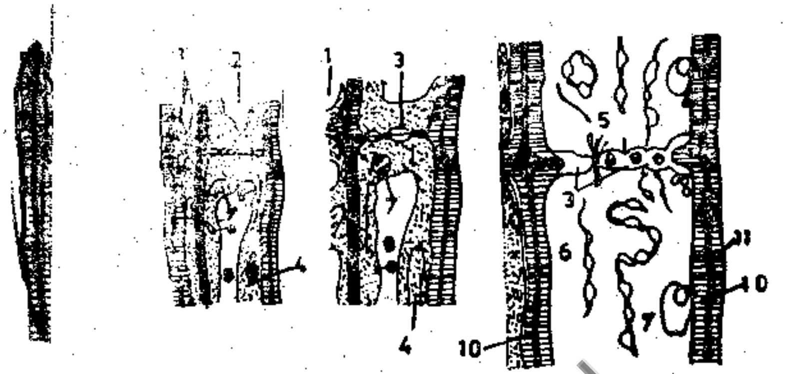


Fig.5.2. The stages of the development of sieve tube seen through electron microscope.
1. Companion cell. 2. Sieve tube 3. Callose 4. Nucleus 5. Endoplasmic reticulum
6. Mitochondrion 7. Plastids 8. Vacuole 9. Tonoplast 10. Middle lamella 11. Cell wall.

The symplast consists of the total protoplasts of the cells, that is the portion of the cells lying within the boundary of the outer differentially permeable membrane of the cell. Vacuole being separated form the cytoplasm by a second differentially permeable membrane would not belong to either of the systems. The protoplasts are connected from cell to cell via small strands of cytoplasm that penetrate the cell wall, called plasmodesmata. This single continuous system of protoplasm of the entire root is considered as symplast. The symplast is otherwise known as "inner space".

Check Your Progress - 1

What is a symplast?

Note: (a) Write the answer in the space given below

(b) Compare your answer with the one given at the end.

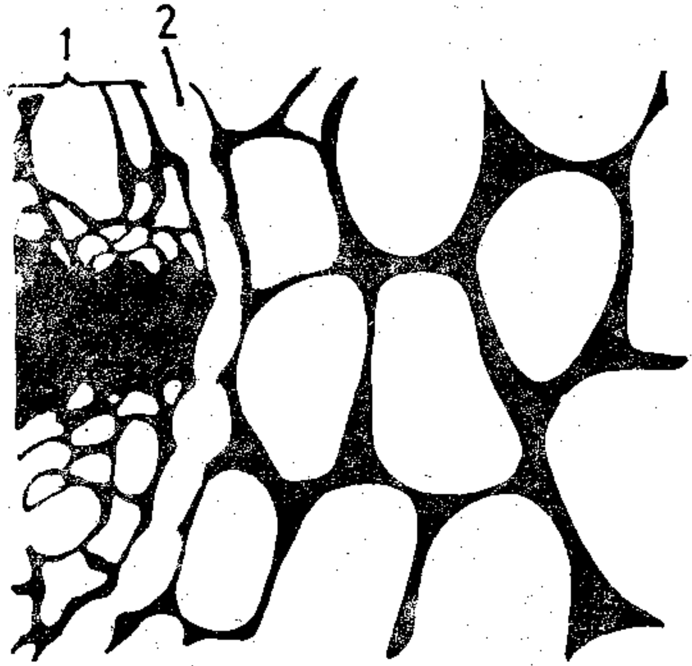


Fig.5.3 The cross section of the root showing the apoplast system. The black portions are the intercellular spaces united with cell walls resulting in the apoplast system 1.Stele 2.Endodermis.

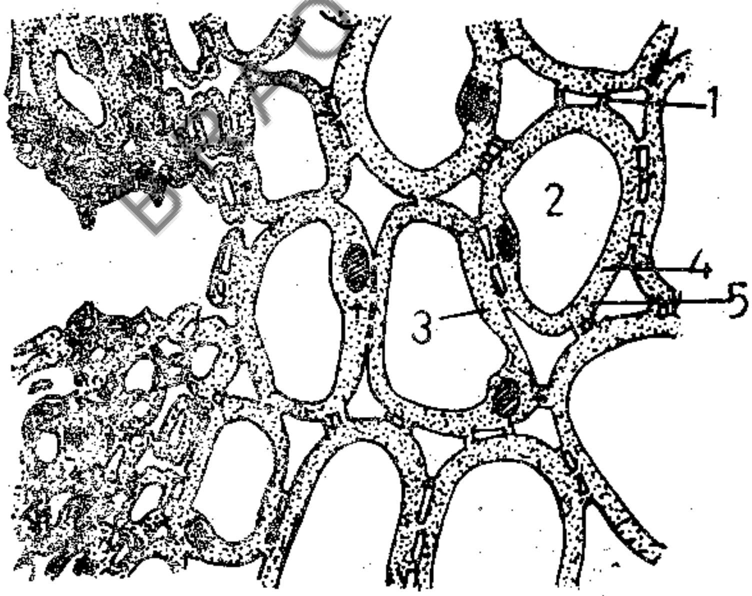


Fig.5.4 The symplast system. The cytoplasm of all the cells is inter- connected with plasmodesmata 1.Plasmodesmata 2.Vacuole 3.Endoplast or tonoplast 4.Mesoplast 5.Ectoplast.

Translocation of Inorganic Solutes

The apoplast - symplast concept led in 1938 to the proposal of a mechanism for water uptake and transport to the stele by American physiologists, *A.S.Grafts* and T.C.Broyer. Soil solution, containing dissolved ions can freely enter the apoplast system or free space outside the endoderrmis by passive absorption. Ions are absorbed into the symplast from the soil solution in the apoplast by an *"active process"*.They are transported through the symplast from cells to cell via intercellular connections (plasmodesmata), across the endodermis and enter into the stele. Once the solution enters the stele they again leak out into the apoplast of the stelar region where the passage of the solution is believed to be a passive process.

According to Crafts and Broyer, the oxygen concentration will be less in the cells in the stelar region, so the availability of metabolic energy will be also less in the endodermis and stele. For this reason, the solutes leak out into the adjacent xylem vessels. The solutes along with water in the xylem vessels are translocated to the distant parts of the plant because of the transpiration pull. The experiments with radio active potassium ($K^{*}42$),in growing *Willow* proved that "K" is principally translocated through xylem vessels. It is also observed that the potassium is laterally translocated from xylem to phloem easily through the cambium by passive and active transport. Some of the salts, especially, sulphates and phosphates along with some amino acids, from the mature leaves are translocated through phloem to growing stem tips and other parts where they are utilised. The potassium and nitrate are also mobile nutrients like phosphorous and sulphur. These mobile nutrients that reach the leaves through xylem will be translocated through phloem to the growing parts. Calcium is not a mobile element and iron is also less mobile and its mobility is influenced by low pH and low phosphorous concentration.

From the observations it is clear that the salts are absorbed by the roots in the form of ions and are principally translocated through xylem to the aerial parts. A portion of it is laterally translocated into the phloem tissue, and to some extent the solutes are again translocated to the lower parts from the senescing leaves. Thus the minerals are being recycled within the plant.

5.6. LONG DISTANCE TRANSLOCATION OF SUBSTANCE THROUGH THE PHLOEM

The important substances that are translocated through the phloem are sugars from the leaves. Sugars are translocated during day time through the phloem to the other parts where they are utilized or to the storage parts like some roots, stems and fruits.

5.6.1. Rate of Translocation

Various methods are adopted to measure the rate of translocation. Viruses are used by some workers for this purpose. Virus is translocated along with the carbohydrates from the source to sink. Viruses are injected at one part and the time taken for the development of the virus-induced disease symptoms at other places is noted. Based on this, the rate of translocation is calculated. The plant growth regulator, 2,4 dichlorophenoxy acetic acid (2,4-D) which induces curvature in petioles is also used to measure the rate of translocation by injecting the 2,4 -D to the upper parts of the plant and noting the time taken for the curvature of the petiole at different heights. Based on these methods and also the use of radio- isotopes the rate of translocation is estimated to be 100 cm to 200 cm per hour. The phloem must be in a living condition for translocation of the solutes.

5.6.2. Methods of Translocation

Recent observations on translocation of solutes reveal that there are various methods of translocation and some substances may be translocated in different ways.

Principally there are two methods of translocation (1) Cytoplasmic streaming (2) Pressure flow hypothesis or mass flow hypothesis.

1. Cytoplasmic Streaming: Cytoplasmic streaming or cyclosis was first described by the Death Botanist Hugo-de-Vries in 1885 as a means for solutes translocation within the cells. In essence, the theory assumes that solute particles are caught up in the circulating cytoplasm of the sieve tube element and carried from one end of the cell to the other. Presumably, these particles pass across sieve plate areas by diffusing through cytoplasmic strands connecting one sieve tube element to other. Curties, supported the phenomenon of cytoplasmic streaming as one of the methods of translocation of solutes in plants. It has been observed that the rates of cyclosis are affected by temperature change and other factors affecting motabolism in the same way as the rates of translocation.

However, experimental evidence shows that as sieve elements mature the rate of cytoplasmic streaming in them decreases and finally stops altogether. Further, it has been observed that, in parenchyma cells, cyclosis does not increase the rate of Cl^- ion translocation, which is in fact independent of streaming rates. Experiments with fluorescent dyes on protoplasmic streaming exhibited unidirectional translocation. This observation is against the view that the translocation of solutes is bidirectional. From the above observations it is concluded that distant translocation of solutes is not dependent on cytoplasmic streaming.

2. Mass Flow Hypothesis or Pressure Flow Hypothesis: Much, (1930) a German scientist proposed the mass flow hypothesis. This hypothesis is based on the assumption that turgor pressure gradient exists between the supplying and receiving tissues, which is a driving force for translocation of solutes to the distant parts. The metabolites are thought to be carried passively along the positive direction of the gradient. In other words, in the pressure flow system, we have a unidirectional flow of solutes and water through the sieve ducts driven by a turgor pressure gradient. He has demonstrated in through a physical model (Fig.5.5).

In this model there are two osmometers A and B permeable only to water. The osmometer "A" contains a higher sugar concentration than "B" and both osmometers are submerged in water. The osmometers or water containers have open connections, that offer little resistance to the flow of solutes and water. Since the model is a closed system and the walls of the osmometers are differentially permeable, water will enter "A" as the concentration of solute is more in "A" than the outer medium and a small amount of water enters "B" as the water potential is less in that.

As the amount of water that entered "A" is more than "B" the turgor pressure increases in "A" and this pressure flows to "B" thus causing the mass flow of water from A to B. Since, the solution in "B" is having less free energy than the outer medium, water from "B" will be forced to outer medium. If the walls of "B" are elastic, a positive water potential will develop in "B" as a result of above conditions. Thus a circulating system is created. Water is induced to flow from A to "B". Solutes being carried along passively. Water is pushed out of B as a result of the negative water potential that has developed and is recirculated via the open connection between the water containers. "A" is then the suppling osmometer and "B" is the receiving osmometer.

If we apply the above system to the plant "A" would represent the supplying cells of the leaf and "B" the receiving cells of some plant organ.

The connecting links between the osmometers and water containers would represent the sieve and xylem ducts respectively. As the sugars are utilised by the cells, the concentration of solutes in the sink will always be less, there is no scope for developing an equilibrium unlike in Munch model.

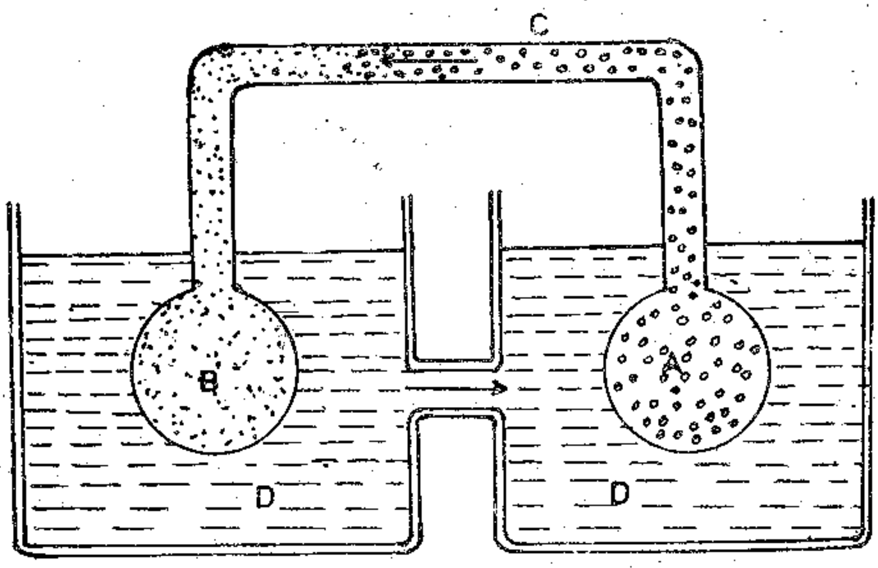


Fig.5.5 A simple physical system demonstrating the mass or pressure flow hypothesis. A&B. Osmometers. C. Tube connecting the two osmometers. D. Solution with less concentration surrounding the osmometers.

Evidence for Pressure flow in Plants: Zimmerman from his experiments proved that, there exists an osmotic pressure gradient from the stem tip to the roots. In other words, the source, that is, the supplying centres-the cells of the leaves are having positive pressure and the sinks-the utilizing centre the cells of roots and other lower parts are having negative pressure.

On defoliation of the plant this pressure gradient disappeared supporting the concept of pressure flow from top to bottom of the plant.

Studies of phloem translocation also gave considerable evidence in support of the pressure flow theory. The common observance of phloem exudate moving out of an incision in the stem of *Borassus* is estimated to be 11 liters per day. That means the rate of translocation in sieve tubes is 100-500 cm per hour.

The apoplast system surrounding the cells in plants is similar to the surrounding medium with less concentration as in Munch model. The apoplast system usually consists of soil water with less concentration, showing less negative water potential.

With this picture, in our mind we can see how sugars are translocated from supplying to receiving organs without any expenditure of energy.

Some facts against pressure flow: The pressure flow accounts only for unidirectional flow of metabolites. But it is generally accepted that bidirectional movement takes place in plants. Crafts has suggested that the leaf may serve two "sinks" one towards the apex and one towards the root, Recent experiments withradioisotopes revealed that same substance or different substance translocated in two directions in the same sieve tube at a time. In a very elegant study of phloem transport in bean plants Biddulph and Cory, using $^{14}CO_2$ feeding and fluorescence techniques, have shown that leaves nearest to the top of plant transport to the stem apex, while leaves in an intermediate position translocate metabolites in both directions.

There is some evidence that the translocation of some substances is under the influence of other inorganic elements. For example (1) Sugar translocation is influenced by the presence of boron. There is evidence that boron deficiency decreases the enzymatic conversion of glucose-

1-P to starch. (2) The translocation of growth regulator 2,4-D has been adversely influenced by phosphorous deficiency. Such type of translocation of substance under the control of other substances is known as "dual transport". Thus the rate of translocation of some solutes is under the influence of some other substances.

The other important argument against the pressure flow is that the pores of sieve plates are plugged with callose, thus interfering with the translocation. But it was assumed that all the sieve plates may not be plugged with callose at one time and the remaining functional sieve plates are enough to carry on translocation efficiently.

Some facts suggested that the translocation of metabolites is not a passive process but requires energy. First of all the pre-requisite to phloem translocation is that sieve tube elements have to be in living condition to translocate. In addition, the translocation of solutes is influenced by temperature and metabolic inhibitors in much the same manner as other physiological processess. These facts suggest that the translocation of metabolites is an active process. Cell to cell movement of solutes in the leaf tissues and final dumping of the solutes into the sieve tube elements could be considered as active process requiring energy. Recently it was pointed out that there exists some specialised cells known as transfer cells, that aid in transferring the solutes from chlorophyll containing cells to sieve tubes. To maintain the cytoplasm and the membrane of sieve tube, energy is required and this energy is available for mature sieve tube elements from the adjacent companion cells as the maturing sieve tubes contain less amount of cytoplasm, vacuoles, mitochondria etc.

Check Your Progress - 2

What are transfer cells?

Note: (a) Write the answer in the space given below.

(b) Compare your answer with the one given at the end.

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5.6.3. Electro-Osmosis Theory

Fensom and Spanner assumed that the translocation is carried out due to electro osmotic potential at sieve plates occuring due to the formation of hydrogen (H^+) and bicarbonate ions (HCO_3^-) during respiration, or it may be due to active transport of potassium (K^+) ions through the differentially permeable membrane of the plates. If a charge potential gradient is established in sive elements by the removal of potassium ions from one end and their subsequent reintroduction at the other by active transport in companion cells, an electro osmotic flow of water would be induced as shown in the figure 5.6.

D.C.Spanner has shown that upto 120 molecules of water per potassium ion can be moved in this fashion. However, it seems unlikely that this electro-osmosis mechanism could be the only one responsible for phloem translocation since the amounts of active transport, or the charge potential necessary to initiate the flow are very great. Such high potentials are not experimentally proved.

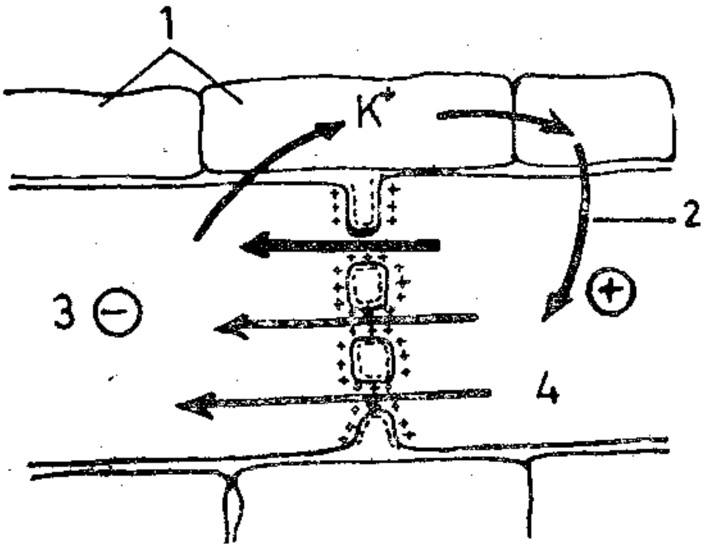

Fig.5.6 The translocation of water, solutes through the perforations of the sieve tube caused by electro-osmosis 1. Companion cells 2. Flow of potassium ions (K^+) from companion cells. 3. Sieve tube 4. Water solutes.

5.6.4. Lateral Transport

So far we have seen that distant translocation of solutes is facilitated by sieve tubes. General studies of translocation patterns shows that materials move laterally also. When the radioactive sugars are fed to a leaf of sunflower plant on one side, the labelled metabolites were translocated laterally in radial manner and were found : the seeds that are in the same radius. When one or two leaves are removed on one side, metabolities from other part of the leaves moved to that side. Zimmerman proved that lateral translocation is also taking place in a tangential direction.

5.7. SUMMARY

In the plants, solutes are translocated from supplying cells (source) to utilizing cells (sink) of various organs. Specialised cells of phloem tissue called sieve tube elements are involved in the translocation of organic solutes. Girdling or ringing experiment proves that phloem is involved in translocation of organic solutes and the direction of translocation of solutes depends upon the source and sink. Principally, the salts, in the form of ions along with soil water are translocated through xylem vessels from roots to the aerial parts. Minerals are recycled within the plant through xylem to various parts. A portion of phloem is involved in the translocation of nutrients including the inorganic nutrients from senescing leaves to growing tips. The rate of translocation of organic nutrients is estimated to be 100 to 200 cm per hour by using radio-active isotopes and other methods. Cytoplasmic streaming and pressure-flow hypothesis or mass flow hypothesis are the two principal methods of translocation. Mass flow or bulk flow hypothesis is otherwise known as "Munch hypothesis". Bidirectional flow of solutes is observed in plants through sieve tubes. Cytoplasmic streaming is a slow process and accounts for translocation of solutes between adjacent cells but does not justify distant translocation. Lateral translocation is also observed in the plants.

5.8. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The total protoplast of cells that is lying within the boundary of the outer plasma membrane of the cells (except the vacuole) in the roots is called symplast.

2. Some cells aid in transferring the solutes from chlorophyll containing cells to sieve tubes. These cells are called transfer cells.

5.9. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. Explain girdling or ringing experiment. What are the conclusion you arrive at from that experiment?
2. What is the tissue involved in the translocations of solutes? Describe its structure with a diagram.
3. Explain cytoplasmic streaming and its role in translocations of solutes.
4. Explain the model given by Munch to demonstrate the pressure flow hypothesis. How are you going to apply the above model to the plant ?
5. Discuss the evidences for and against pressure flow hypothesis in plants.

II. Answer the following questions in about 10 lines each.

1. Explain the structure of sieve tubes.
2. Explain the terms source and sink.
3. Explain the terms apoplast and symplast.
4. Explain the method of cytoplasmic streaming.
5. Explain the lateral transport of solutes in plants.

UNIT-6: STRUCTURE, NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Contents

6.1. OBJECTIVES

By the end of this unit you will be able to:

1. list out the properties of the enzymes,
2. describe the method of nomenclature of enzymes,
3. list out the different groups and sub-groups of enzymes,
4. define the cofactors, holoenzymes etc.,
5. list out the different factors that effect enzymatic activity, and explain their role,
6. describe the enzymes structure and mechanism of action,
7. list out the different inhibitors that inhibit the enzymes, and
8. define isozymes.

6.2. INTRODUCTION

Soluble enzymes are proteins that catalyse various metabolic reactions of living organisms. Enzymes are biological catalysts of the cell occurring in several hundreds and carry out the various biochemical reactions including biosynthesis of enzyme proteins. Their rate of synthesis is under genetic control. Their activity is, however, strongly controlled by substrate concentration pH and temperature. They are exceptional catalysts because of their efficiency in the turnover number (number of substrate molecules metabolized per enzyme molecule per minute) i.e., rapid rate at which they catalyse reactions under optimal conditions. They catalyse a broad spectrum of reactions and are specific to the nature of reaction catalysed and to the structure of the substrate utilized.

According to Dixon and Web (1964)an enzyme is "a protein with catalytic properties due to its power of specific activation". Buchner brothers (1897) extracted crude enzymes for the first time from yeast cells and concluded that these enzymes catalyze the fermentation of sugar to alcohol. The protein nature of the enzyme was first shown by James Summer (1926), who isolated urease in a crystalline form from jack bean. Northrop and his associates (1930) confirmed the protein nature of enzymes by isolating and crystallizing, many proteolytic enzymes. There are about 2,000 different enzymes many of which have been purified and crystallized.

6.3. PROPERTIES OF ENZYMES

Like inorganic catalysts enzymes assist and accelerate biochemical reactions. Some of the important properties of enzymes are,

1. Enzymes are needed in small concentrations to convert a large amount of substrate to product. The two terms 'substrate' and 'product' designate the starting and ending substances of a chemical reaction respectively.

   A single enzyme molecule can work over and over again to convert thousands of molecules of substrate into product. One unit of enzyme is defined as "the amount of enzyme which will catalyse the transformation of 1 mole of substrate per minute under defined conditions". Specific activity is expressed as units of enzyme per milligram of protein.

2. Enzymes remain unaffected by the reaction which they catalyse and they are stable due to protein nature.

3. Although an enzyme will speed up a reaction, it will not affect the equilibrium of the reaction.

4. The specificity of the catalytic function of an enzyme is due to its protein nature. The region of the protein which binds with the substrate and thus participating in the conversion of substrate to product, is known as active site.

How does an enzyme enhance the rate of chemical reactions? This can be answered by describing what happens when substance 'A' is converted to substance 'B', first in the absence of the enzyme and then in the presence of the enzyme.

In a given population of molecules of substance 'A' at specific temperature, the majority of them possess average kinetic energy. Some of the molecules possess more or lower energy than the average kinetic energy because of collisions between them. These are known as energy-rich and energy-poor molecules. A chemical reaction such as A–>B takes place because a certain fraction of 'A' molecules possess more energy than the rest of the population and it is sufficient to bring them up to the top of the energy level, to a reactive form known as transition state.

The energy over and above the average level that is required for 'A' to react and then be converted to 'B' is called the activation energy of the reaction. The activation energy of a reaction is " the amount of energy in calories required to bring all the molecules in one mole of a substance at a given temperature to the transition state at the top of the energy barrier". At this juncture, there is equal probability for them to undergo reaction to form products (B) or to fall back into the pool of unreacted molecules of 'A'. The rate of any chemical reaction is proportional to the concentration of the transition state species.

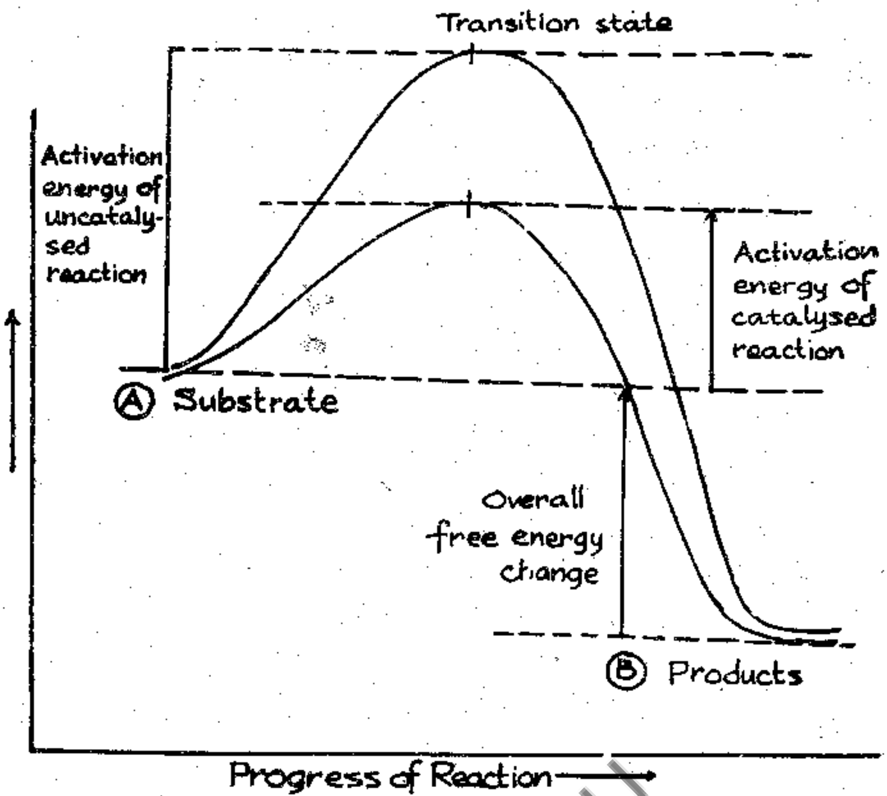

Fig.6.1. Energy diagrams of catalyzed chemical reactions.

The rate of a chemical reaction will be high if a large fraction of 'A' molecules is in the energy-rich transition state and the rate will be less if only a small fraction of 'A' is in the transition state. But the rate of a chemical reaction can be increased by two ways. One is to increase the temperature by which the molecules attain higher energy level to enter the transition state. The second way to increase the reaction rate is to add a catalyst (enzyme). Enzymes function by lowering required activation energy. They achieve this by combining with the substrates to produce a transition state complex which has a lower energy of activation than the uncatalyzed reaction.

The enzyme substrate complex, ES, then reacts to form the product 'P' releasing the free enzyme. This freed enzyme can combine with another molecule of 'A' and repeat the cycle. In this way enzymes lower the activation energy of chemical reactions are allowing a much larger fraction of the molecules in a given population to react per unit time in the presence of the enzyme (catalyst).

6.4. NOMENCLATURE & CLASSIFICATION OF ENZYMES

Systematic nomenclature as well as knowledge of trivial systems is of importance in the study of enzymes. Most trivial names give the nature of the substrate involved and the nature of the catalysed reaction. Most such names end with the suffix "ase". For e.g., dehydrogenases, phosphatases, transferases, mutases, synthetases etc.

Enzymes are also named by adding the suffix "ase" to the name of the substrate as ATP ase, ribonuclease, aconitase etc. Some names bear no relationship to the substrate or type of reaction catalyzed or several names are employed for one and the same enzyme. This system has its own limitations. Some of the enzyme names have been approved by the Enzyme Commission as acceptable alternatives.

With the discovery of thousands of enzymes it was necessary to standardise the classification and nomenclature of enzymes. Hence the Commission on Enzymes of the International Union of

Biochemistry (1964) have proposed a systematic classification and nomenclature for the enzyme catalysed reactions by employing numbers specifying groups and sub-groups. According to this classification, enzymes are divided into six broad groups.

6.4.1. Oxidoreductases

These enzymes catalyse oxidation-reduction reactions by involving hydrogen transfer. The include three different sub-groups.

1. Dehydrogenases: Transfer hydrogen atoms from substrate to NAD^+.
2. Reductases: Add hydrogen atoms to substrate.
3. Oxidases: Transfer hydrogen atoms from substrate to oxygen.

6.4.2. Transferases

Transfer chemical group such as amino, or phosphate group from one molecule to another (catalyse group transfer reactions).

1. Phosphorylases: Add orthophosphate to substrate.
2. Transaminases: Transfer amino group from the substrate to other molecule
3. Kinases: Transfer phosphate from ATP to substrate

6.4.3. Hydrolases

Cleave bonds by the addition of water (catalysing hydrolytic reactions).

1. Phosphatases: Remove phosphate group from the substrate.
2. Peptidases: Involve in the cleavage of peptide bonds.

6.4.4. Lyases

These enzymes catalyse the addition of groups to double bonds or vice versa.

1. Decarboxylases: Remove CO_2 from substrate.
2. Aldolases: Catalyse aldol condensation or the reverse involved in the breakdown of hexoses into 3-carbon units.

6.4.5. Isomerases

These enzymes bring about isomerizations and include the racemases and epimerases which catalyse the interconversion of the stereoisomers of amino acids and sugars respectively.

6.4.6. Ligases

Catalyse the linking of two molecules coupled with the breakdown of ATP. The energy liberated by the ATP is used for the condensation. These enzymes are involved in some important reactions like the linkage of amino acids of tRNA.

Check Your Progress - 1 & 2

1. To which group of enzymes, the enzymes that are involved in the cleavage of peptide bonds belong?
2. Name the sub-group of the enzymes that are responsible for the removal of CO_2 from substrate?

Note:(a) Write your answers in the space given below.

(b) Compare your answers with those given at the end.

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6.5. COFACTORS

Majority of enzymes require an additional component in addition to the enzyme protein for the catalytic reaction to proceed. These ion components are known as cofactors. Co-factor is one that can be easily removed from the protein part of the enzyme by dialysis. Some enzymes contain only polypeptides such as pancreatic ribonuclease. Catalytically active enzyme together with its cofactor is known as holoenzyme. Protein part of the enzyme is known as apoenzyme. The cofactors are heat-stable whereas apoenzyme is denature by heat. The cofactors are broadly divided into two groups. 1. Metal ion activators and 2. Coenzymes.

6.5.1. Metal ion activators

These are simple inorganic ions such as K^+, Fe^{++}, Zn^{++}, Mn^{++} etc., They may be either loosely or firmly bound to the enzyme protein. In some cases metals have been built into the structure of the enzyme and will not be removed without destroying the structure. Metaloflavoproteins, cytochromes and ferredoxins fall under this category i.e., Prosthetic group. In other instances metals react reversibly with proteins to form metal-protein complexes that constitute the active catalyst.

Table 6.1. Metal ion requirements of some enzymes

Enzymes	Metal Ion
Cytochrome oxidase,	Fe^{++} or Fe^{+++}
Catalase, Peroxidase	
Carbonic anhydrase,	Zn^{++}
Alcohol dehydrogenase,	
DNA polymerase	
Isocitrate dehydrogenase,	Mn^{++}
Arginase,	
Nitrite reductase	
Cytochrome oxidase,	Cu^{++}
Polyphenol oxidase	
Nitrate reductase	Mo
Pyruvate kinase,	K^+
Aldolase,	
Acetyl CoA synthetase.	

6.5.2. Coenzymes

Coenzymes are complex organic molecules which play more active role in the reaction by carrying a specific chemical group. They may change their structure during the course of the reaction but become regenerated in subsequent reactions. A further distinction can be made between coenzymes.

The coenzymes which are tightly bound to the enzyme molecules are known as prosthetic group. These include haem groups which are covalently bound to the polypeptide chains of a number of enzyme molecules.They function as electron carriers undergoing alternate oxidation and reduction. Co-enzyume Q (ubiquinine) is an important quinone taking part in the electron transport chains of respiration and photosynthesis.

Coenzyme A (CoA) is made up of a molecule of the vitamin panthothenic acid and a molecule of ATP. It works as a group transfer agent in the decarboxylation of pyruvate and x-ketoglutarate in the oxidation metobolism and also in the oxidation of fats and acetate. Coenzyme-I is Nicotinamide Adenine Dinucleotide (NAD) taking part in electron chain transport in respiration in association with dehydrogenases. Coenzyme-II, is Nicotinamide Adenine Dinucleotide Phosphate (NADP) taking part mostly in the photosynthetic electron transport reactions. Many coenzymes are loosely attached to the enzyme molecule which move from one enzyme to another to fulfill their catalytic function, e.g., NAD, FAD & ATP.

Check Your Progress - 3

Define coenzymes.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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6.6. FACTORS AFFECTING ENZYME ACTIVITY

Enzyme activity is controlled mainly by substrate concentration, enzyme concentration, temperature and pH at which the reaction is proceeding.

6.6.1. Substrate Concentration

The enzyme catalysed reaction shows a saturation effect with increasing substrate concentration when the enzyme concentration is kept constant. At very low concentrations of substrate the rate of the reaction is very low, but with the increase in substrate concentration, the reaction rate will be increased. Further increase in substrate concentration, will cause only a marginal increase in reaction rate. Finally, a point will be reached beyond which the reaction rate will approach but never reach a plateau the, maximum rate (Vmax). At this state the enzyme is saturated with its substrate at all its active sites and this can function no further.

Michaelis & Menten (1913) postulated that the Enzyme (E) combines reversibly with substrate (S) to form Enzyme-substrate complex (ES) which eventually breaks down to yield product and the free enzyme.

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

In an enzyme catalysed reaction, the enzyme exists in two forms, the free form (E) and the combined form bound with the substrate (ES). The rate of the reaction will be maximum when all the enzyme is in ES form. This condition is observed at higher concentrations of substrate, since, by the law of mass action the equilibrium of the first reaction will be pushed towards the right side (forward reaction).

$$E + S \rightleftharpoons ES$$

The ES complex continuously breaks down to yield product and free enzyme.

But if the concentration of substrate is high enough, the free enzyme will immediately combine with other substrate molecules. So, a steady state is achieved in which the enzyme is always saturated with its substrate and the reaction rate will be maximum.

Michaelis & Menten defined a constant, Km, and it is useful in establishing the relationship between the substrate concentration and velocity of the enzyme catalysed reaction. According to Michaelis and Menten, constant (Km) may be defined as the substrate concentration at which the given enzyme yields half its maximal velocity.

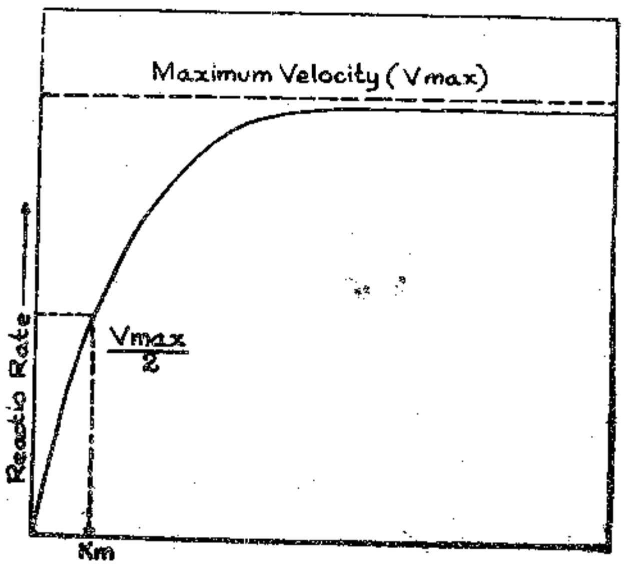


Fig.6.2. Effect of substrate concentration on the rate of enzyme catalyzed reaction, assuming that enzyme concentration is constant.

The characteristic shape of the substrate saturation curve (hyperbolic curve) for an enzyme can be expressed mathematically by the Michealis & Menten equation.

$$V_o = \frac{Vmax\ (S)}{Km + (S)}$$

Where Vo = observed velocity at given substrate concentration (S,)

Vmax = maximum velocity

Km = Michaelis & Menten constant

Michaelis - Menten equation is characteristic of any given enzyme for a specific substrate under defined conditions of pH and temperature.

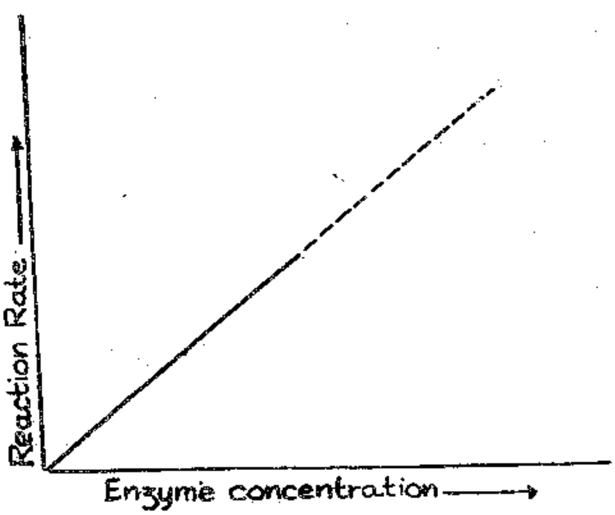


Fig.6.3. Effect of enzyme concentration on reaction rate with high substrate concentration.

6.6.2. Enzyme Concentration

To see the effect of different concentrations of enzyme on reaction rate of the enzyme activity, low concentration of enzyme is used with high concentrations of substrate. The rate of the reaction is directly proportional to enzyme concentration.

However, if the substrate concentration is relatively low, increa in enzyme concentration, increas the rate of reaction to a point and then remains constant.

6.6.3. Temperature

For majority of the enzymes, within temperatures upto $50^{0}C$, usually, the rate of a chemical reaction is approximately doubled for every $10^{0}C$ increase in temperature. This is called Q_{10} of a reaction. With regard to enzyme catalysed reaction, this will be followed upto $25^{0}C$. The increase in the reaction rate is due to the increase in kinetic energy of both substrate and enzyme molecules and this will result in increase in chances of collisions between enzyme and substrate molecules. Majority of enzymes have higher activity between 25 and $37^{0}C$. Above $40^{0}C$ onwards the enzyme activity will be de creased. It is related to the stability of the enzyme and in volves inactivation of the enzyme with increase in temperature. Higher temperature causes the change in three dimensional shape of enzyme protein resulting in loss of catalytic activity.

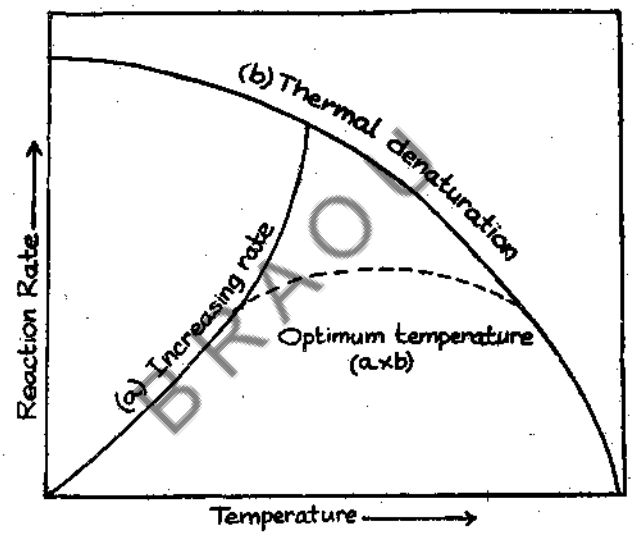


Fig.6.4. Effect of temperature on reaction rate of an enzyme catalysed reaction.

6.6.4. Effect of pH

Enzymes are sensitive to small changes in pH. Many enzymes have maximum activity at a pH near 7. Their catalytic activity decreases as the pH is shifted away from this optimum. The pH curve is shown in fig.6.5. pH influences enzyme activity in a number of ways.

1. The pH with the extreme values brings about changes in protein structure that alter the stability of the enzyme.
2. pH changes the ionisation of the enzyme, thus affecting the affinity of the enzyme for the substrate.
3. It alters the ionisation of the substrate and affects the binding of the substrate to the enzyme.
4. pH changes the ionisation of enzyme-substrate complex.

Factors 2 and 3 will affect Km value whereas factor 4 will affect the Vmax of the reaction and not Km.

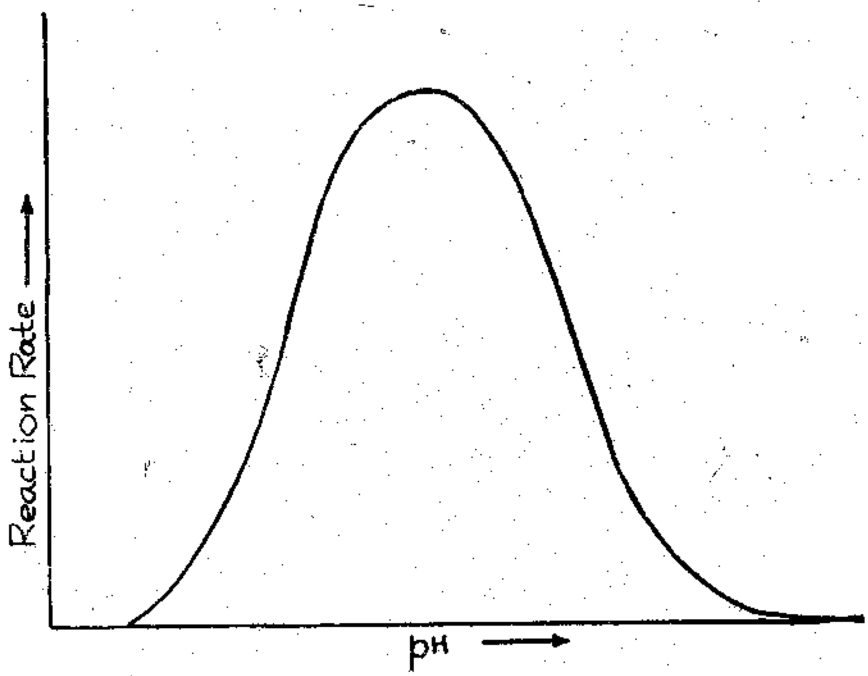


Fig.6.5. Effect of pH on enzyme catalysed reaction.

6.7. ENZYME STRUCTURE AND MECHANISM OF ACTION

The molecular weight of enzymes ranges from about 10,000 to over one million and contains many thousands of amino acids. Substrate molecules are very small compared to the enzymes so only a small part of the enzyme (polypeptide chain) will be in contact with the substrate in enzyme- substrate complex. The part of the enzyme molecule at which activation and reaction of the substrate occurs is known as active centre or active site of the enzyme.

The catalytic ability of an enzyme depends on its three-dimensional shape. The catalysis of an enzyme catalysed reaction consists of three steps in which individual enzyme molecules participate over and over again.

1. The substrate recognises the enzyme and the two come together in a specific arrangement- enzyme - substrate complex.
2. By the catalytic activity of certain groups on the enzyme surface, the substrates will be converted to products.
3. The products leave the enzyme.

The mechanism of chymotrypsin action is shown in Fig.8.6. Chymotrypsin is a digestive enzyme which catalyses the hydrolysis of protein in the intestine of human beings.

The substrate fits on the active site of the enzyme like pieces in a three dimensional jigsaw puzzle. The substrate and enzyme lock together to form the enzyme-substrate complex. The two are held together by weak bonds such as hydrogen bonds.

The conversion of substrate to product depends on certain amino acid R groups which protrude into and are an integral part of the active site.

Each enzyme catalyses only one type of reaction, since the site of each enzyme is designed to hold a particular substrate and to catalyse one reaction very effectively. It will not have the correct shape and charge of catalytic R groups necessary to promote totally different reactions.

The active site consists of two parts: a hydrophobic binding site which holds aromatic R groups and a catalytic site, where peptide bond cleavage occurs. Three amino acid R groups are responsible for catalysis: The R groups of serine, at position 195 in the amino acid chain, histidine and aspartic acid at position 57 and 102 respectively (After T.W.Goodwin, J.I.Harris & B.S.Hartley, 1964).

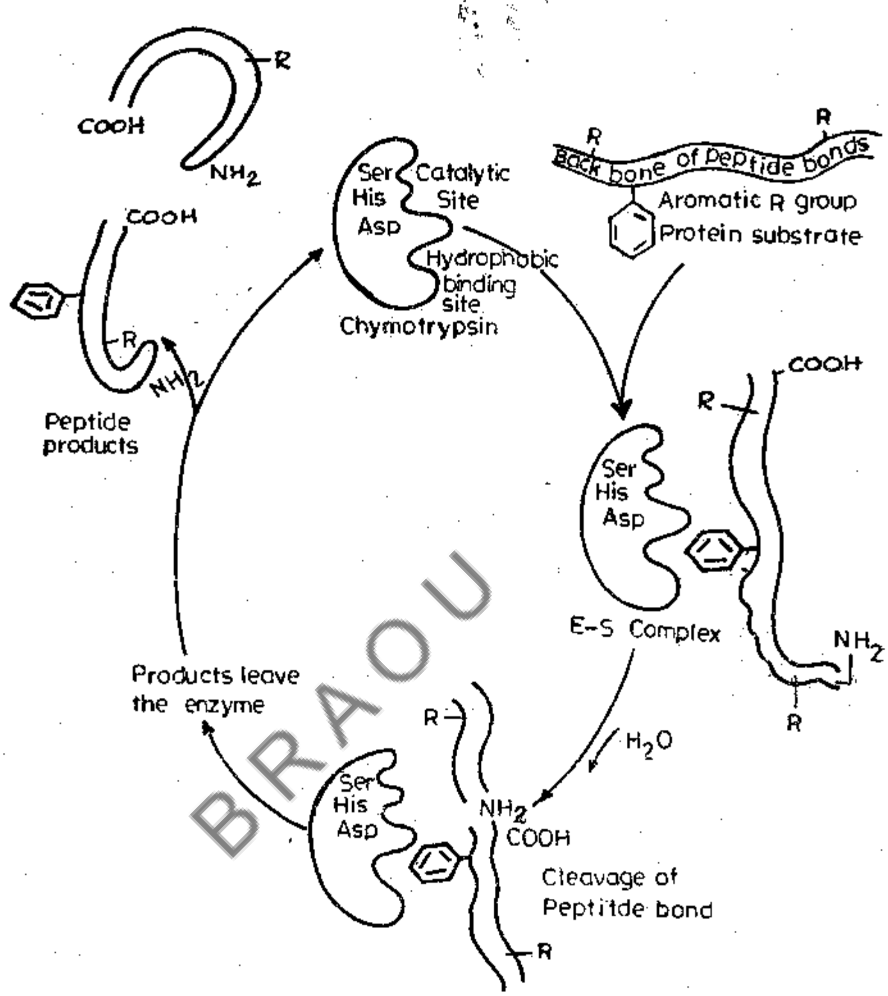


Fig.6.6. Mechanism of chymotrypsin action.

6.8. INHIBITORS OF ENZYMES

The compounds which have the ability to combine with certain enzymes and block the catalytic protein, are known as inhibitors of enzymes.

Inhibitors are divided into two classes depending on the nature of inhibition. 1. Irreversible inhibitors, 2. Reversible inhibitors.

6.8.1. Irreversible Inhibitors

Irreversible inhibitors form covalent bonds with a specific functional group, usually an amino acid residue, which is associated with the catalytic activity of the enzyme. There are some inhibitors which physically block the active site.

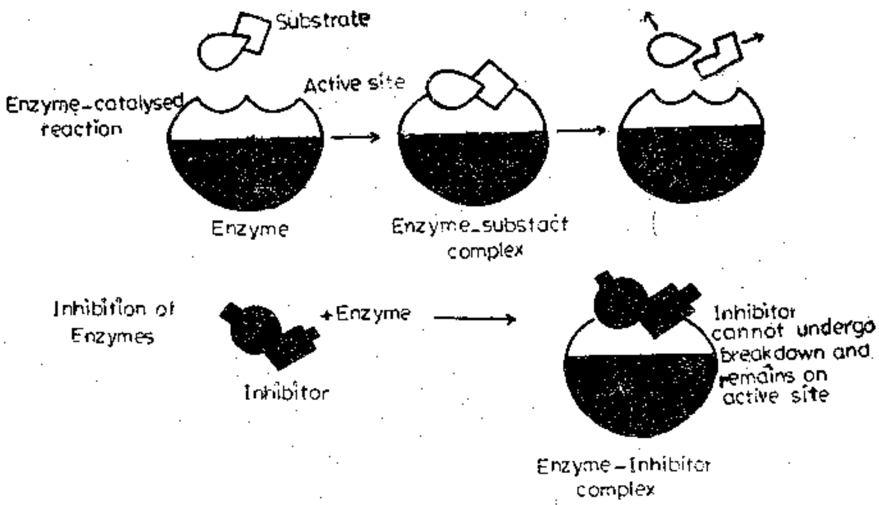


Fig.6.7. Characterstics of enzyme inhibtion.

These inhibitors cannot be removed by dialysis and this usually means that the functional groups of the enzyme molecule have been altered or destroyed.

$$
\begin{array}{c}
E + S \rightleftharpoons ES \rightleftharpoons E + P \\
+ \\
I \\
\downarrow \\
EI
\end{array}
$$

Example: Iodoacetate which reacts with sulfahydral groups of an enzyme such as triose phosphate dehydrogenase.

$$E\text{-}SH + ICH_2COOH \longrightarrow E\text{-}S\text{-}CH_2COOH + HI$$

6.8.2. Reversible Inhibitors

The reversible inhibition means that the enzyme activity can be restored by removing the inhibitor by dialysis. The inhibitor involves the equilibrium between inhibitor anqud free enzyme which can be anly sed by Michaelis-Menten Kinetics.

The reversible inhibitors are classified into two main types (a) Competitive Inhibitors, (b) Non Competitive Inhibitors.

(a) Competitive Inhibitors: Compounds that compete with the substrate for binding to the active site but once bound, cannot be transformed by the enzyme are known as competitive inhibitors.

$$
\begin{array}{c}
E + S \rightleftharpoons ES \rightleftharpoons E + P \\
+ \\
I \\
\uparrow\downarrow \\
EI
\end{array}
$$

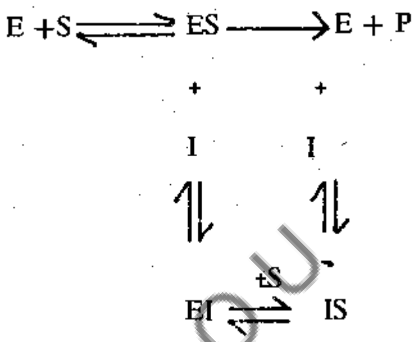
ES and EI complexes are formed but EIS complexes are never formed. So the inhibition can be reversed or relieved by increasing the substrate concentration.

Ex: The classical example of the competitive inhibition is the inhibition of the enzyme, succinic dehydrogenase by malonic acid. Succinic dehydrogenase catalyses the oxidation of succinic acid to fumaric acid.

Succinate Malonate

The two molecules each having two ionisable carboxyl groups compete for the same site on the enzyme molecule.

(b) Non Competitive Inhibitors: Compounds that reversibly bind with either the enzyme or the enzyme - substrate complex are known as non competitive inhibitors.



Non competitive inhibition differs from competitive inhibition in that the inhibitor can combine with ES, and S can combine with EI to form the inactive complex EIS. So this type of inhibnition is not completely reversed by increasing the substrate concentration.

Ex: The inhibition of L-threonine dehydratase by L-isoleucine.

Check Your Progress - 4

What are inhibitors of enzymes? And what are the different classes recognised in them?

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

6.9. ISOZYMES

An enzyme which has multiple molecular forms found in the same organism catalysing the same reaction is known as 'Isozyme'. Isozymes differ from one another in having different amino acid composition or sequence and have different kinetic properties. One of the first enzymes found to have multiple forms and the most thoroughly studied isoenzyme is lactic dehydrogenase, which catalyses the reversible oxidation-reduction reaction.

$$\text{Lcatate} + NAD^+ \rightleftharpoons \text{Pyruvate} + NADH + H^+$$

Lactate dehydrogenase occurs in five possible forms in organs of many vertebrates. Two important types of LDH are seen in heart and skeletal muscles and they are known as HLDH and MLDH respectively. The heart LDH contains four identical monomers which are known as H sub units and the muscle LDH also consists of four identical M sub units. The two sub units, H and M have the same molecular weight (35,000) but differ in the amino acid composition. Although isoenzymes catalyse the reaction, they differ significantly in their K_m values for their substrates as well as their Vmax values. Isoenzymes are now known for a great many different enzymes. There are about one hudred different enzymes participating in cellular metabolism which occurs in multiple forms.

6.10. QUANTITATIVE ASSAY OF ENZYMATIC ACTIVITY

The amount of enzyme in a given solution or tissue extract can be assayed quantitatively in terms of the catalytic effect in produces. For this purpose. It is necessary to know:

1. The overall stoichiometry of the reaciton catalysed.
2. Whether the enzyme requires the addition of cofactors such as metal ions or coenzymes.
3. Its dependence on substrate and cofactor concentrations i.e., Km for both substrate and cofactor.
4. Its optimum pH.
5. A temperature zone in which it is stable and has high activity.
6. A simple analytical procedure for determining the disappearance of the substrate or the appearance of the reaction products.

The quantitative assay of enzyme activity is carried out most conveniently when the substrate or product is coloured or absorbs light in the ultraviolet region which can be followed with a spectrophotometer.

It is possible to purify the enzymes and obtain them in homogenous forms by using a sequence of seperation procedures such as density gradient centrifugation, molecular-exclusion chromatography, free or zone electrophoresis, ion-exchange chromatography etc.

6.11. SUMMARY

Various metabolic reactions of living organisms are catalyzed by enzymes. Enzymes are proteins and are generally referred to as biological catalysts. Biosynthesis of these enzymes is under genetic control. Under optimal conditions they catalyze reactions at rapid rates. They are specific to the nature of reaction catalysed and to the structure of the substrate utilized. A small concentration of enzyme can convert a large amount of substrate to product. Enzymes remain unaffected by the reaction and are not consumed during the process. Generally, enzymes are named after the substrate or type of reaction catalysed and end with a suffic 'ase'. The Commission on Enzymes of the International Union of Biochemistry (1964) have proposed a numerical classification. Enzymes are divided broadly into six groups. Some enzymes need addition of metal ion or a coenzyme to catalyse the reaction. Various factors affect the rate of enzymatic activity. Certain compounds act as inhibitors blocking the catalytic protein by combining with the enzyme. An enzyme may have multiple molecular forms catalysing the same

reaction. These are the isozymes. An enzyme can be quantitatively assayed by using colorimeter or spectrophotometer. They can also be purified by employing certain techniques.

6.12. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The enzymes that are involved in the leavage of peptide bonds belong to the group hydrolases.
2. The enzymes that are responsible for the removal of CO_2 from the substrate belong to the sub-group decarboxylases.
3. The complex organic molecules that play an active role in the reaction by carrying a specific chemical group are called coenzymes.
4. Inhibitors of enzymes are the compounds that are able to combine with certain enzymes and block the catalytic protein. Inhibitors are devided into two classes. They are (1) Irreversible Inhibitors and (2) Reversible Inhibitors.

6.13. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. What are enzymes? Explain the role of enzymes in plants.
2. Give an account of the nature of enzymes and their classification.
3. Enumerate the properties of enzymes.
4. Describe the structure of enzymes and explain their mode of action.
5. Distinguish between cofactors, coenzymes and prosthetic groups.

II. Answer the following questions in about 10 lines each.

1. Write about isoenzymes.
2. Give an account of non-competitive inhibitors.
3. Write about reversible inhibitors.
4. Explain the factors affecting enzymatic activity.
5. What are competitive inhibitors? Give some examples.

UNIT-7: PHOTOSYNTHETIC PIGMENTS AND PHOTOCHEMICAL REACTIONS

Contents

7.1. OBJECTIVES

By the end of this unit you will be able to:

1. list out the various pigments that are involved in photosyntheis,
2. list out the differences between chlorophyll a and b
3. describe the biosynthesis of chlorophyll,
4. list out the various carotenoid pigments and describe their functions,
5. define quantum efficiency,
6. describe the Emerson enhancement effect,
7. describe the process of oxygen evolution in photosynthesis,
8. describe the three kinds of photophosphorylation viz., cyclic, noncyclic and pseudocyclic photophosphorylation.

7.2. INTRODUCTION

All living organisms need energy to sustain. The radiant energy is derived from sunlight via the process of photosynthesis. In obligate photoautotrophs as higher plants, algae and some bacteria, light is the sole exogenous energy source used for the synthesis of organic materials. In comparison, heterotrophic organisms are absolutely dependent upon organic compounds formed by the autotrophs for their carbon and energy requirements. Thus, photosynthesis can be defined as a biological process where organic matter is synthesized with the help of light.

7.3. PHOTOSYNTHETIC PIGMENTS

Photoautotrophic organisms are unique in possessing coloured complex organic molecules, pigments, which trap the light and convert radiant energy into chemical energy. These pigments act as reaction centers (RC) in absorbing the light which is in the form of electromagnetic energy and convert it into chemical energy. They are also referred to as antenna pigments. One can study the different types of pigments by employing method such as paper chromatography or by chemical separation of chloroplast pigments. These techniques help in separating the different types of pigments such as chlorophyll a, b, carotenoids and phycobilins. The pigments are grouped into functional "units" with photochemical conversion centers and other catalysts and are located in membrane-bounded flattened sacs (thylakoids). However, in higher plants a number of thylakoids are stacked into a granum. 10-30 such grana are found in special organelles-chloroplasts.

The various photosynthetic pigments can be classified into three major groups 1) Chlorophylls (water insoluble); 2) Carotenoids (water insoluble and 3) Phycobilins. Phycobilins are water soluble because of their attachment to water soluble proteins.

Check Your Progress - 1

What are the major groups into which the various photosynthetic pigments are classified?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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7.3.1. Chlorophyll Pigments

Chlorophyll A: is the primary photosynthetic pigment present in all higher plants and algae. It is the major pigment and plays an active role in photosynthesis. Several forms are recognized: Chl a 660, 670, 680, 685, 690, 700, 720, the number indicating their respectiv read absorption maxima.

Chlorophyll b: is present in all the higher plants and green algae and is a locally oxidized derivative of Chl a. It is normally identified by its yellow-green colour while Chl a is identified by blue-green colour. It has been shown that there are two forms of Chl b--640 and 650. Molecular structure, molecular formula and empirical formula of Chl a and b are given in Fig.7.1.

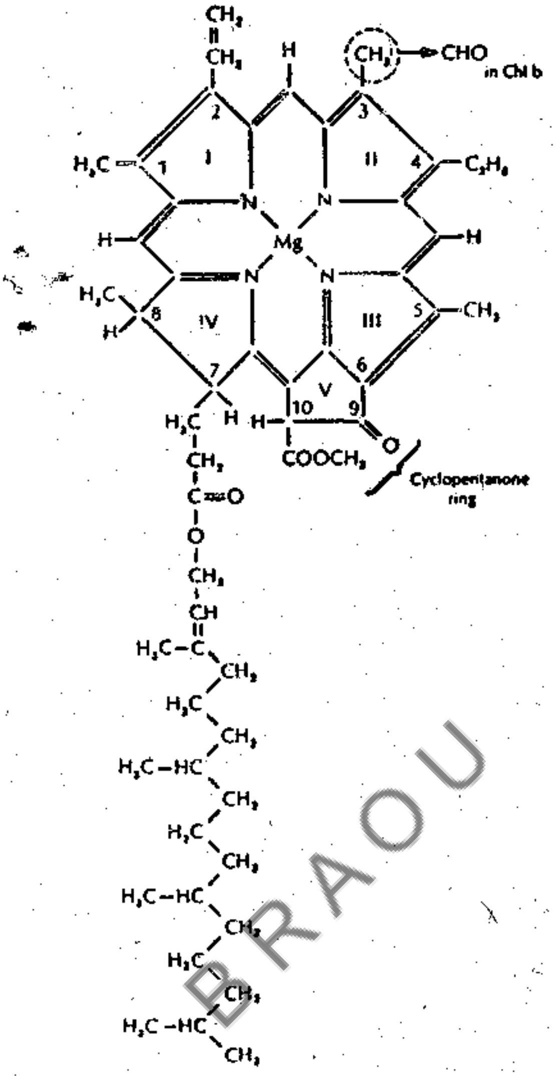

Fig.7.1. The molecular structure of chlorophyll a&b. To form chlorophyll b an aldehyde group replaces the methyl group at the C_3 atom. Empirical Formula: Chl a $C_{55}H_{72}O_5N_4Mg$, Chl $C_{55}H_{70}O_6N_4Mg$

Molecular Structure of Chlorophyll: The structure of a chlorophyll molecule roughly resembles a "tennis-racket" having a large flat head (the porphyrin part) and a long handle or tail (the phytol part). It is a typical porphyrin derivative possessing a cyclic tetrapyrrolic structure in which one pyrrole ring is partially reduced. The four pyrrole rings are joined into a single master ring by CH "bridges". A non ionic magnesium atom is held at the centre of the tetrapyrrole by two covalent and two coordinate bonds. A peculiar and important characteristic of clorophyll, in addition to the four pyrrole rings is the presence of a fifth isocyclic ring which carries a carbonyl group (C=O). This V additional ring may be the "nerve centre" of the chlorophyll molecule, most closely associated with its photocatalytic action in photosynthesis. The phytol which is esterified with the carboxyl group on the C_7 atom of the chlorophyll molecule is a long chain alcohol containing one double bond. To form Chl b the methyl group at position 3 is replaced by an aldehyde group. In its natural state it is bound to protein to form a lipo-protein complex.

The German chemists Richard Willstatter and Hans Fisher (1940) received Nobel Prize for the development of the chlorophyll structure. Robert B.Woodward of Harvard (1965) received

Nobel Prize for total *in vitro* synthesis of chlorophyll.

Although Chl a&b closely resemble each other in molecular structure, definite differences in their physical and chemical properties have been observed.

The ratio of Chl a to b may vary between about 2 and 6 in different species and in the same species grown under different conditions. Apparently many plant species form more Chl b when grown under low light intensity in order to increase their light harvesting capacity.

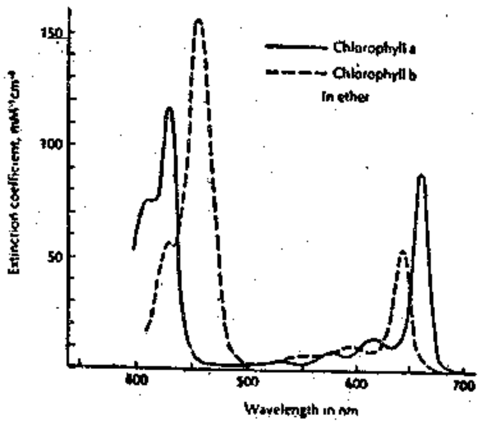

Fig.7.2. Absorption spectra of Chlorophyll a&b in ether. These are the absorption spectra of extracts of chlorophylls in organic solvent.

Petroleum ether is a good solvent for Chl a and methyl alcohol for Chl b. Chl a is blue-green in colour and Chl b yellow-green.

A pheophytin is chlorophyll without Mg and a phaeophorbide is without both Mg and the phytol. Acid conditions promote the loss of Mg from chlorophyll.

All phototrophic bacteria with anoxygenic (anaerobic) photosynthesis, which have only one photosystem, possess bacteriochlorophylls in their membrane bound photosynthetic apparatus. It is the main light harvesting pigment of photosynthetic bacteria. It is a tetrahydroporphyrin.

Bacteriochlorophyll a: This is most widely distributed among the purple bacteria, Chromatiaceae and Rhodospirillaceae. In organic solvents it has absorption maxima at 360, 590 and 760 nm. For e.g., *Chromatium* has peaks at 800, 850 and 870nm. The Bchl a of all known purple bacteria is Bchl ap in which the propionic acid side chain is esterified with phytol (cloe and Pfenning, 1974). In *rhodospirillum rubrum* Bchl a is esterified with geranyl- geraniol (Bchl a Gg) (Kunzler and Pfenning, 1973).

All green bacteria contain small amounts of Bchl ap located in the photosynthetic reaction centers in the cytoplasmic membrane near the chlorobium vesicles.

Check Your Progress - 2

What are the differences between chlorophyll a&b with regard to their solubility and colour?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Biosynthesis of Chlorophyll: As mentioned earlier chlorophylls contain tetrapyrrole ring and Mg chelated with nitrogen atom of pyrrole rings. The biosynthesis of chlorophyll molecule takes place through several steps and they are light mediated. Briefly, the synthesis involves the formation of the pyrrole (porphobilinogen) by condensation of two molecules of δ-aminolevulinic acid. Four pyrrole molecules complexed with Mg and a protein give rise to protochlorophyllide. This is reduced in the presence of light to give rise to protochlorophyll, which after phytol estirification and in the presence of light forms chlorophyll-a molecule.

7.3.2. Carotenoid Pigments

They are widely distributed and invariably occur with chlorophylls in photosynthetic organisms where they function both as photoreactants and with varying degrees of efficiency in tapping light energy for photosynthesis. The total number of carotenoids in all these classes of organisms is about 60. Carotenoids belong to a large group of compounds called terpenoids. They are divided into 2 classes: 1) the carotenes which are hydrocarbons and their oxygen derivatives 2) the xanthophylls. Common names used for different species end with-ene and those for xanthophylls with -in.

According to Goodwin (1966), the major carotenoids of higher plants are β- carotene, lutein, viloxanthin and neoxanthin. β-carotene is the most prevalent carotene and lutein the most abundant xanthophyll. The chloroplast carotenoids occur in two granal chromoproteins corresponding to PS I and PS II.

In some higher plants, small amounts of α-carotene may accompany β - carotene. Lutein, is a derivative of α-carotene.

Function in Photosynthesis: Like chlorophyll, carotenoid also occur in their natural state as protein complexes. Chlorophylls and carotenoids may be attached to the same protein forming a complex, known as photosynthin.

Carotenoids play two distinct roles in photosynthesis.

1. Chlorophyll gets bleached in the presence of oxygen and light. This is called photo-oxidation. Photo oxidation of chlorophyll is inhibited in the presence of carotenoids.
2. Carotenoid absorb yellow and green wave lengths of light and transfer the energy of the light to chlorophyll molecules. In otherwords, carotenoids are light gathering pigment molecules which act as "antennae" in receiving light.

Check Your Progress - 3

Name the four major carotenoids of higher plants.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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7.3.3. Phycobiliproteins

These are water soluble pigments containing open pyrrole chains found occurring in cyanophytes, phaeophytes and Rhodophytes. They are of two kinds-(a) phycoerythrin (red pigment) and (b) phycocyanins (blue). They are found together, but their relative ratios vary greatly. These pigments absorb blue, yellow and green lights and pass on the energy by resonance transfer to chlorophyll a molecule. Like carotenoids, phycobilins are called accessory pigments.

Phycobiliproteins are water-soluble complexes of protein and plant bile pigments, called phycobilins. The first complete primary structure of a biliprotein (C-phycocyanin) from *Mastigocladus laminosus* was determined by Frank et al. The main function is light harvesting and energy transfer to chlorophyll a. It is now generally accepted that this process is a step- wise migration of energy from phycoerythrin to phycocyanin to allophycocyanin to Chl a. Like carotenoids phycobiliproteins are classified as accessory pigments.

Chlorophyll is called "the most important organic compound on earth" because of its role as a light activated enzyme (photo enzyme) in photosynthesis. All other pigments seem to serve only as physical energy suppliers and are referred to as accessory pigments.

7.4. PHOTOCHEMICAL REACTIONS

Biological solar energy conversion is carried out by certain bacteria, algae and higher plants because of the existence of photosynthetic mechanism. Evolutionary studies revealed that the first prokaryotic cell could have evolved some 4 billion years ago. It is presumed that the first living cell was a photoautotroph and carried out simple photoreactions. Subsequently, a biological diversity developed in these forms with respect to autotrophic energy production.

Light is a form of radiant energy and travels in discret corpuscles or bundles known as photons or quanta. Photon or light quantum is a discrete package of energy. It is directly proportional to the frequency of the radiation. Sun is the source of the electromagnetic radiation and it forms a spectrum. Part of the spectrum visible to us is known as visible spectrum. It extends from about 400 to 700 nm and the colours range in order from violet to red (Fig.10.1). Light of a single wavelength (or a single frequency) is called monochromatic. The visible spectrum is also active in photosynthesis. Therefore, it is called photo-synthetically active radiation (PAR). Green plants utilise light at a wave length of 400 to 450 and 650 to 700 nm for maximum photosynthetic efficiency i.e., blue and red light of the visible spectrum are more efficient.

7.4.1. Absorption Spectrum and Action Spectrum

The absorption of different wavelengths of visible radiation and their degree of absorption can be estimated by passing different wavelengths of this light through a culture of *Chlorella* (an unicellular green alga) and *Ulva* Thallus representing the green leaf of the plant. The absorption of different wave lengths of light is due to different pigments found in the chloroplast i.e. chlorophylls a,b, carotenes, and xanthophylls absorbing specific portions of transmitted light. The chlorophylls have peaks of absorption at blue and red end of visible spectrum. The carotenoids have their peaks in green part of the spectrum (450 to 520 nm). The absorption of transmitted light of particular wavelength is also a characteristic of the substance absorbing the light. The absorption of light at different wavelengths of chloroplast pigments (for that matter by any substance) is called absorption spectrum.

The effectiveness of different wavelengths of light in the photosynthesis can also be estimated by measuring O_2 evolution in *Chlorella* cells enclosed in a glass container through *Ulva* thallus by transmitting different wavelengths of light through them. The activity or function of different wavelengths of light in photosynthesis (or in any process) is called action spectrum.

A comparison of absorption spectrum with action spectrum, also indicates the relative contribution of different wavelengths for photochemical reactions (light reactions) of photosynthesis.

In case of photosynthesis, it so happens that the absorption spectrum and action spectrum overlap each other. It means that blue and red light which are absorbed (wavelengths between 400 to 450 and 650-700 nm respectively) to a maximum degree are also having their maximum effect in photosynthesis and its photochemical events. The photochemical reaction require blue and red light in definite number of units of light called quanta (quantum singular). The energy carried by a quantum of blue light is 70 k cal/einstein and that of red light is 40 k cal/einstein. But for photo- chemical reactions the basic requirement is for a specific number of quanta and the energy for quantum is important. In other words, red light quanta are as effective as blue light quanta, the extra energy of blue light quanta being wasted.

7.4.2. Quantum Efficiency

The efficiency of a quantum of light of any wave length, in carrying out the function in terms of number of CO_2 molecules reduced or number of O_2 molecules evolved is called quantum yield or quantum efficiency.

7.4.3. Emersom Effect

With the help of action spectra one can study the details of the function of individual wavelengths of light i.e., its influence of the effect of monochromatic light on O_2 evolution etc. Such studies have indicated the decline of the quantum yield occurring at wavelengths beyond 680 nm in green algae. Because, this area of spectrum is in the red region the decrease quantum yield is called red drop. Study on this "red-drop" clarified many complexities involved in two photosystems. Emerson and his co-workers (1957) found during the study of red-drop mechanism in green algae, *Chlorella*, that light beyond 680 nm was quite inefficient (in far-red light) and could bemade efficient with a simultaneous illumination of a shorter wave length of light. That is, if a beam of 700 nm light simultaneously given along with a beam of 650 nm light, the oxygen production is enhanced to higher degree than the sum of O_2 produced with the two separate beams of light. This discovery of Emerson, called Emerson enhancement effect is a great land mark in the study of photochemical reaction concerned with photosynthesis. The observations made Emerson to suggest that photosynthesis involves two photo-chemical reactions and coordination between them.

Check Your Progress - 4

What is meant by red drop?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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7.4.4. Hill Reaction

Another great discovery in photosynthesis is the evolution of O_2 from dry leaf powders in solution exposed to light. This was demonstrated by Robert Hill (1937). The oxygen evolution could be prolonged with the addition of iron salts, like ferric oxalate, sugars and magnesium salts to the solution to the suspended leaf material. This is called Hill reaction. Work on these lines proved that this reaction was due to photochemical oxidation of water.

7.4.5. Oxygen Evolution

Joseph Priestly (1776) discovered for the first time that plant could produce oxygen. He demonstrated that plants could improve the quality of air. In the subsequent years many scientists from varied fields of science contributed to the understanding of the mechanisms of photosynthesis. They showed the participation of light CO_2, H_2O, and the chlorophylls in the mechanism. The overall photosynthetic reaction could be written as

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

Such a generalised equation could give an impression that O_2 could be derived from either CO_2 or water. For quite some time, this question remained a challenging one leading to the proposal of many theories on photosynthesis. Some scientists considered that O_2 comes from CO_2 and some considered that chlorophyll-CO_2 complex as the immediate precursor to O_2. Some others showed that photosynthetic oxygen comes from water. This problem was solved by Ruben et al (1941) by using labelled water and carbondioxide containing oxygen ^{18}O separately in the experiments with *Chlorella* (unicellular green alga) cells. They found that the amount of labelled oxygen evolved during photosynthesis was found to be identical to the oxygen content of labelled water and not the oxygen content of CO_2. This showed that oxygen indeed comes from water and not from CO_2.

The oxygen evolving part is associated with pigment system II (PS II) the composition of which is described later. Apart from the pigments, manganese, and chloride ions are found to be necessary for the photolysis of water and evolution of oxygen by PS II.

After conducting several experiments, Otto Warburg has suggested that 4 quanta of light energy are required for reducing one molecule of carbon dioxide or for releasing one molecule of oxygen. But by improved techniques Emerson found that 8 quanta are required to reduce one molecule of CO_2. This is in accord with his two pigment scheme, each photosystem absorbing 4 photons of light.

7.4.6. Photosynthetic Unit

Earlier scientists have believed that intact chloroplasts are required for absorption of light. But it is recently demonstrated by several workers that even fragments of chloroplasts can carry on light reactions. Park & Biggin (1964) suggested that the small spheres present in the thylakoid membranes of chloroplasts are the photosynthetic units. These photosynthetic units are called quantasomes. Each quantasome contain about 250 chlorophyll molecules. Its molecular weight is about 2 millions. These quantasomes are particles seen in electron micrographs. But now it is proved that these are not the minimum unit structures of the chloroplast to carry on photosynthesis. Fragments of lamellae containing at least 2000 chlorophyll molecules can alone synthesize ATP.

The whole process of photosynthesis can be divided into two stages (1) the photochemical steps involving the electron transfer, to which is coupled ATP and $NADPH_2$ formation and (2) the biochemical reactions involving the incorporations of CO_2 into carbohydrates.

The photochemical reactions are often referred to as the primary events of photosynthesis. The earliest steps of this complex conversion involve the absorption of a photon by the light harvesting (antenna) pigments, the migration of the excitonic energy among other antenna pigments until it is trapped by the specialized reaction centre (RC).

Following the absorption of light, the initial photochemistry occurs at RC complexes, where the excitation energy is trapped and drives the transfer of an electron from a high potential primary donor (Chl or Bchl) to a low potential electron acceptor. This electrochemical energy, stored in the initial charge separation is to drive all subsequent electron and proton transfer reactions in photosynthesis.

Based on the experimental results, Emerson suggested that photosynthesis involves two photochemical reactions. The photosynthetic pigments are distributed in two systems and can be called photosystem I (PS-I) and phtosystem II (PS-II).

Both photosystem I and II can absorb quanta of light. The RC of PS-II absorbs light at 680 nm and that is why the pigment is called P_{680}. The RC for PS-I is P_{700} and has an absorption maximum at approximately 700 nm and therefore it is called P_{700}. In PS-I, the primarry electron acceptor is assumed to be 'X' and in PS-II, similarly it is 'Z'. The charge separation occurs in the form of a transfer of an electron from the excited RC chlorophyll to the primary acceptor.

Photosystem-I: This system essentially consists of 200 harvesting chlorophyll (more of chl.a and some chl.b) molecules, 50 carotenoid molecules and specially bound chlorophyll molecule, P_{700}. A few molecules of cytochrome f,m cytochrome b_{563}, plastocyanin and membrane bound ferredoxin molecules also form a part of PS-I. Studies with electron spin resonance and spectroscopy have shown ferredoxin to be the primary electron acceptor of PS-I. It may be found lightly bound to chloroplast lamella.

Photosystem-II: Photosystem-II is mainly involved in O_2 evolving mechanism. PS-II consists of 200 chlorophylls, (more of chlorophyll b molecules), 50 carotenoids, a trapping chlorophyll, P_{680}, an unidentified electron donor (Z), a primary electron acceptor (Q), that might be plastoquninone, Mn, Cl atoms and cytochrome B_{559} also form a part of PS-II.

It is assumed that P_{680} chlorophyll role in PS-II is similar to that of P_{700} in PS-I.

Check Your Progress - 5

What are the primary electron acceptors of PSI & PS-II?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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7.4.7. Photophosphorylation

Arnon and his associates (1954) have shown for the first time that isolated chloroplasts can product ATP when exposed to light in the presence of ADP and inorganic phosphate. They called this phosphorylation as photophosphorylation. This is different from the oxidative phosphorylation taking place in mitochondria during respiration.

Three different kinds of photophosphorylation have been discovered. They are:

1. Cyclic phtophosphorylation.
2. Non-cyclic phtophosphorylation.
3. Pseudocyclic photophosphorylation.

Cyclic Photophosphorylation: If the chloroplasts are illuminated with wave lengths of light greater than 680 nm only pigment system-I operates and cyclic photophosphorylation takes place. In cyclic photophosphorylation the electrons first flow from its reaction centre, i.e., P_{700} to ferredoxin. From ferredoxin the electrons pass back to P_{700} through cytochrome b_6, cyt.f and plastocyanin. As the electrons pass from P_{700} back to P_{700} through ferredoxin, cyt.b_6, cyt.f, plastocyanin, it is called cyclic photophosphorylation (Fig.7.3).

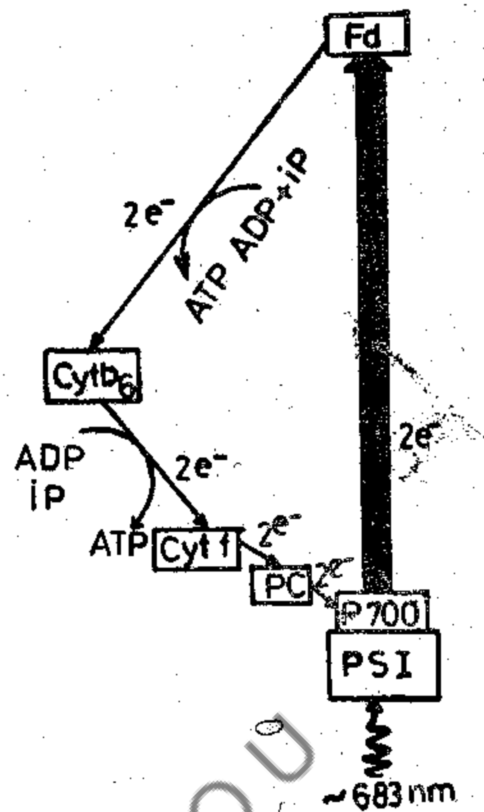

Fig.7.3. Cyclic photophosphorylation (The unidentified compound X is omitted)

Two ATP molecules are synthesized in cyclic photophosphorylation. One ATP molecule is synthesized when the electrons pass from ferredoxin to Cyt.b_6 and another when they pass from Cyt.b_6 to Cyt.f.

Non-Cyclic Photophosphorylation: This involves photosystem-I and photosystem-II. The schematic representation of non-cyclic photo phosphorylation is given in fig.7.4.

The two photosystems are connected by several intermediates. It is seen from the figure that light raises the electrons from PS-II to plastoquinone. From plastoquinone these electrons pass to PS-I through Cytochrome b_6, Cyt.f and plastocyanin. As the electrons are flowing from photosystem-II to photosystem-I, the intermediates are alternately reduced and oxidised. While the electrons are moving from Cyt.b_6 to Cyt.f, some of the energy is used to generate ATP. The electrons which arrive from photosystem-II to photosystem-I are at a lower energy level. The second photochemical event raises the electrons from PS-I to Ferredoxin. This reduced Ferredoxin reduces NADP to NADPG + H^+. The electrons lost by PS-II are replaced by water. The details of the transfer of electrons from water to PS-II is not fully known.

It is believed that manganese containing enzyme, chloride ions and several electron acceptors are involved in the transfer of electrons from water to PS-II. The scheme of the liberation of electrons from water is shown below.

$4\,H_2O$ —
- → $4H^+$ → to $NADP^+$ which received electrons in non-cyclic photophosphorylation.
- → $4\,OH^-$ —
  - → $4e^-$ to PS-II
  - → O_2
  - → $2H_2O$

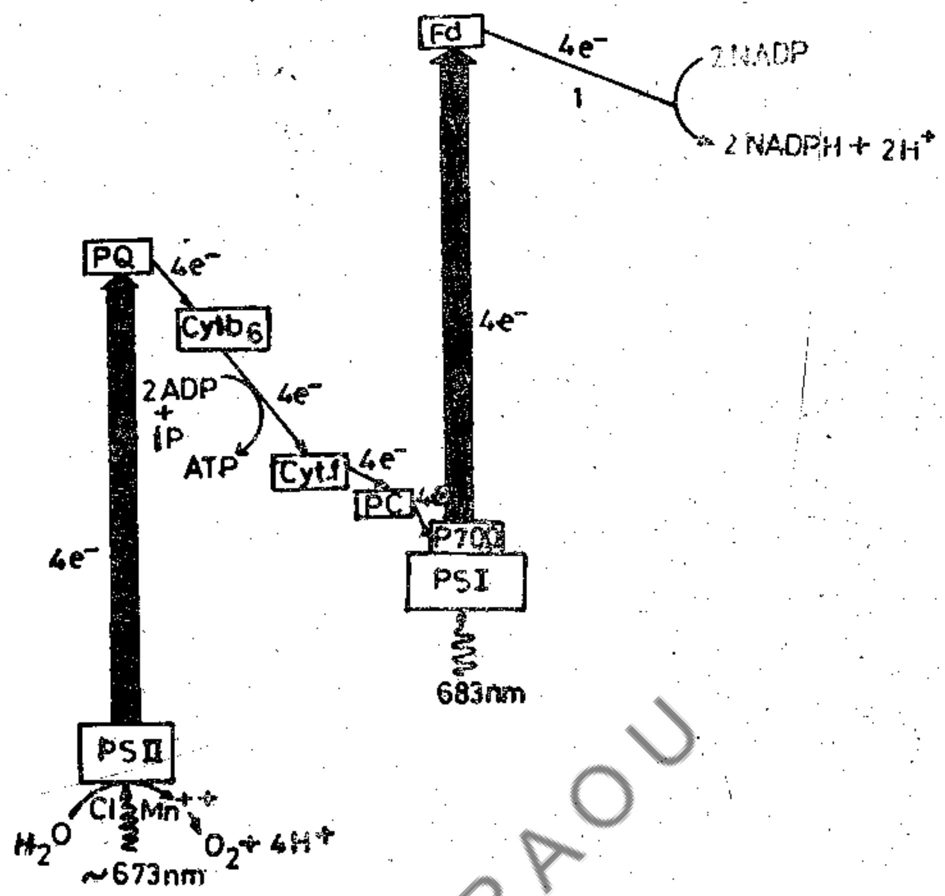

Fig.7.4. Non-cyclic photophosphorylation (The unidentified compounds Z or Q or Y are omitted).

The NADP which receives electrons in non-cyclic photophosphorylation receive protons (H^+) formed by the dissociation of water.

In this photophosphorylation the electron flow is unidirectional i.e., the electrons released from PS-II does not come back. That is why this phosphorylation is called Non-Cyclic Phosphorylation.

To get one molecule of oxygen 4 water molecules are used. Eight photons are required to pass these 4 electrons from water to NADP. In non- cyclic photophosphorylation for every 4 electrons 2 molecules of ATP&2 molecules of NADP are formed.

Pseudo-Cyclic Photophosphorylation: This photophosphorylation was demonstrated by Arnon & his coworkers (1954). He observed that if illuminated, the chlorophyll molecules produce ATP from ADP and iP in the presence of FMN or vitamin K and water (Fig.7.5).

FMN is an auto-oxidisable hydrogen acceptor and the reduced FMN is reoxidised by oxygen. This process occurs repeatedly and produce ATP continuously.

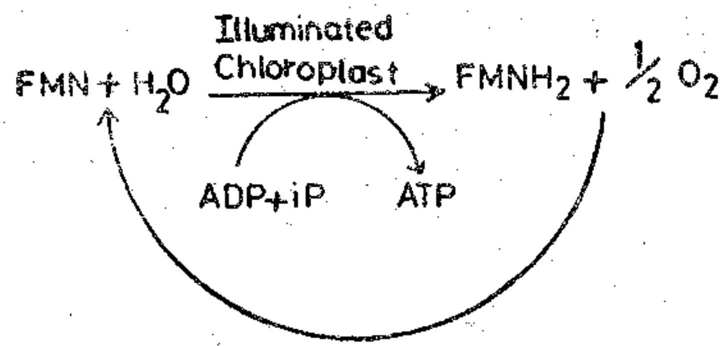

Fig.7.5: Pseudocyclic phosphorylation.

7.5. SUMMARY

Photosynthesis is a light-driven process. Photoautotrophs possess pigments which are coloured organic molecules. These pigments carry out the conversion of light energy into chemical energy. Reduced pyridine nucleotides and adenosine triphosphate are produced in the presence of light which are used to reduce atmospheric CO_2 to carbohydrates, proteins, lipids etc. Three major photosynthetic pigments are recognised. 1) Chlorophylls 2) Carotenoids and 3) Phycobilins. Chlorophylls and carotenoids are water-insoluble and can be extracted from most plant tissues by acetone-water mixtures or methanol. Phycobilins are water soluble. All oxygen-evolving photosynthetic organisms including algae possess chlorophyll a. chlorophyll b is also found in higher plants. Chlorophyll a and b closely resemble in molecular structure but differ in their physical and chemical properties. A chlorophyll molecule has a flat porphyrin head and a long phytol tail. Major chlorophyll of photosynthetic bacteria is bacteriochlorophyll a, however, green-sulfur bacteria have bacteriochlorophyll c, d and e (chlorobium chlorophyll). Biosynthesis of chlorophyll is partly light-mediated.Dark synthesis of chlorophyll can also occur solely through enzymatic activity. Carotenoids are widely distributed and divided into two classes 1) carotenes and 2) xanthophylls. They play two distinct roles 1) in the absorption and transfer of light energy and 2) in protecting the chlorophyll from photo-oxidation. Phycobilins are narrowly distributed and found only in certain algal groups and cyanobacteria. Their main function is light harvesting and energy transfer. Photosynthesis is a biological process where photosynthetic cells absorb light energy and convert it into a usable form of chemical energy. Photosynthetic bacteria, algae and higher plants which contain chlorophylls derive energy from sunlight to drive the photochemical reactions and hence they are photoautotrophs. Light quantum or photon is a discret package of energy and on absorption by a suitable pigment, brings about a photochemical reaction. There are two distinct photosynthetic photochemical processes associated with two pigment system. They are photosystem-I (PS-I) and photosystem-II (PS-II). The reaction center of PS-I is P_{700} which has an absorption maximum at approximately 700 nm. Photosystem-II reaction center is P_{680} and absorbs light at 680nm. PS-I and PS-II are connected in series by electron and hydrogen carrying components. Some of the additional energy produced during photochemical reactions is stored in the form of ATP. Formation of ATP from ADP during this process is termed "photophosphorylation. Ruben et al (1941) have successfully demonstrated that O_2 liberated in photosynthesis came from water and not from CO_2.

7.6. CHECK YOUR PROGRESS - MODEL ANSWERS

1. The photosynthetic pigments are classified into 3 major groups. They are (a) Chlorophylls (b) Carotenoids and (c) Phycobilins.

2. Chlorophyll a is a soluable in petroleum ether and chlorophyll b in methyl alcohol. The chlorophyll a is blue green in colour whereas chlorophyll b is yellow green in colour.

3. β-Carotone, lutein, viloxanthin and neoxanthin are the four major carotenoids of higher plants.

4. At wave length beyond 680 nm there is a decline in the quantum yield. This decrease in the quantum yield is called red drop because this area of spectrum is in the red region.

5. The primary electron acceptor in PS-I is ferredoxin whereas it is plastoquinone in PS-II.

7.7. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Describe the role of light in the synthesis of assimilatory power.
2. Explain photosystem I & II.
3. Explain cyclic photophosphorylation and compare it with that of non-cyclic photophosphorylation.
4. Write about the electron carriers in Z-scheme.

II. Answer the following questions in 10 lines each.

1. Explain Hill reaction.
2. Write about the absorption spectrum & action spectrum.
3. Write briefly about the photolysis of water.
4. Explain the Emerson enhancement effect.
5. Write briefly about the non-cyclic photophosphorylation.
6. Write about the pseudocyclic phosphorylation.

UNIT-8: C_3, C_4, CAM PATHWAYS AND FACTORS INFLUENCING PHOTOSYNTHESIS

Contents

8.1. OBJECTIVES

By the end of this unit you will be able to:

1. list out and describe the CO_2 fixation pathways,
2. distinguish between the carboxylation phase, reductive phase and RUBP regeneration phase in Calven Cycle,
3. list out the differences between the anatomy of C_3 and C_4 Plants,
4. list out the families in which the C_4 pathways are reported to occur,
5. describe the significance of C_4 pathway,
6. describe the CAM pathway in the succulents and its significance.

8.2. INTRODUCTION

Photosynthesis is the most important bio-chemical process on the earth. Green plants are the primary harvesters of solar energy. The green plants trap the solar energy into chemical energy. For every gram atomic weight of carbon taken during photosynthesis, potential energy amounting to 477 kilo joules (114 K calaries) is obtained. Thus plants are considered as primary producers of food and energy in the ecosystem.

The photosynthesis involves the following two phases.

1. The absorption and retention of light energy in the form of ATP.
2. Conversion of light energy into stable chemical potential (carbohydrates) and its storage.

In the preceding unit you have studied in detail about the photochemical reactions that involve the traping of the light energy by the chlorophyll pigments and retension of light energy in the form of chemical energy i.e. Adenosine triphosphate (ATP). The ATP along with reduced NADP constitute the assimlatory power which is necessary for reduction of CO_2 into carbohydrates in dark reaction.

8.3. CO_2 FIXATION PATHWAYS

Currently, three different types of carbon dioxide fixation pathways are known. They are C_3 pathway (Calvin cycle), C_4 pathway and Crassulacean acid metabolism (CAM). The C_3 and C_4 pathways derive the name based on the initial product of CO_2 fixation. In Calvin cycle the first product is a 3 carbon compound, the PGA (Phosphoglyceric acid), whereas in C_4 pathway the first product is a 4-carbon compound, Oxaloacetic acid. It has become a common practice to refer to the plants possessing the C_3 pathway as C_3 plants and those having C_4 pathway as C_4 plant. The succulent plants on the contrary exhibit crassulacean acid metabolism (CAM) as an adaptation to xerophytic habitats.

8.3.1. C_3 Pathway (Calvin Cycle)

The C_3 pathway constitute mainly the sequence of reactions starting from intake of CO_2 from outer atmosphere by the plants and its reduction to carbohydrates in the presence of the assimilatory power i.e., reduced NADP and ATP, which are formed during light phase. For each molecule of CO_2 assimilation, two molecules of NADP and three molecules of ATP are necessary to accomplish the synthesis of carbohydrates. In 1954 Melvin Calvin and his associates succeeded in finding out the intermediates formed during the conversion of CO_2 to carbohydrates using labelled carbondioxide i.e., $^{14}CO_2$. They could, thus, trace the sequence of reactions leading to the formation of the end product. The CO_2 assimilation which is otherwise known as the dark reaction of photosynthesis occurs in a cyclic sequence which is named after Calvin as Calvin cycle. Melvin Calvin received Nobel prize for his work in 1961.

The overall C_3 pathway consists of 3 distinct phases.

1. Carboxylation phase.
2. Reductive phase
3. RuBP regeneration phase.

Carboxylation phase: The phase constitute the fixation and reduction of CO_2. A carbon compound called Ribulose-1, 5 biphosphate (RuBP) is the initial CO_2 acceptor in Calvin cycle. Ribulose bi-phosphate reacts with CO_2 to form two molecules of phosphoglyceric acid (PGA) in an enzymatic reaction catalyzed by ribulose-bis-phosphate carboxylase.

$$\text{Ribulose 1, 5-bisphosphate} + CO_2 \xrightarrow[\text{RuBP carboxylase}]{+H_2O} \text{2 phosphoglyceric acid}$$

As the initial product of CO_2 fixation is a 3 carbon compound, plants with this CO_2 fixation pattern are often referred to as C_3 plants. The site of this reaction is the stroma of the chloroplast.

Reductive phase: In this phase PGA is reduced to glyceraldehyde-3 phosphate by $NADP^+H^+$ (in the presence of ATP and the reaction is catalised by *triose phosphate dehydrogenase.*

$$\text{3 Phosphoglyceric acid} + NADPH + H^+ + ATP \xrightarrow[\text{dehydrogenase}]{\text{Triose phosphate}} \text{glyceraldehyde-3-phosphate} + ADP + NADP$$

A part of the triose i.e.,glyceraldehyde-3-phosphate is transformed into its isomer dihydroxy acetone-3-phosphate with the help of the enzyme *triose phosphate isomerase.*

$$\text{Glyceraldehyde-3-P} \underset{\text{Isomerase}}{\overset{\text{Phosphotriose}}{\rightleftharpoons}} \text{Dihydroxyacetone-3-P}$$

Out of the trioses formed 1/6 trioses are converted into carbohydrate (hexose) and the rest of 5/6 trioses are recycled to regenerate acceptor- RuBP.

Hexose Formation: In this step, one molecule each of dihydroxy acetone-3- phosphate and glyceraldehyde-3-phosphate condense together to form fructose 1,6-diphosphate. Then one phosphate radical is removed to produce fructose-6-phosphate as follows:

$$\text{Glyceraldehyde-3-phosphate+dihydroxy acetone-3-phosphate} \xrightarrow{\text{aldolase}} \text{Fructose-1, 6-diphosphate.}$$

$$\text{Fructose 1,6-diphosphate} \xrightarrow[+\ H_2O]{\text{Phosphatase}} \text{Fructose-6-phosphate}$$

Some of the Fructose-6-phosphate give rise to glucose and ultimately sucrose is formed and it is said to be the first stable sugar produced during photosynthesis.

RuBP regeneration phase: In this step fructose-6-phosphate reacts with glyceraldehyde-3-phosphate to give rise to one molecule each of erythrose-4-phosphate and xylulose-5-phosphate with the help of *transketolase.*

$$\text{Erythrose-4-phosphate+Dihydroxyacetone-3-phosphate} \xrightarrow{\text{Transketolase}} \text{erythrose-4-phosphate} + \text{Xylulose-5-phosphate}$$

The erythrose-4-phosphate and dihydroxyacetone-3-phosphate molecules condense together in the presence of aldolase to produce sedoheptulose- 1,7diphosphate. Later in the presence of the enzyme phosphate the sedoheptulose-1, 7-diphosphate is converted into

$$\text{Erythrose-4-phosphate} + \text{Dihydroxyacetone-3-phosphate} \xrightarrow{\text{Aldolase}} \text{Sedoheptulose 1,7 diphosphate}$$

There is also the formation of one molecule each of xylulose-5-phosphate and ribose-5-phosphate by the reaction between sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate, in the presence of transketolase. These 5 carbon compounds are transformed into ribulose-1,5 bis-phosphate (RuBP) as shown below.

$$\text{Sedoheptulose-7-phosphate} + \text{Glyceraldehyde-3-phosphate} \xrightarrow{\text{Transketolase}} \text{Xylulose-5-phosphate}$$

$$\text{Ribose-5-phosphate} + \text{Xylulose-5-phosphate} \xrightarrow[\text{Phosphopentose isomerase}]{\text{Phosphopentose epimerase}} \text{Ribulose-1-5 bisphosphate}$$

Check Your Progress - 1&2

1. What is the initial carbondioxide acceptor in Calvin cycle?
2. What is the enzyme that is responsible for the conversion of fructose 1,6 diphosphate to fructose 6-phosphate?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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The essential of Calvin cycle are shown below.

1. 6 Carbondioxide + 6 Ribulose 1, 5 bisphosphate $\xrightarrow[\text{+ Water}]{\text{Carboxydismutage}}$ 12 Phosphoglyceric acid

2. 12 Phosphoglyceric acid + 12 ATP + 12 NADPH + H^+ $\xrightarrow[\text{Triose phosphate dehydrogenase}]{\text{Phosphoglycerokinase}}$

$\longrightarrow$ 12 Glyceraldehyde 3-P + 12ADP + 12 NADPH + 12 iP

3. Glyceraldehyde-3-Phosphate (triose) $\underset{\text{isomarase}}{\overset{\text{Triose phosphate}}{\rightleftharpoons}}$ Dihydroxyacetone phosphate (triose)

4. 6 Triosephosphate (3 phosphoglyceraldehyde) +3 Dihydroxyacetone phosphate $\xrightarrow{\text{Aldolase}}$
$\longrightarrow$ 3 Fructose 1,6 bisphosphate

5. 3 fructose bisphosphate $\xrightarrow{\text{Phosphatase}}$ 3 fructose-6-phosphate + 3 iP

6. 2 fructose-6-phosphate + 2 triosephosphate $\xrightarrow{\text{Transketolase}}$ 2 Xylulose-5-phosphate + 2 erythrose-4-phosphate

7. 2 erythrose-4-phosphate + 2 triosephosphate $\xrightarrow{\text{Transaldolase}}$ 2 sedoheptulose-di-phosphate

8. 2 Sedoheptulose-di-phosphate $\xrightarrow{\text{Phosphatase}}$ 2 Sedoheptulose-7-phosphate + 2 iP

9. 4 Xylulose-5-phosphate $\xrightarrow{\text{Phosphoketopento epimerase}}$ 4 ribulose-5-phosphate

10. 2 ribose-5-phosphate $\xrightarrow{\text{Phosphoribose isomerase}}$ 2 ribulose-5-phosphate

11. 6 ribulose-5-phosphate $\xrightarrow{\text{Phosphopentokinase}}$ 6 ribulose-di-phosphate

The Calvin cycle is given in Fig.8.1

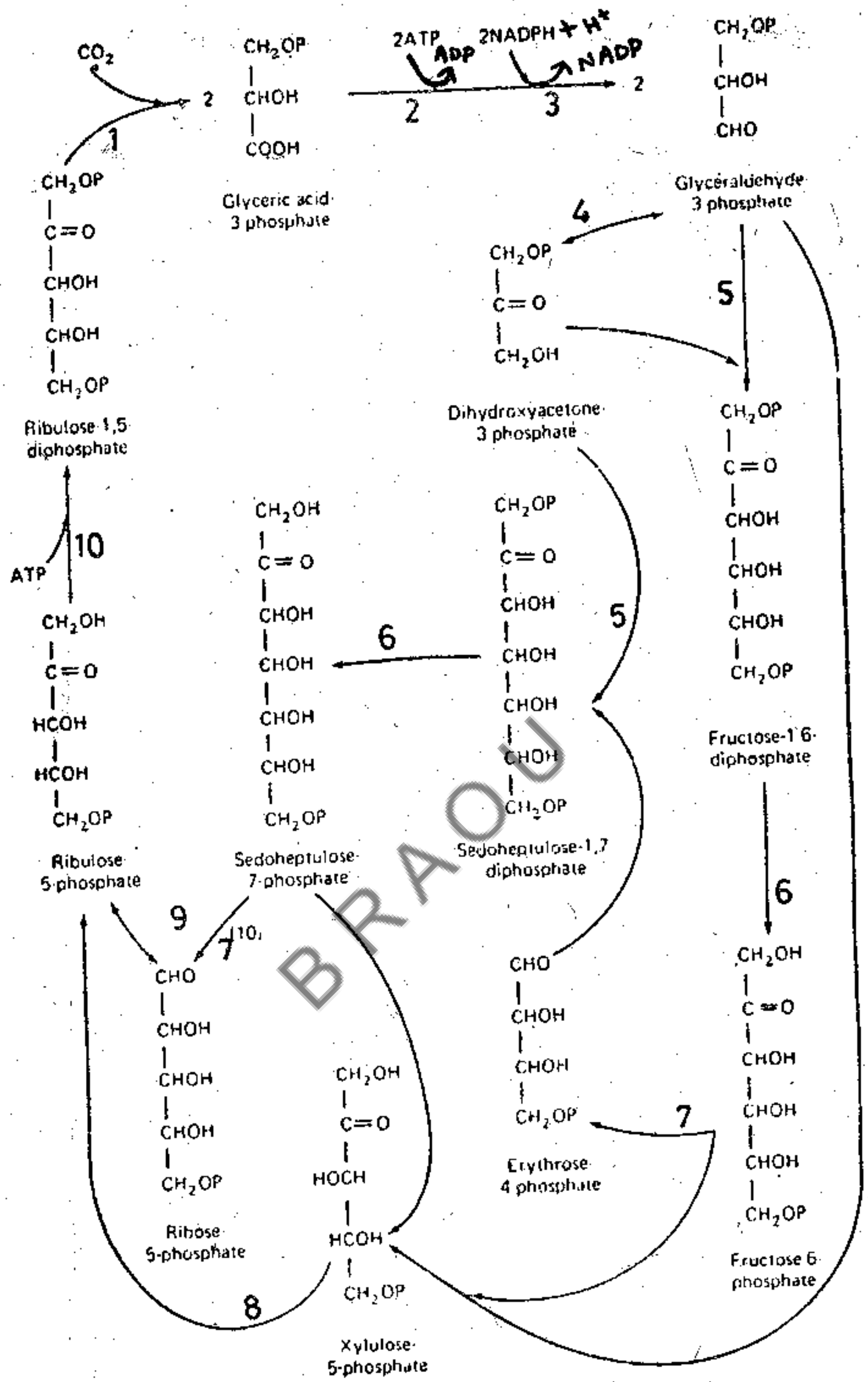

Fig.8.1. The Calvin cycle is photosynthesis or C_3 pathway of carbon fixation. 1. Ribulose bis-phosphate carboxylase 2. Phosphoglycero kinase 3. Triose phosphate dehydrogenase 4. Triose phosphate isomerase 5. Aldolase 6. Phosphatase 7. Transketolase 8. Epimerase 9. Pentophosphate isomerase 10. Phosphoribulo kinase.

8.3.2. C_4 Pathway

In 1954 Kortschak and others observed an alternate pathway for CO_2 fixation known as C_4 pathway. According to their observations, using labelled carbon dioxide ($^{14}CO_2$). C_4 dicarboxylic acids are formed as primary products of photosynthesis in sugarcane instead of PGA, a C_3 compound. When $^{14}CO_2$ was supplied to sugarcane leaves, in a short period 3-four carbon compounds (oxaloacetate, malate and aspartate) were produced initially. The oxaloacetate being unstable breaks down readily to malate and aspartate. When the period of exposure is extended, the labelled carbon appeared in 3 phosphoglyceric acid. Later, M.D.Hatch and C.R.Slack (1966) have extended these experiments to several plants and then proposed the alternative pathway for CO_2 fixation in photosynthesis.

In C_4 plants, the CO_2 is first accepted by PEP (Phospho-enol-pyruvate) a 3-carbon compound and as a result 4-carbon product oxaloacetic acid is formed. This product being unstable breaks down readily to malate and aspartate through transamination. These C_4 acids are enzymatically degraded to yield free CO_2 and pyruvate. The pyruvate (3-carbon compound) is phosphorylated to regenerate PEP. It is believed that the CO_2 produced by the degradation of C_4 acids in C_4 cycle is utilized by RuBP carboxylase system in Calvin cycle and the CO_2 is reduced to carbohydrates.

Check Your Progress - 3

What is the primary acceptor of CO_2 in C_4 plants?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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The essentials of C_4 pathway are given below:

1 . Phosphoenolpyruvic acid + CO_2 $\xrightarrow{\text{PEP Carboxylase}}$ Malic acid, Oxaloacetic acid, Aspartic acid (C_4 dicarboxylic acids)

2. C_4-Dicarboxylic acid $\longrightarrow$ CO_2 + Pyruvate

3. Pyruvic acid + ATP $\xrightarrow{\text{Pyruvic kinase}}$ Phosphoenolpyruvic acid + ADP

4. CO_2 + RuBP $\xrightarrow{\text{Calvin } C_3 \text{ cycle}}$ Carbohydrates + RuBP.

In summary, C_4 plants have an additional CO_2 fixing sequence which is co-ordinated in the C_3 cycle and RuBP carboxylase. Thus CO_2 is fixed by PEP and is finally given to RuBP carboxylase within the leaf. The carbon dioxide reduction through C_4 pathway is given in Fig.8.2.

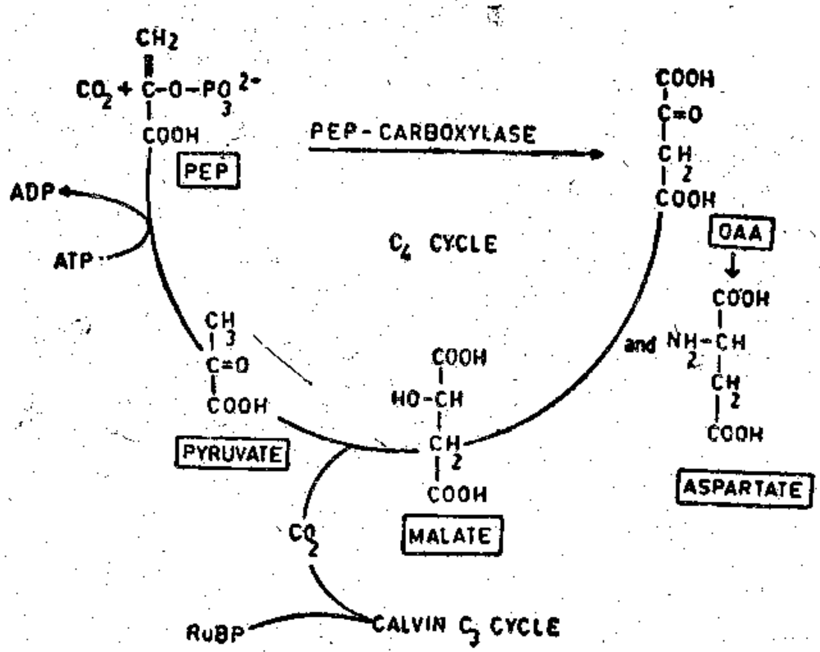

Fig.8.2. Carbondioxide reduction through C_4 pathway. The reactions of the pathway were proposed by Hatch and Slack. Lower part of the diagram is the site of interaction of C_4 and C_3 cycles which operate in the same plant species.

Anatomical Difference between C_3 and C_4 Plants: The C_4 cycle is almost always accompanied by characteristic anatomical specialization called '*kranz*' anatomy. (Kranz in German means a wreath or garland). Kranz leaves have smaller air spaces, dense mesophyll parenchyma, and many vascular bundles surrounded by conspicuous garland of cells having many chloroplasts. The bundle sheath cells are usually prominently loaded with starch. Stomata are often more numerous. Leaves possessing the 'Kranz' anatomy can often be recognized by eye because of their dark green veins, where as the veins of C_3 leaves are usually pale as the bundle sheath cells of C_3 plants are devoid of chloroplasts.

Check Your Progress - 4

What is the difference between the bundle sheath of C_3 & C_4 plants?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Chloroplast dimorphism: Extreme structural dimorphism is found in chloroplast of C_4 dictos as well as monocots. Pronounced size dimorphism also exists. The chloroplasts of mesophyll cells are smaller and show granal nature while the bundle sheath chloroplasts are large, predominantly agranal (without grana) and are specialized for starch accumulation. In *Zea mays* (a C_4 plant) the mesophyll cell chloroplasts had grana. While the bundle sheath cell chloroplasts are without grana. In sugarcane also the structural dimorphism in chloroplasts has led to the notion that structural dimorphism of chloroplasts is a common feature of all C_4 plants.

The C_4 cycle in essence is a device for absorbing CO_2 in the mesophyll cells by the enzyme PEP carboxylase which has greater affinity for CO_2 than RuBP carboxylase, and transferring it as the β-carboxyl of a C_4 acid, to the inner bundle sheath cells of the leaf, where it is made available for C_3 carbon dioxide fixation by RuBP carboxylase and reduction. The outline of C_4 photosynthesis is given in Fig.8.3.

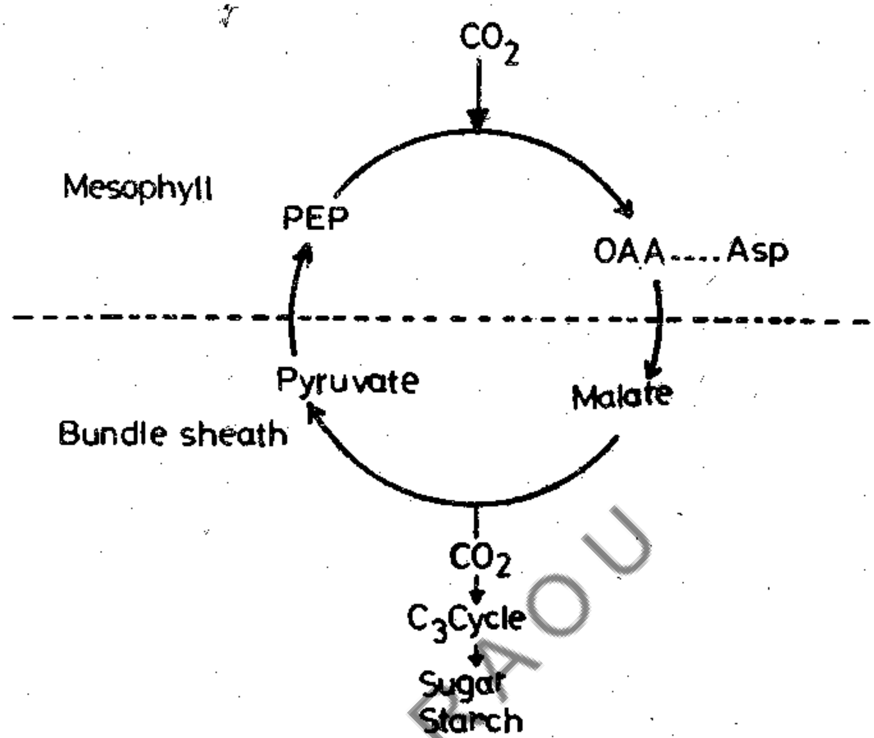

Fig.8.3. Special separation of C_3, C_4 pathways in C_4 plants.

TABLE 8.1: Characteristics of C_4 and C_3 Plants

Characteristics	C_4 Plants	C_3 Plants
Primary CO_2 acceptor	PEP carboxylase	RuBP carboxylase
First product of photosynthesis	C_4 acids (Oxalaloacetate, malate, aspartate)	C_3 acids PGA
Photorespiration	Nil or Low	High
Light	Not saturating at normal light intensities	Saturates at 100-200 Wm^{-2} or less in shade plants
Temperature optimum	30-40°c	10-25°c
CO_2 compensation point	Less than 10 ppm	About 70 ppm
Leaf structure	Mesophyll arranged radially around chlorenchymatic bundle sheaths	Laminar mesophyll parenchymatic bundle sheaths.
Chloroplasts	Mesophyll-granal bundle sheath cells-agranal or granal	Granal
Chlorophyll a/b	Ca 4:1	Ca 3:1
Photosynthesis depressed by O_2	No	Yes
Net photosynthetic capacity	High to very high	Slight to high
Redistribution of assimilation products	Rapid	Slow
Dry matter production	High	Medium
Photosynthetic rate ($mgCO_2\ dm^{-2}h^{-1}$)	40-85	Less than 30

In C_4 plants, C_4 acids and PEP carboxylase are seen in the chloroplasts of mesophyll cells whereas bundle sheath cells are abundant in Calvin cycle compound RuBP carboxylase. The characteristics of C_3 and C_4 plants is given in Table 8.1.

C_4 cycle provides a powerful mechanism in C_4 plants for concentrating CO_2 at the site of RuBP carboxylation in the bundle sheath chloroplasts. RuBP carboxylase, has affinity for CO_2 and O_2 also. Therefore in the presence of O_2 and low levels of CO_2 it fixes less amounts of CO_2. But if the level of CO_2 is raised it fixes CO_2 actively. In C_4 plants the CO_2 is supplied to the chloroplasts of bundle sheath cells, *in situ* through C_4 compounds which are decarboxylated. The level of CO_2 is maintained sufficiently high for the activity of RuBP carboxylase. Another important point is that the chloroplasts in bundle sheath cells do not possess grana. Therefore photosystem II, which liberates O_2 is absent. So, RuBP carboxylase does not act as oxygenase, but act as carboxylase alone. That is one of the reasons for the C_4 plant not having photorespiration.

Distribution of C_4 Plants: The C_4 plants exhibit higher net photosynthetic rates when compared to those of C_3 plants. The occurrence of C_4 pathway has been shown in more than 1000 angiosperm species belonging to atleast 18 plant families including Poaceae and Cyperaceae among monocots and among dicot taxa belonging to Portulaccaceae, Amaranthaceae, Chenopodiaceae, Nyctaginaceae, Aizoaceae, Euphorbiaceae, Caryophyllaceae, Zygophyllaceae, Boraginaceae and Asteracea etc. In some genera, only certain species alone show C_4 pathway. Even within the species there can be C_3 and C_4 and intermediates also. However the C_4 plants are taxonomically widely distributed, that is they do not belong to any single taxonomic group. Among the dicotyledons the largest concentration seems to have occurred in the Centrospermae. As the majority of C_4 plants are tropical, it is presumed that C_4 pathway was evolved (later to Calvin cycle) in plants belonging to diverse families exposed to temperature stress and other environmental conditions of tropics.

Check Your Progress - 5

Name five families in which the C_4 pathway is reported to occur.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Significance of C_4 Pathway: 1. The significance of two photosynthetic carbon cycles operating in the same individual, appears to assist the mechanism by which CO_2 in the environment is utilized more effectively.

2. C_4 pathway may confer remarkable ecological adaptations in certain plants with respect to their behaviour and survival under extreme conditions of temperature and drought.

3. The discovery of the C_4 pathway of photosynthesis has given a deeper understanding of the intricacies of photosynthesis.

4. This discovery also has led to the hypothesis that increased productivity might be achieved by manipulating plants.

5. A knowledge of C_4 characteristics of plants may help in selecting the efficient photosynthetic plants.

8.3.3. Crassulacean Acid Metabolism (CAM) Pathways:

Many species having succulent leaves as well as stems, have crassulacean acid metabolism. In these plants the stomata remain closed in the day and open at night, as an adaptation to prevent the water loss during day time through transpiration. It was also recognised that the leaves of plants belonging to the families Crassulaceae, Euphorbiaceae, Orchidaceae, Bromeliaceae and Cactaceae display a diurnal pattern of organic acid formation. During the day the total organic acid content of leaves decreases, accompanied by an increase in pH of the leaf cell sap. During night the organic acid content increases and the pH of the cell sap decreases. All plants which show these rhythms are referred to as CAM plants.

Crassulacean acid metabolism (CAM) plants fix carbondioxide in the dark primarily through PEP carboxylation leading to the synthesis of C_4 acids, predominantly malic acid. This is called dark carbondioxide fixation. CAM plants have parenchyma cells which are large and vacuolated. These vacuoles are used for storing malic acid and other organic acids in large amounts. These acids keep CO_2 temporarily in bound form. During the day when the stomata are closed, the CO_2 is released from these organic acids which break down into PGA and release CO_2 and carbohydrates are synthesised through the Calvin cycle.

In other words CO_2 requirement for photosynthesis is satisfied through the internal reservoir of CO_2 in the form of malate during night. Thus they have separated the uptake of CO_2 (during night when stomata are open) and its reduction (during day time when light is available). This separation of these two steps in photosynthesis in time is called temporal separation. The diurnal change in leaf organic acid content is accounted for almost entirely by the appearance of malic acid in the dark and its disappearance in the light. The leaves of CAM plants also contain the enzymes of reductive pentose phosphate cycle in the normal chloroplasts. The CO_2 fixed in malate during dark period via PEP carboxylase is ultimately converted to hexoses, starch and other compounds in the day. Pathway for these dark and light reactions are presented in Fig.8.4.

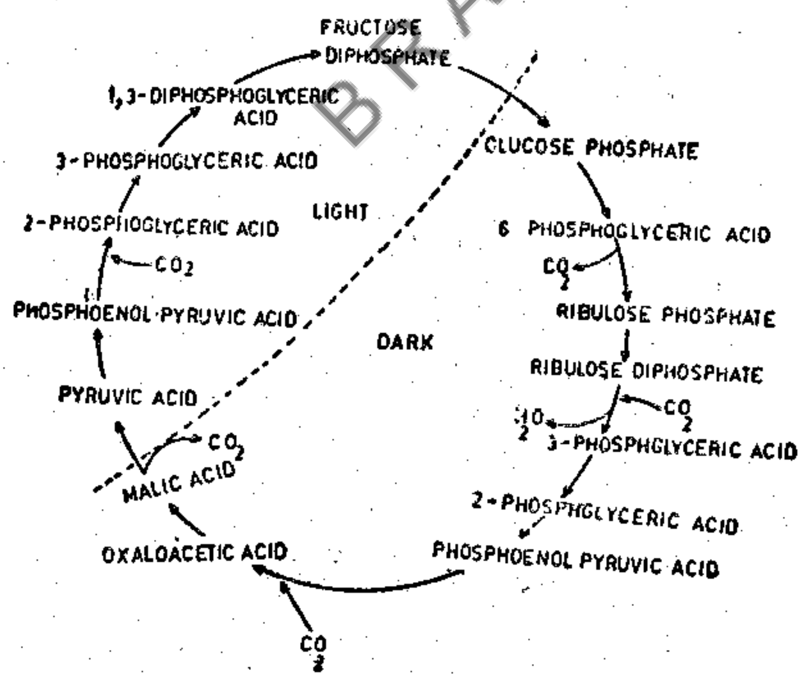

Fig.8.4. Diagrammatic representation of CO_2 fixation by reactions which occur in leaf cells of CAM plants. e.g., *Sedum telephium*.

Check Your Progress - 6

What are CAM Plants?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

Significance of CAM Pathway: 1. It increases water conservation efficiency of the succulent plants by preventing excessive evaporation of water, as the stomata are closed during day time and open in the night. 2. It is an adoptation of succulent plants to extreme hot climates. 3. CAM plants can also obtain a CO_2 compensation point of zero at night and in this way accomplish a steeper gradient for CO_2 uptake compared with C_3 plants.

Recent studies have shown significant similarities between C_4 pathway and CAM pathway. In both process PEP carboxylase is the efficient fixer of atmospheric CO_2. However they differ in regard to time (CAM) and space (C_4) of carboxylation, yet Calvin cycle being the final pathway in both for carbohydrate synthesis. In C_4 plants, PEP carboxylation and Calvin Cycle are spatially separated in different leaf cells-C_4 pathway in mesophyll cells and Calvin cycle in bundle sheath cells, whereas in CAM plants, these are separated temporarily in time by way of PEP carboxylation occurring in darkness followed by RuBP carboxylation in light. Both pathways are found in leaf mesophyll cells. These relationships are given below.

Table-8.2. **The relationships of C_4 & CAM Pathways**

C_4 Pathway		CAM Pathway	
Mesophyll cells	Bundle sheath cells	Darkness	Light
PEP carboxylation (C_4 cycle)	RuBP carboxylation Calvin cycle	PEP Carboxylation C_4 cycle	RuBP carboxylation Calvin cycle

Only a relatively few species of plants have been thoroughly studied, other new and different routes of CO_2 assimilation may yet be discovered.

8.4. FACTORS INFLUENCING PHOTOSYNTHESIS

The rate of photosynthesis is controlled by many factors, both internal and external. Of the external factors, light (quality and intensity), CO_2 concentration, temperature, oxygen, water, wind speed and nutrient level of the soil have profound influence. Besides these factors, certain internal factors, chlorophyll content, stomatal behaviour, concentration of carboxylating enzymes and leaf water content influence the photosynthetic rates. Most of these internal factors are influenced by environmental factors. It is appropriate to mention that several of these factors interact to influence the rate of photosynthesis.

For example, percent of CO_2 entry is regulated by stomatal mechanism. Increase in CO_2 enhances photosynthesis, but such an increase may also cause closure of stomata. Therefore, it may be understood that no single factor should be taken as solely responsible for an increase in photosynthetic rate. "Blackman" defined his law of limiting factors as follows. "When a process is conditioned as to its rapidity by several factors independently, the rate of process is limited by the pace of the slowest factor". The slowest factor implies a factor which is present in intensity or quantity that is less than what is required for the process, when two or more factors act at the same time. To explain this principle, Blackman presented the example.

For instance, a leaf is subjected to a light intensity sufficient to decompose 5 ml of CO_2 per hour and only 1 ml of the gas is available, photosynthesis will continue at a certain rate, since light intensity is not a limiting factor. Any increase in light intensity will not result in an increase in the rate of photosynthesis. If the concentration of CO_2 is increased, the rate of photosynthesis goes up with the same light intensity. Therefore, it is evident that in this case CO_2 is the limiting factor. Increase in CO_2 till it attains 5 ml level will result in an increase in the rate of photosynthesis. At this concentration of carbondioxide, light energy is just sufficient to decompose it but not more. If the CO_2 concentration is increased further, the rate of photosynthesis does not increase, since light now becomes the limiting factor. When the light intensity is increased, then a higher concentration of CO_2 will be decomposed and the photosynthetic rate increases till light again becomes a limiting factor. Fig.8.5. represents the whole concept in a detailed way.

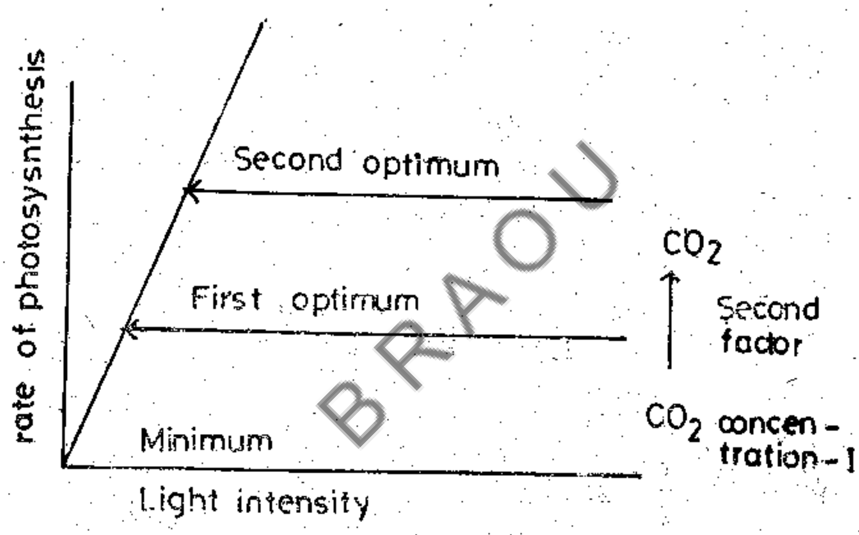

Fig.8.5. Photosynthesis at different intensities of light and carbondioxide concentrations.

Blackman's discovery provided a means to explain the effect of external factors individually on the rate of photosynthesis by keeping all other factors constant and not limiting the rate of the process. The following text explain the influence of main factors.

Modern man's population explosion has led to continuous attempts to increase the food supply. This ofcourse cannot exceed the amount of photosynthate produced by green plants. It is, therefore, of practical importance to know what are the limits to the production of photosynthate, because any attempt to exceed these limits is useless. Inspite of the high efficiency of modern agriculture, only about 0.2 percent of the annual sun-shine is stored by photosynthesizing plants.

8.4.1. External Factors

Light: The photosynthetically active radiation is at wave length from 400 to 700nm. Light varies in intensity, quality and duration.

Quality: The action spectrum for photosynthesis in leaves show two major peaks, one in the red and the other in the blue. In these regions chlorophylls absorb maximum light as stated above. Most effective wave- lengths differ with different plants. It is of interest to note that plants show high photosynthesis in the blue and red light, while red algae do so in green light and brown algae in blue light. The blue green algae have action spectrum peak in orange light.

Duration: In general, a plant will accomplish more photosynthesis when exposed to longer periods of light. It has also been found that uninterrupted and continuous light for relatively long periods of time, may be sustained without any damage to the plant. If we remove the source of light, the rate of CO_2 fixation falls to zero immediately.

Intensity: When CO_2 and temperature are not limiting and light intensity is low, the rate of photosynthesis increases with an increase in its intensity. The increased intensity of light causes no increase in the photosynthetic rate because of some other limiting factor or may be due to destructive effects of high light intensity. At a point, saturation may be reached, when further increase in light intensity fails to induce increase in photosynthesis. Optimum or saturation intensities may vary with different plant species, for example C_3 and C_4. C_3 plants become saturated at levels considerably lower than full sunlight but C_4 plants are not saturated in full sunlight.

When the intensity of light falling on a photosynthesizing organ is increased beyond a certain point, the cells of that particular organ become vulnerable to chlorophyll catalyzed photo-oxidations. Consequently, these organs begin to consume O_2 instead of CO_2 and CO_2 is released. Photo-oxidation is maximal when O_2 is present or when carotenoids are absent or CO_2 concentration is low.

Temperature: Temperature has a marked effect upon the rate of photosynthesis under conditions where light intensity and carbondioxide concentration are not limiting. This observation led Blackman to postulate the occurrence of "dark reactions". For most plants, photosynthesis increases from just above 0^oC to 35^oC, although often at "temperatures above 30^oC the initial high rate is not maintained and this is markedly so above 35^oC. One major factor in the decline in the rate of photosynthesis at these high temperatures is the denaturation of enzyme proteins. It is of interest to know that some tropical plants can photosynthesize at temperatures above 40^oC and that some algae, indigenous to hot springs, can grow at temperatures upto 80^oC. Therefore, light intensity, temperature and CO_2 concentration are all important factors. Since temperature is the limiting factor, a small increase in temperature will produce a corresponding increase in the rate of photosynthesis.

The effect of temperature is pronounced as in respiration. When temperature is the limiting factor, a rise of 10^oC may increase the rate of photosynthesis 2 or 3 times or sometimes even more. The reason being that all the reactions of the Calvin cycle are temperature-dependent and the rate of diffusion of CO_2 to the chloroplasts is accelerated by high temperature. This is typical of ordinary chemical process. Photochemical reactions show little change. For example, a 10^oC rise in temperature usually increases the rate of photochemical reactions by only about one tenth. The optimum temperature photosynthesis for the maximum rate falls between $20\text{-}30^oC$.

Between C_3 and C_4 plants, the former have optimal rates from $20\text{-}26^oC$ the latter show optimal rates from $35\text{-}40^oC$. C_4 photosynthetic mechanism has undoubtedly a significant advantage for plants in some tropical environments. Similarly, temperature also influences the light (optimum $30\text{-}35^oC$) and dark respiration (optimum $40\text{-}45^oC$).

Carbon dioxide concentration: The measured carbon dioxide absorption is really a sum of the excess of photosynthesis over respiration. Therefore, it is a measurement of the net rate of photosynthesis. To get the "actual" rate of photosynthesis it would be necessary to add to the measured carbon dioxide absorption, the carbon dioxide evolution of the same plant when kept in dark. Usually, under conditions of bright days, the rate of photosynthesis is limited by the atmospheric CO_2 concentration of 0.03-0.032 percent. In fact, increase in rates of photosynthesis are observed with increase in CO_2 concentration upto at least one percent. CO_2 fertilisation is, sometimes, a commercially useful proposition for some glass house crops.

If a plant is illuminated in a closed container, the CO_2 concentration inside the container eventually attains a steady level. This is known as the CO_2 compensation point. The final level represents the balance attained between CO_2 fixing and CO_2 releasing reactions. The CO_2 releasing reactions do occur, if leaves which have previously been photosynthesizing in labelled CO_2 are transferred to unlabelled CO_2. Labelled CO_2 can be detected in the atmosphere surrounding the leaves. This experiment also emphasizes the fact, that, when photosynthesis is measured as CO_2 uptake or even as oxygen evolution the value obtained represents only an apparent photosynthesis. It is the balance between reaction resulting in CO_2 and oxygen uptake and evolution. Plants vary in their ability to utilize high concentrations of CO_2. Particularly, in tomatoes, high CO_2 concentration, above the physiological range, results in harmful effect causing leaf injury also.

Oxygen: Oxygen has been shown to inhibit photosynthesis. It has been shown that rate of oxygen uptake and of CO_2 evolution is increased in the light. This phenomenon is called photorespiration. This decreases the overall efficiency of photosynthesis. This involves a reaction sequence quite different from "normal" respiration. In photorespiration, glycolic acid is serving as the principal compound. Plants exhibiting photorespiration usually have a CO_2 compensation point of around 50 ppm. The C_3 plants show high photorespiration. High O_2 concentration stimulates the photorespiration. The rate of photosynthesis increases by 30-50 percent when oxygen concentration in the air is reduced from 200 percent to 0.5 percent when CO_2, light and temperature are not the limiting factors. Oxygen is inhibitory to photosynthesis, because it would facilitate rapid rate of respiration, utilizing common intermediates, thus reducing photosynthesis. Plants which do not exhibit photorespiration (C_4 plants) have CO_2 compensation points very close to zero and are capable of photosynthesizing at very high rates.

Higher than normal oxygen concentrations have long been known to inhibit photosynthesis. This is called the Warburg effect, as mentioned earlier. In certain terrestrial plants, even the oxygen of normal air strongly inhibits photosynthesis. This can be explained as an inhibitions only of net photosynthesis, resulting from an increase in photorespiration by the O_2, induced production of glycolate. The O_2 would, therefore, increase the loss from the sugar phosphate pool rather than specifically inhibiting an enzyme system. Oxygen also destroys the excited state of chlorophyll and thereby inhibits photosynthesis.

Water: Besides light, temperature, CO_2 and O_2 concentration, water may affect the rate of photosynthesis. Water is an essential raw material in photosynthesis. Normally, less than one percent of the water absorbed by a plant is considerable drop in photosynthesis (usually from field capacity to permanent wilting co-efficient). Low moisture supply may lead to a cessation of photosynthesis. The inhibitory effect is primarily attributed to increased dehydration of protoplasm and also stomatal closure. The removal of water from protoplasm also affects its colloidal state, impairs enzyme efficiency besides causing damage to structure of chloroplasts. Water stress may cause drying of the cell walls of mesophyll cells, reducing their permeability to CO_2. It may also accumulate sugars, thus resulting in increase in respiratory rate and decrease in the rate of photosynthesis.

Nutrients: The efficiency of photosynthesis is commonly based on availability of essential minerals for plant growth. Those include Mg, Fe, Cu, Mn, Cl, P and are closely associated nutrients with enzyme systems as they are integral components for all key reactions of photosynthesis. The deficiency of any of these elements would have serious effect on photosynthetic activity.

Air pollutants are also known to result in decreased rate of photosynthesis. Certain gaseous substances react with cellular constituents and bring about the death of the affected tissue. Sometimes, the entry of CO_2 into the leaf is prevented, low concentrations of certain gases (sublethal doses) may temporarily inhibit photosynthetic activity without causing permanent damage.

Check Your Progress - 7

Name the important external factors that affect the rate of photosynthesis?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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8.4.2. Internal Factors

Chlorophyll: Chlorophylls constitute about 65 percent in chloroplast segments besides carotenes (6 per cent) and xanthophyll (29 percent). Percentage distribution is highly variable. Chlorophyll-a and chlorophyll-b are most frequent in higher plants.

Photosynthetic activity coincides with the amount of chlorophyll, maximum absorption peaks of chlorophyll-a and chlorophyll-b. Although several other pigments in addition to chlorophylls participate in the absorption of radiant energy used in photosynthesis, only chlorophyll a participates directly in the reactions leading to the conversion of radient energy to chemical energy. Photosynthetic prospect of the leaf is dependent upon the presence of chlorophyll pigment.

Stomatal behaviour: Carbondioxide reaches the chloroplasts via the open stomata. Stomatal resistance can help regulate photosynthesis. Both transpiration and photosynthesis show close dependence upon stomatal resistance, under stable conditions. In other words, both water and CO_2 exchange are under stomatal control.

Stomatal opening is affected by light quality as well as intensity. Blue light is far more effective than red in promoting stomatal opening. Rapid stomatal responses require sudden changes in osmotic potential of guard cells as provided by potassium ion flux, selective absorption of potassium ions by guard cells under illumination may be the primary mechanism for stomatal opening whereas sustained opening depends on a shift in starch- sugar interconversion initiated by the alteration in potassium ion concentration. External factors, like light intensity and quality, photoperiod, CO_2 concentration, water, can affect photosynthesis via stomatal resistance.

Enzymes: Enzyme activity can govern not only the rate of photosynthesis, but sometimes also the direction of the metabolic events. Enzyme assays on cell free systems suggest that the activity of the major carboxylating systems in C_3 plants (RuBP carboxylase) is really insufficient to support even the limited rate of CO_2 assimilation observed in the intact leaf. This key photosynthetic enzyme is also sensitive to end product inhibition. Enzymes of the C_4 fixation pathway may also exert a regulatory role, besides catalyzing reactions, through the primary CO_2 fixing system, PEP carboxylase, as it is also inhibited by its own reaction product, oxaloacetate. Similarly, another enzyme of the C_4 pathway, pyruvate dikinase, can be selectively inhibited. This enzyme is light-sensitive. Another critical enzyme is the NADP linked glyceraldehyde-3-phosphate dehydrogenase, which is also located in the chloroplast. This enzyme shows a rapid increase in activity following illumination.

Hormones: The effects of hormones on leaf photosynthesis are complex. Gibberellins and cytokinins can increase the rate,of photosynthesis. The treated plants also showed higher carboxylating activity, apparently due to enzyme activation rather than de novo synthesis.

Check Your Progress - 8

What are the hormones that increase the rate of photosynthesis?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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8.5. SUMMARY

Based on CO_2 fixation pathways, plants are classified as C_3, C_4 and Crassulacian Acid Metabolism (CAM) plants. In C_3 pathway or Calvin cycle the initial product is a 3 carbon compound, Phosphoglyceric acid (PGA) and carbondioxide acceptor is RuBP. The first formed products in C_4 pathway or Hatch Slack Pathway are oxaloacetate, malate and aspartate (4 carbon compounds). The CO_2 acceptor is phosphoenolpyruvic acid (PEP). The Crassulacian acid metabolism is observed in succulents (Xerophytes), where the CO_2 absorption and assimilation are separated temporarily. The C_4 plants exhibit a characteristic Kranz anatomy of their leaves and dimorphism in the chloroplasts located in mesophyll cells and bundle sheath cells. C_4 plants are characterised with high photosynthetic rates on the other hand C_3 plants show low CO_2 fixation ability. The factors influencing the rate of photosynthesis can be divided into internal and external. External or environmental factors include light, carbondioxide concentration, temperature, water, oxygen concentration and nutrient supply. Internal factors refer to photosynthetic enzyme systems, leaf resistance, hormones and leaf age.

8.6. CHECK YOUR PROGRESS : MODEL ANSWERS

1. In Calvin cycle Ribulose 1,5 bisphosphate (RuBP) is the initial CO_2 acceptor.
2. Phsophatase is the enzyme that is responsible for the convension of fructose 1,6 diphosphate to fructose-6 phosphate.
3. Phosphoenol pyruvate is the primary acceptor of CO_2 in C_4 plants.
4. The bundle sheath of C_3 plants is usually pale green due to the absence of chloroplasts whereas it is dark green in C_4 plants due to the presence of chloroplasts.
5. Any five of the following families: Portulaccacea, Amarantaceae, Chenopodiacea, Nictaginacea, Aizoacea, Euphorhiacea, Caryophyllacea, Zygophyllacea, Boraginacea and Asteracea.
6. In some plants the total organic content decrease during the day and the pH of the leaf cell sap increases. During nights the organic content increases whereas the leaf cell sap pH decreases. All those plants which show this type of rhythms are called CAM plants.
7. Light, temperature CO_2, O_2, H_2O and nutrients are the important external factors that affect the rate of photosynthesis.
8. The hormones that increase the rate of photosynthesis are gibberellins and cytokinins.

8.7. MODEL EXAMINATION QUESTIONS

I. Answer the following question in 30 lines each.

1. Explain C_3 cycle of carbon assimilation.
2. What is Hatch-Slack mechanism? Explain how it is a more efficient way of carbon fixation?
3. Explain the dark fixation of carbon dioxide in CAM plants.
4. What are the factors that affect photosynthesis in land plants? Explain CO_2 as a factor affecting photosynthesis?
5. What is Black man's Law of limiting factors? Explain it with reference to light and CO_2 concentrations.
6. How does water stress affect photosynthesis?

II. Answer the following questions in 10 lines each.

1. Distinguish between C_3 & C_4 pathways of carbon assimilation in terms of enzymes concerned.
2. Explain the C_3 pathway taking place in the bundle sheath chloroplasts of C_4 plants.
3. What are the adaptations of CAM plants? What is their significance?
4. How do shade plants get the light for photosynthesis?
5. Explain how light acts as a limiting factor in photosynthesis?
6. Explain the role of stomata in photosynthesis.
7. What are the factors that affect photosynthesis in aquatic plants? Explain it with reference to quality of light and availability of CO_2.

UNIT-9: GLYCOCYSIS AND FERMENTATION

Contents

9.1. OBJECTIVES

By the end of this unit you will be able to:

1. describe the different steps in glycolysis,
2. define the fermentation process carried by some micro-organisms,
3. describe and differentiate between the alcohol fermentation and lactic acid fermentation.

9.2. INTRODUCTION

The process of liberation of chemical energy from organic substances is called respiration. There are two kinds of respiration - aerobic and anaerobic. Respiration which takes place in the presence of oxygen is called aerobic respiration and respiration in the absence of oxygen is called anaerobic respiration. Usually the reserve substances are utilised in respiration. When the reserve substances are exhausted, the organic cell constituents are also utilised.

During the process of photosynthesis, light energy is converted to chemical energy and stored in the bonds of complex organic molecules. Most of the stored energy in the plants is usually found in the form of starch and glucose. The breaking up of the bonds of these compounds releases a considerable amount of energy which is useful to the plant. The energy released during the oxidation of organic compounds is stored in the form of adenosine triphosphate (ATP).

By considering glucose as the substrate, the equation of the entire aerobic respiration is written as:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6H_2O + 690 \text{ K cals of energy.}$$

During this process, only 38 ATP molecules are formed and each ATP molecules contains 12 K cals. of energy. This shows that only 456(38 x 12) K cals of energy is trapped in ATP molecules and the remaining 234 K cals. of energy is wasted as heat.

Before the series of reactions in respiration are discussed, a mention must be made about oxidation and reduction. The removal of electrons from a compound usually accompanied by the removal of hydrogen is called oxidation. The addition of electrons to a compound usually accompanied by the addition of hydrogen is called reduction.

9.3. GLYCOLYSIS

Although glucose is considered as a respiratory substrate, it should be phosphorylated before it could act as a primary substrate in glycolysis. In the initial phase of respiratory metabolism glucose is converted to pyruvic acid. The series of reactions and the enzymes involved are the same in all living cells of micro-organisms, higher plants and also animals. The sequence of reactions in the conversion of glucose to pyruvic acid was first elucidated in yeast and muscle cells. In mammalian skeletal muscle, the pathway actually starts with glycogen, a reserve polymer of glucose. Literally glycolysis means degradation of glycogen. Because of these early investigations, the pathway is referred to as glycolysis. This pathway is also called as EMP pathway in honour of Embden, Meyerhof and Parnas (German Biochemists) who discovered it. This pathway takes place outside the mitochondria in cytoplasm.

The equation for the overall reaction of glycolysis is written as:

$$\underset{\text{Glucose}}{C_6H_{12}O} \longrightarrow \underset{\text{Pyruvate}}{2C_3H_4O_3} + 4H + 2\ ATP$$

The above equation states that one molecule of glucose is converted into two molecules of pyruvate. The sequence of steps in glycolysis is given in Fig.9.1.

Glucose
1 ↓
Glucose -6- phosphate
2 ↓
Fructose - 6 - phosphate
3 ↓
Fructose - 1,6-diphosphate
4 ↓   5 ↓
3-phosphoglyceraldehyde ⇌ Dihydroxy acetone phosphate
6 ↓
1,3-diphopshoglyceric acid
7 ↓
3- phosphoglyceric acid
8 ↓
2- phosphoglyceric acid
9 ↓
phosphoenol pyruvate
10 ↓
pyruvic acid

Fig.9.1. Degradation of one molecule of glucose to 2 molecules of pyruvic acid by glycolysis. 1.Hexokinase 2.Phosphohexoisomerase 3. Phosphofructokinase 4.Aldolase 5.Triose phosphate isomerase 6.Phosphoglyceraldehyde dehydrogenase 7.Phosphoglycerokinase 8.Phosphoglycero-mutase 9.Enolase 10.Pyruvic kinase.

Check Your Progress - 1

Who have discovered glycolysis?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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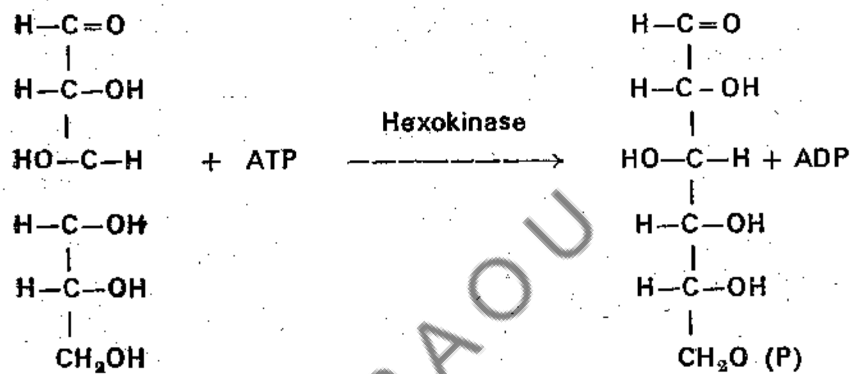
Glycolysis may be subdivided into two major steps. 1. The conversion of glucose into fructose 1,6-diphosphate and 2. The splitting up of fructose 1,6-diphosphate into two three-carbon compounds, which are eventually converted to pyruvic acid. All the reactions of glycolysis occur in the cytoplasm and these reactions do not require oxygen.

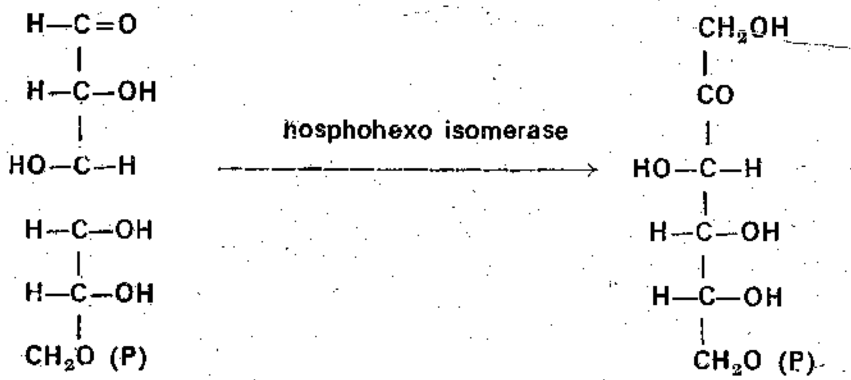
(1) First, the 6th carbon of glucose is phosphorylated in the presence of ATP and the enzyme hexokinase. The products of this reaction are Glucose 6- phosphate and ADP.



Glucose   Glucose-6-phosphate

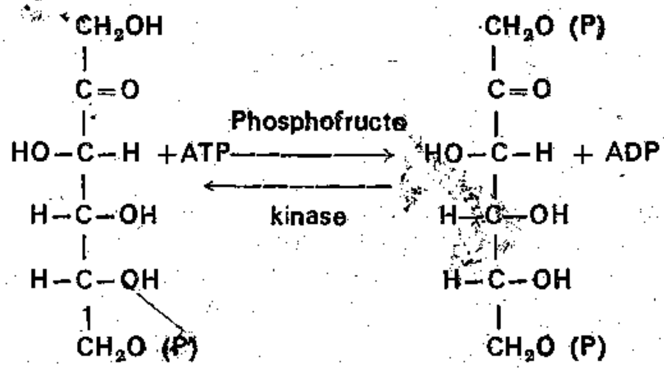
Glucose 6-phosphate is the starting point for glycolysis. In other words glucose should be phosphorylated before, it could take part in glycolysis.

(2) In the next reaction Glucose-6 phosphate is converted to fructose 6- phosphate in the presence of the enzyme phosphohexoisomerase.



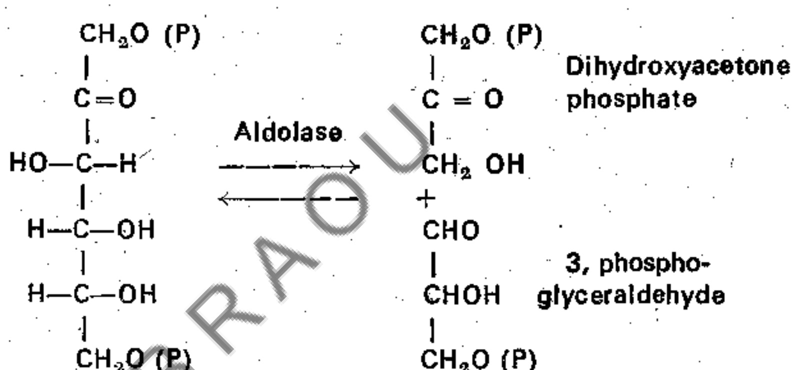
Glucose-6-phosphate   Fructose-6-phosphate

(3) The first carbon of fructose 6-phosphate is phosphorylated in the presence of ATP and the enzyme phosphofructokinase.



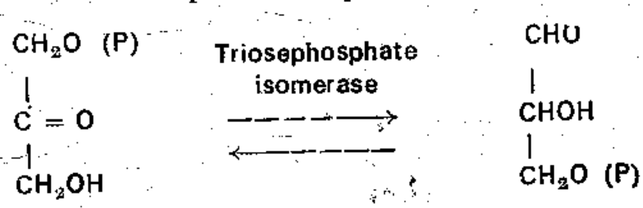
Fructose-6-phosphate Fructose-1, 6-diphosphate

(4) Fructose-1, 6-diphosphate thus formed, is split up into two three- carbon compounds in the presence of an enzyme *aldolase*. The two three- carbon compounds are 3-phosphoglyceraldehyde and dihydroxyacetone phosphate.



Fructose 1, 6, diphosphate

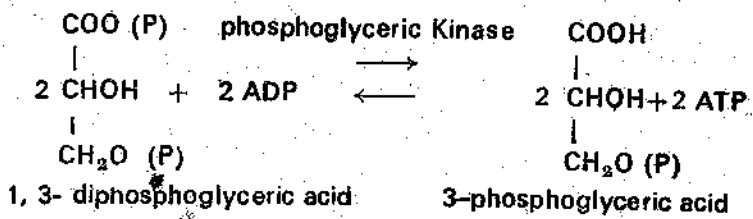
Dihydroxyacetone phosphate and 3-phosphoglyceraldehyde are interconvertible i.e., an quilibrium exists between these two compounds in the presence of an enzyme *triose phosphate isomerase*



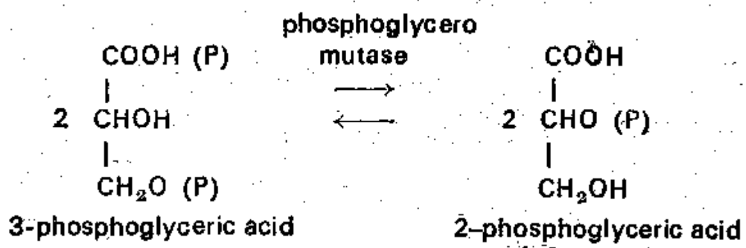
Dihydroxyacetone phosphate 3- phosphoglyceraldehyde

(5) In the next step, the two molecules of 3-phosphoglyceraldehyde are converted to diphosphoglyceric acid in the presence of an enzyme phosphoglyceraldehyde dehydrogenase. In this reaction, the addition of inorganic phosphate (phosphoric acid = H_3PO_4) and reduction of NAD takes place.

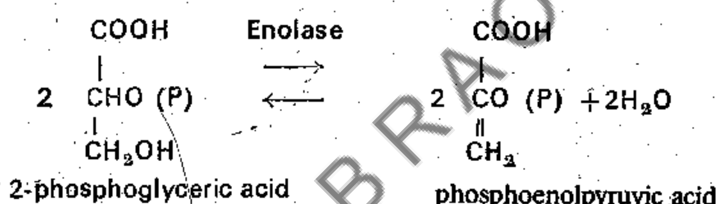
(6) 1,3-diphosphoglyceric acid is converted to 3-phosphoglyceric acid in the presence of an enzyme *phosphoglycerokinase* and ADP.



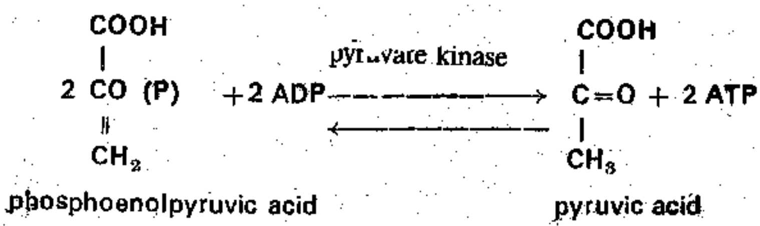
(7) The 3-phosphoglyceric acid formed in the above reaction is transformed into 2-phospho glyceric acid by the activity of the enzyme phosphoglyceromutase.



(8) The removal of water from 2-phosphoglyceric acid results in the formation of phosphoenolpyruvic acid in the presence of an enzyme enolase.



(9) In the last reaction of glycolysis phosphoenolpyruvic acid is converted to pyruvic acid in the presence of ADP and an enzyme pyruvate kinase.



To sum up, for each molecule of glucose consumed in glycolysis, 2 molecules of pyruvic acid and 2 molecules of NADH $+H^+$ are formed (Reaction 5). Also 2 molecules of ATP are consumed (Reaction 1&3) and 4 molecules of ATP are regenerated (Reactions 6&9). So, there is a net gain of 2 molecules of ATP.

In addition to this, the intermediate products of glycolysis are also used in the synthesis of complex constituents of plants. For example, glyceraldehyde formed as an intermediate may be converted to glycerol which will in turn be used in the synthesis of lipids.

Except the first two reactions, all the other reactions of glycolytic pathway are reversible. So, by the reversal of glycolysis, a plant cell can synthesise fructose 1,6-diphosphate from pyruvic acid. Fructose 1,6- diphosphate thus formed may be dephosphorylated to fructose-6-phosphate by the activity of dephosphorylating enzymes (phosphatases). Then fructose-6- phosphate may be converted to glucose, sucrose or starch. This shows that glycolysis is not only a pathway of carbohydrate degradation to pyruvic acid but also useful in the synthesis of glucose, sucrose and starch from pyruvic acid.

Check Your Progress - 2,3 & 4

2. Which is the enzyme that is responsible for the conversion of dihydroxy acetone phosphate to 3-phosphoglyaraldehyde.
3. Name the reaction in which 2 moles of ATP is formed?
4. One molecule of ATP is utilised in the conversion of __________ to __________ where the enzyme involved is prosphofructokinase.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end of this unit.

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9.4. FERMENTATION

The term fermentation (literally a chemical change followed by effervescence) is loosely applied to several processes carried by some microorganisms such as bacteria, yeasts and certain other fungi. Some examples of fermentation are alcohol fermentation, lactic acid fermentation, citric acid fermentation, oxalic acid fermentation etc. All the fermentations are of commercial importance. In this lesson alcohol and lactic acid fermentations are discussed.

9.4.1. Alcohol Fermentation

Alcohol fermentation is the type of respiration (anaerobic respiration) carried on by yeast cells. In this process, alcohol and carbon dioxide are produced. The production of alcohol through fermenta tion has been known to man from ancient times. Pasteur (1857) discovered that alcohol fermentation was due to anaerobic respi ration of yeast cells. Buchner (1897) discovered that the enzyme zymase extracted from yeast cells can catalyse the process. Enzyme zymase is a complex of several enzymes.

Pyruvic acid produced in plants undergoes different metabolic fates depending upon the presence or absence of oxygen. Under aerobic conditions (i.e., in the presence of oxygen) the enzymes present in mitochondria convert pyruvic acid to carbon dioxide and water. However, certain anaerobes such as yeasts, convert pyruvic acid to alcohol and carbon dioxide in two steps.

In the first step pyruvic acid is decarboxylated into acetaldehyde and carbon dioxide in the presence of an enzyme pyruvic acid decarboxylase.

$$\underset{\text{pyruvic acid}}{\begin{array}{c} CH_3 \\ | \\ C{=}O \\ | \\ COOH \end{array}} \xrightarrow[\text{decarboxylase}]{\text{Pyruvic acid}} \underset{\text{Acetaldehyde}}{CH_3.CHO} + CO_2$$

In the second step, acetaldehyde is reduced to ethyl alcohol by the reducing agent NADH+H$^+$. This reaction is catalysed by the enzyme alcohol dehydrogenase.

$$\underset{\text{Acetaldehyde}}{CH_3.CHO} + NADH + H^+ \xrightarrow[\text{dehydrogenase}]{\text{Alcohol}} \underset{\text{Ethyl alcohol}}{CH_3.CH_2\,OH} + NAD$$

In addition to glucose, yeasts can ferment sucrose and moltose also as they contain the enzymes sucrase and maltase. The above disaccharide sugars are first converted to hexose sugars. The enzyme amylase is lacking in the yeasts. So they cannot ferment starch which has to be hydrolysed into hexose sugars.

9.4.2. Lactic Acid Fermentation

A different course is followed in lactic acid bacillus, animal tissues and some plant cells. In this process lactic acid is produced instead of alcohol and carbon dioxide. This process is best known for its souring effect on milk. The pyruvic acid molecules formed in glycolysis are directly reduced by NADH+H$^+$ to lactic acid. This reaction is catalysed by the enzyme lactic acid dehydrogenase.

$$\underset{\text{pyruvic acid}}{H_3C{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}COOH} + NADH + H^+ \xrightarrow[\text{dehydrogenase}]{\text{lactic acid}} \underset{\text{Lactic acid}}{H_3C{-}\underset{\underset{\displaystyle H}{|}}{\overset{\overset{\displaystyle OH}{|}}{C}}{-}COOH} + NAD$$

It is worth mentioning here that the products of fermentation (both ethanol and lactic acid) still contain so much of energy locked up within their structures. The net gain of fermentation is only 2 ATP molecules which is equal to 24(2 x 12) K cals. of energy. A molecule of glucose contains 690 K cals of energy and in aerobic respiration 456 K cals. of energy is trapped ATP moles. So fermentation is considered to be relatively an inefficient process.

Check Your Progress - 5

Which is the enzyme responsible for the conversion of acetaldehyde to ethyl alcohol?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end of this unit.

9.5. SUMMARY

Respiration is of two types-aerobic and anaerobic. Respiration taking place in the presence of oxygen is called aerobic respiration and respiration in the absence of oxygen is called anaerobic respiration. In respiration, glucose is converted to carbondioxide and water in the presence of oxygen. Although glucose is considered to be the precursor of glycolysis, it should be photophosphorylated before it enters the glycolytic pathway. It undergoes a series of reactions in the presence of several enzymes to produce pyruvic acid. The removal of electrons from a compound usually accompanied by the removal of hydrogen is called oxidation and the addition of electrons followed by the addition of hydrogen is called reduction. Liter ally glycolysis means degradation of glycogen. Glycolytic pathway was first discovered in mammalian skeletal muscles. Where it actually starts with glycogen. Glycogen is a reserve polymer of glucose in animals and certain fungi. Glycolysis is subdivided into two major steps. In the first step glucose is converted to fructose 1,6-diphosphate. In the second step fructose 1,6-diphosphate is converted to 2 molecules of pyruvic acid. All the reac tions of glycolysis take place in cytoplasm. For each molecule of glucose consumed in glycolysis, 2 molecules of pyruvic acid, 2 molecules of $NADH+H^+$ and 2 molecules of ATP are formed. Most of the reactions of glycolytic pathway are reversible. Glucose may also be produced from 2 molecules of pyruvic acid. Literally, fermentation means a chemical change followed by effervescence. Some examples of fermentation are lactic acid fermentation, alcohol fermentation, malic acid fermentation, citric acid fer mentation etc. In alcohol fermentation, pyruvic acid formed in glycolysis is converted to carbon dioxide and ethyl alcohol. in lactic acid fermentation, pyruvic acid is directly reduced to lactic acid.

9.6. CHECK YOUR PROGRESS : MODEL ANSWERS

1. Glycolysis was discovered by Embden, Meyerhoff and Parnas.
2. Triose phosphate isomerase is the enzyme responsible for the conversion of dihydroxy acetone phosphate to 3-phosphoglycerlade hyde.
3. In the conversion of 1,3-diphosphoglyceric acid to 3-phosphoglyceric acid 2 moles of ATP is formed.
4. Fructose 6-phosphate, Fructose 1,6 diphosphate.
5. Alcohol delydrogenase is the enzyme responsible to ethyl alcohol.

9.7. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines.

1. Write an account of Glycolysis. Why did the name glycolysis come to this pathway?
2. What is fermentation? Write an account of lactic acid & alcohol fermentations.
3. Give an account of glycolysis. Add a note on the differences between aerobic respiration & anaerobic respiration.

II. Answer the following questions in 10 lines.

1. What are the differences between aerobic respiration & Fermentation?
2. What are oxidation & reduction? Give one example for each one of them.
3. What are the reactions involved in the conversion of glucose to fructose 1,6-diphosphate?
4. What are the sites of ATP production and ATP utilisation in glycolysis?
5. What is fermentation? And write about the Lactic acid fermentation.
6. What is fermentation? How is alcohol produced in alcohol fermentation?

UNIT-10: KREBS CYCLE AND ELECTRON TRANSPORT SYSTEM

Contents

10.1. OBJECTIVES

By the end of this unit you will be able to:

1. describe the various steps in the conversion of pyruvic acid to carbondioxide and water in krebs cycle,
2. list out the different carriers of electrons in the respiratory chain,
3. define and differentiate transphasphorylation and oxidative phospharylation.

10.2. INTRODUCTION

Pyruvic acid formed at the end of glycolysis is further oxidised during Krebs cycle and electron transport. Krebs cycle is named after *Hans Krebs*, an English biochemist, who played a major role in the discovery of the reactions. This cycle is also known as *Citric acid* cycle because citric acid is one of the intermediates. As citric acid is a tricarboxylic acid, this cycle is also known as tricarboxylic acid cycle (TCA cycle). Pyruvate is completely oxidised to CO_2 and water by means of Krebs cycle and electron transport system. Before entering into the TCA Cycle, pyruvic acid undergoes oxidative decarboxylation and form acetyl coenzyme A (Acetyl CoA). The reaction is very complex. It requires the presence of atleast five essential cofactors and a complex of enzymes. The cofactors are thiamine pyrophosphate (TPP), Mg ions, NAD^+, coenzyme A and lipoic acid. The reaction involves four steps.

Check Your Progress - 1

Kreb's cycle is also called as TCA cycle - Why?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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10.3. REACTIONS IN KREBS CYCLE

In the first step, pyruvic acid forms a complex with TPP and this will be followed by decarboxylation.

$$\text{Pyruvate} + \text{TPP} \longrightarrow \text{TPP Complex} + CO_2$$

In the second step, the TPP complex reacts with another cofactor lipoic acid and forms acetyl lipoic acid complex and TPP. In this reaction TPP is regenerated.

$$\text{TPP complex} + \underset{\text{(oxidised form)}}{\text{Lipoic acid}} \longrightarrow \text{Acetyl lipoic acid complex} + \text{TPP}$$

In the third step, acetyl lipoic acid reacts with CoA and forms acetyl CoA and reduced form of lipoic acid.

$$\text{Acetyl lipoic acid complex} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \underset{\text{(reduced form)}}{\text{Lipoic acid}}$$

In the final step reduced form of lipoic acid is oxidised by NAD^+

$$\underset{\text{(reduced form)}}{\text{Lipoic acid}} + NAD^+ \longrightarrow \underset{\text{(Oxidised form)}}{\text{Lipoic acid}} + NADH + H^+$$

In summary, the above four steps are usually shown by the following equation.

$$\text{Pyruvate} + \text{CoA} + NAD^+ \longrightarrow \text{Acetyl CoA} + \text{CoA} + Co_2 + NADH + H^+$$

The sequence of steps in Krebs Cycle is given below in Fig.10.1. All the reactions of Krebs cycle take place within the mitochondria, particularly on the cristae.

(1) The first reaction of Krebs Cycle is the condensation of acetyl CoA with oxaloacetic acid (oxaloacetate). This reaction is catalysed by a condensing enzyme and results in the formation of citric acid and CoA.

$$\underset{\text{Acetyl CoA}}{\begin{array}{c} CH_3 \\ | \\ C=O \\ | \\ CoA \end{array}} + \underset{\text{oxaloacetate}}{\begin{array}{c} O=C-COOH \\ | \\ H_2-C-COOH \end{array}} \xrightarrow[\text{enzyme}]{\text{Condensing}} \underset{\text{Citric acid}}{\begin{array}{c} H_2-C-COOH \\ | \\ HO-C-COOH \\ | \\ H_2-C-COOH \end{array}} + \text{CoA}$$

(2) Citric acid thus formed is dehydrated to cis-aconitic acid in the presence of the enzyme aconitase.

$$\underset{\text{citric acid}}{\begin{array}{c} H_2\ C-COOH \\ | \\ HO-C-COOH \\ | \\ H_2-C-COOH \end{array}} \xrightarrow[\text{Aconitase}]{-H_2O} \underset{\text{Cis-aconitic acid}}{\begin{array}{c} H_2-C-COOH \\ | \\ C-COOH \\ \| \\ H-C-COOH \end{array}}$$

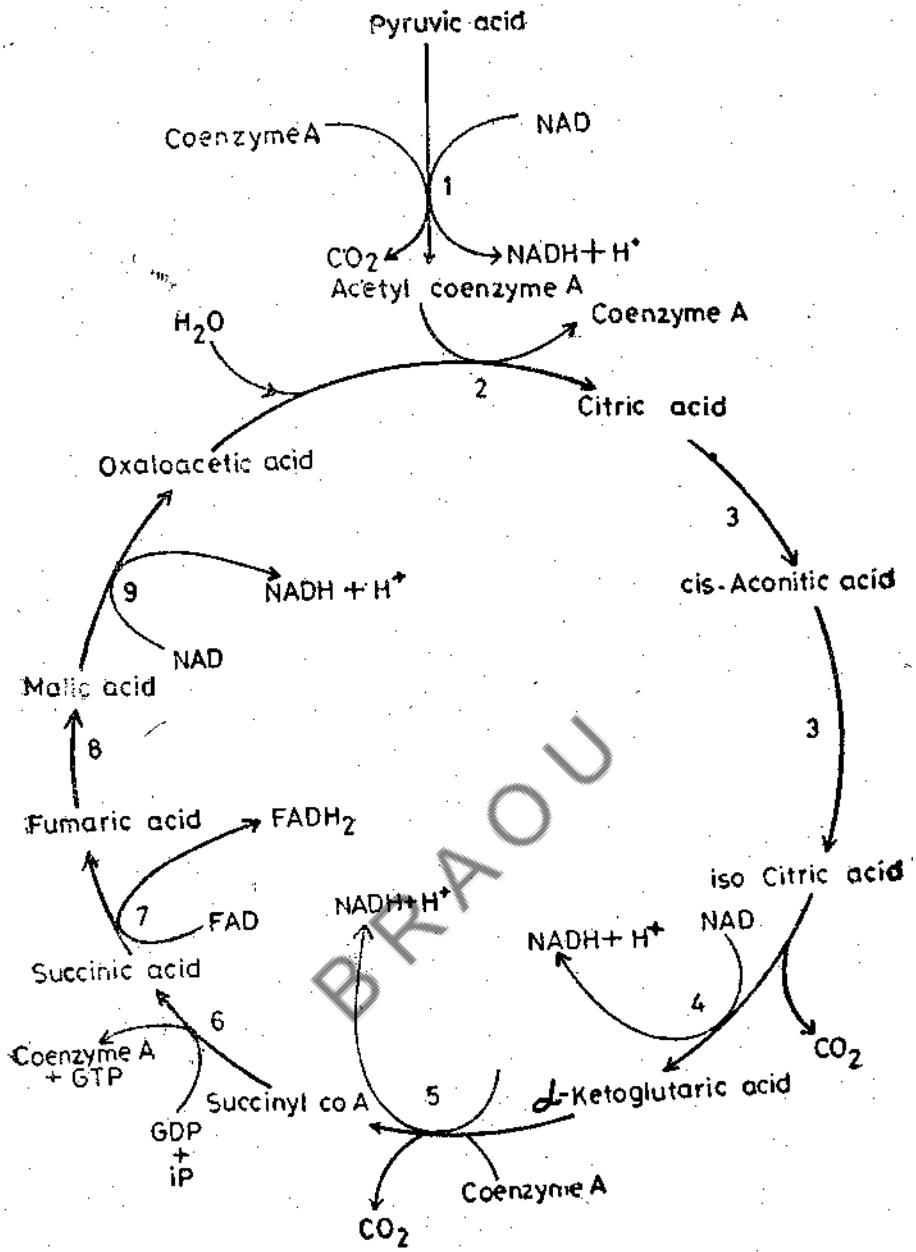
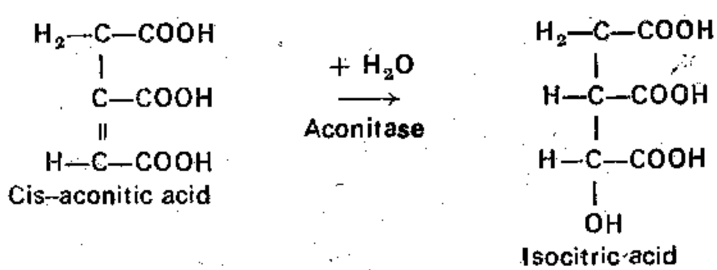
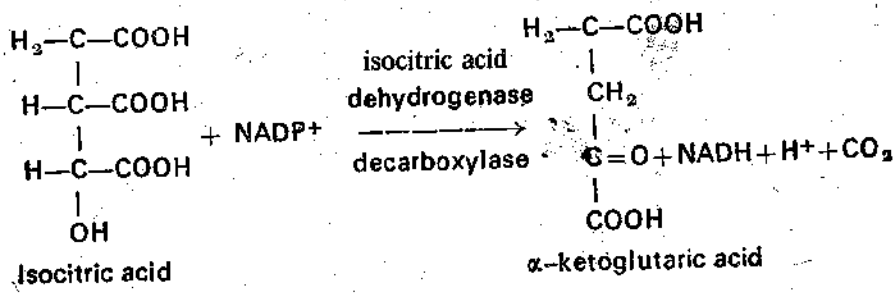


Fig.10.1 Krebs cycle or TCA cycle. 1. Pyruvic acid dehydrogenase and decarboxylase with TPP as coenzyme 2.Condensing enzyme 3.Aconitase 4.Isocitric acid dehydrogenase & decarboxylase 5α-Ketoglutaric dehydrogenase & decarbnoxylase 6.Succinyl kinase 7.Succinic dehydrogenase 8.Fumarase 9.Malic dehydrogenase.

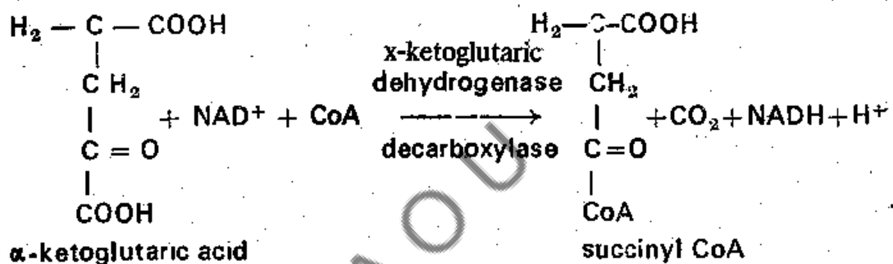
(3) Cis-aconitic acid is hydrated to form isocitric acid in the presence of the same enmzyme aconitase.



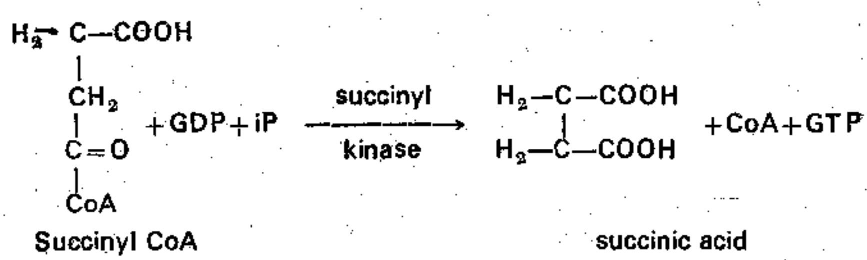
(4) In the presence of the enzyme isocitric acid dehydrogenase & decarboxylase and NAD isocitric acid is dehydrogenated and decarboxylated to α-ketoglutaric acid. Two electrons and two hydrogen ions are removed from isocitric acid and these are taken up by the coenzyme NAD^+ to form $NADH+H_+$.



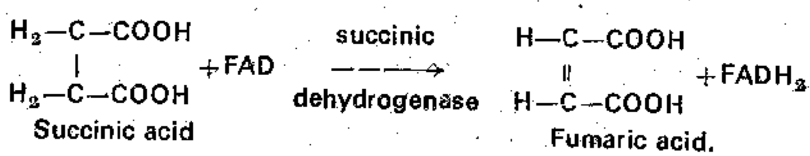
(5) α-ketoglutaric acid is decarboxylated and dehydrogenated to succinyl CoA in the presence of α-ketoglutaric dehydrogenase and decarboxylase. NAD^+ is the oxidising agent.



(6) Then, succinyl CoA is converted to succinic acid. In this conversion process, the energy locked up in succinyl CoA is released. This energy converts guanosine diphosphate (GDP) and inorganic phosphate into guanosine triphosphate (GTP).



(7) In the presence of the enzyme succinic dehydrogenase succinic acid is converted or dehydrogenated to fumaric acid. In this reaction Coenzyme FAD (Flavin adenine dinucleatide) is the oxidising agent.



(8) In the next reaction fumaric acid is converted (dehydrated) to malic acid in the presence of water. This reaction is catalysed by the enzyme fumarase.

$$\begin{array}{l} COOH \\ | \\ CH \\ \| \\ CH \\ | \\ COOH \\ \text{Fumaric acid} \end{array} + H_2O \xrightarrow{\text{fumarase}} \begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CH_2 \\ | \\ COOH \\ \text{Malic acid} \end{array}$$

(9) In the last reaction, the oxaloacetic acid consumed in the first reaction is regenerated from malic acid by dehydrogenation. This reaction is catalysed by the enzyme malic dehydrogenase. The oxidising agent here is NAD^+

$$\begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CH_2 \\ | \\ COOH \\ \text{Malic acid} \end{array} + NAD^+ \xrightarrow[\text{dehydrogenase}]{\text{malic}} \begin{array}{l} COOH \\ | \\ C{=}O \\ | \\ CH_2 \\ | \\ COOH \\ \text{Oxaloacetic acid} \end{array} + NADH + H^+$$

To sum up, in one complete turn of Krebs cycle, one molecule of two carbon acetyl unit is consumed and two molecules of carbondioxide are released. At each of the four reactions in the cycle two hydrogen atoms (2 protons and 2 electrons) are released. At 3 places NAD^+ is reduced to $NADH+H^+$ and at the 4th place FAD is reduced to $FADH_2$. In the conversion of succinyl CoA to succinic acid one molecule of GTP is formed. Oxaloacetic acid which is used in the first step is regenerated in the final reaction. The overall reactants and products of Krebs cycle are:

$$\text{Acetyl unit} + 3NAD^+ + FAD + GDP + iP \longrightarrow 2CO_2 + 3\,(NADH + H^+) + FADH_2 + GTP$$

With this information, if we sum up, all the reactions of glycolysis and Krebs cycle yield 6 molecules of carbondioxide, 2 molecules each of ATP and GTP, 10 reduced NAD(NADH $+H^+$) and 2 $FADH_2$. The reactants and products are:

$$\text{Glucose} + 2\,ADP + 2\,GDP + 4\,iP + 10\,NAD + 2\,FAD \longrightarrow 6CO_2 + 2\,ATP + 2\,GTP + 10\,(NADH + H^+) + 2\,FADH_2$$

It is worth mentioning here that for each molecule of glucose entering the pathway, the Krebs cycle will turn twice.

Till now, only 2 molecules of ATP and two molecules of GTP are produced. However the electrons carried by the 10 reduced NAD and 2 reduced FAD molecules contain most of the energy obtained from the oxidation of glucose to 6 molecules of carbon dioxide. This energy is released during the transfer of electrons to oxygen through a system of series of steps called electron transport system.

Check Your Progress-2,3&4

2. Which is the enzyme that is reponsible for the conversion of succinyl COA to succinic acid?

3. Fumaric acid is convered to malic acid by the enzyme ________.

4. How many NADH + H^+ are produced in the TAC cycle starting from acetyl COA?

Note: (a) Write your answer in the space given below:

(b) Compare your answer with the one given at the end.

10.4. ELECTRON TRANSPORT SYSTEM

The Krebs cycle cannot operate continuously unless the reduced coenzymes (NADH $+H^+$ and $FADH_2$) are oxidised. NADH$+H^+$ and $FADH_2$ are only the temporary torage products for electrons. The oxidation of these reduced NAD^+ and FAD occurs by transfer of electrons from these two to molecular oxygen via a system of mitochondrial enzymes and electron carriers. This process of continuous electron transport system is referred to as *respiratory chain*.It is also called *oxidative phosphorylation* because phosphorylation (i.e., ATP production) and oxidation are coupled and ATP is produced along with the oxidation.

The electron transport system comprised of many hydrogen and electron carriers. At one end of the chain the reduced NAD donates electrons to the oxidised form of first carrier. By accepting these electrons, the first carrier is reduced. The oxidised second carrier is reduced by accepting the electrons from the first carrier. This process of sequential reduction and oxidation of the intermediate carriers proceeds until electrons from the terminal carriers are passed to molecular oxygen, which is reduced to water.

The enzymes and electron carriers of the respiratory chain are located in the inner membranes of mitochondria. The cytochromes and flavoproteins are the electron carriers in the respiratory chain. The cytochromes are proteins with iron in a porphyrin ring. The cytochromes are also called haemoproteins since the iron-porphyrin complex is called heme. Iron has two states-ferrous and ferric. Ferrous iron is a reduced state and ferric iron is an oxidised state.

First, NADH $+H^+$ donates electrons to the first carrier i.e., oxidised flavin. In this reaction oxidised flavin is reduced. The electrons from this reduced flavin are passed on serially to Cyt.Q(or ubiquinone) Cytochrome b, Cytochrome c_1, Cytochrome c, Cytochrome a, Cytochrome a_3 and finally to molecular oxygen. The schematic representation of the respiratory chain is given below in Fig.10.2.

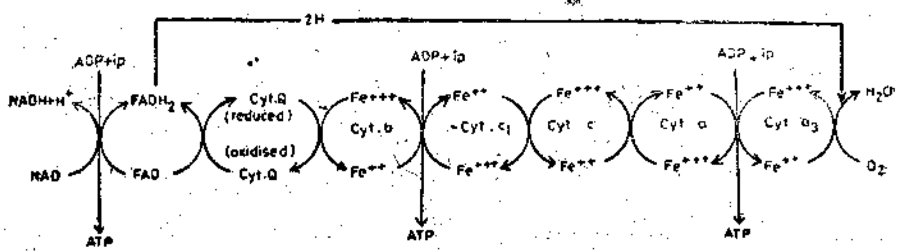


Fig.10.2. A schematic representation of the respiratory chain (There is a doubt about the participation of Cyt.Q in the electron transport)

NADH dehydrogenase and succinate dehydrogenase are the two flavoproteins which act as carriers in the electron transport system. NADH dehydrogenase contain FMN (Flavin mononucleotide) as prosthetic group and succinate dehydrogenase contain FAD (Flavin adenine dinucleotide) as prosthetic group. FMN accepts 2H from NADH $+H^+$ and is reduced to $FMNH_2$ and FAD accepts 2H from succinate and is reduced $FADH_2$. The pair of hydrogens of $FMNH_2$ or $FADH_2$ are split into 2 protons ($2H^+$) and 2 electrons. Only the electrons are passed through the cytochromes and the protons are released into the medium. At the end of the respiratory chain, oxygen accepts electrons from cytochrome a_3 and protons from the medium to form water. Water formed in the metabolic reactions of respiration is often called as respiratory water. This water represents only a small fraction of the total water in plant cells.

Cytochrome a and cytochrome a_3 are together called cytochrome oxidase. Attempts to separate cytochrome a & a_3 by scientists have not been successful. Unlike other cytochromes, cytochrome oxidase (Cyt.a & a_3) contains copper in addition to iron. Like iron, copper also has two states cuprous (Cu^+) and cupric (Cu^{++}). Cuprous is a reduced state and cupric is an oxidised state. In the respiratory chain both iron and copper undergo reversible changes.

Check Your Progress - 5

What is meant by oxidative phosphorylation?

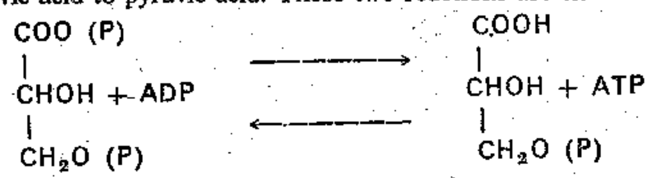
Note: (a) Write your answer in the space given below:

(b) Compare your answer with the one given at the end.

Generation of ATP: The living cells generate ATP from ADP in two different ways (1) Transphosphorylation and (2) Oxidative phosphorylation.

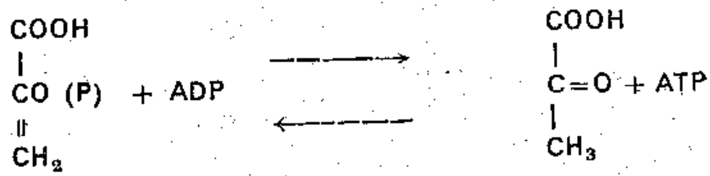
In a transphosphorylation reaction, the phosphate group of one already phosphorylated compound is transferred to ADP. You have already come across two such reactions in glycolysis (1) in the formation of 3 phosphoglyceric acid from diphospho-glyceric acid & (2) in the conversion of phosphoenol pyruvic acid to pyruvic acid. These two reactions are shown below.

(1)



Diphosphoglyceric acid — 3 phosphoglyceric acid

(2)



Phosphoenol pyruvic acid — Pyruvic acid

In oxidative phosphorylation, the oxidation of $NADH+H^+$ or $FADH_2$ takes place. This usually occurs in respiratory chain. Three molecules of ATP are synthesized for each molecule of $NADH+H^+$ when oxidised to NAD^+ in the respiratory chain. The overall reaction is:

$$(NADH + H^+) + 3ADP + 3\,iP + 1/2O_2 \longrightarrow NAD^+ + 4H_2O + 3ATP$$

It is already mentioned that at the end of Krebs cycle, for every molecule of glucose which entered into the respiratory cycle 10 $NADH+H^+$, $2FADH_2$, 2 ATP, 2 GTP and 6 molecules of carbon dioxide are formed. The Guanosine triphosphate (GTP) produced is subsequently converted to ATP by a mitochondrial enzyme that readily interconverts various nucleoside triphosphates.

$$GTP + ADP \longrightarrow ATP + GDP$$

For each $NADH+H^+$, 3 molecules of ATP are produced in the respiratory chain.

The three points of the production ATP molecules are:

1. $NADH + H^+ \longrightarrow$ Flavoprotein
2. Cyt.b $\longrightarrow$ Cyt.C_1
3. Cyt.a $\longrightarrow$ Cyt.a_3

So, far 10 molecules of $NADH+H^+$, a total of 30 molecules of ATP are formed. For each molecule of $FADH_2$, 2 molecules of ATP are formed. So far $2FADH_2$ formed at the end of Krebs cycle a total of 4 ATP molecules are formed. So, a total of 34 ATP molecules are formed in the respiratory chain. It is already mentioned that 4 ATP molecules are formed at the end of Krebs cycle. If we add all these for every glucose molecule a total of 38 ATP molecules are produced. But Jensen & Salisbury (1984) reported that the $NADH + H^+$ produced in glocolysis enters the electron transport system after the first step that generates 1 ATP molecule. So, this $NADH_2$ produced in glycolysis generates only 2 ATP molecules in respiratory chain. This needs further confirmation. According to these people only 36 molecules of ATP are formed for every molecule of glucose.

The total energy available in glucose is found to be 690 K cals. Each ATP molecule contains 12 K cals of energy. The energy stored in ATP molecules during complete oxidation of 1 glucose molecule is 456 (38 x 12) K cals. So, only 66% of the energy is trapped in the ATP molecules and the remaining 34% escapes out as heat energy.

At this stage it is worth mentioning the role of ATP (adenosine triphosphate) in living cells. In some metabolic reactions ATP is an essential participant. ATP is required in the production of materials essential to cellular maintenance and growth including proteins and nucleic acids. It is also necessary for the synthesis of storage materials such as fats and polysaccharides. ATP is found to be necessary in active transport and protoplasmic streaming. It reacts withg other compound by donating its terminal phosphate group to it.

Besides giving ATP by oxidative phosphorylation the Krebs cycle is also important in giving a number of intermediates which act as ports of entry to ammonia to form amino acids. For example, (a) α-ketoglutaric acid combines with ammonia to give rise to glutamic acid (b) oxaloacetic acid combines with ammonia to form aspartic acid. Thus, Krebs cycle provides carbon skeletons for the important aminoacids.

Check Your Progress - 6

How many K cals of energy is present in each ATP molecule? And how many ATP molecules are released in the breakdown of one glucose molecule?

Note: (a) Write your answer in the space given below:

(b) Compare your answer with the one given at the end.

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10.6. SUMMARY

Pyruvic acid undergoes oxidative decarboxylation and forms Acetyl coenzyme A (Acetyl CoA). Citric acid is formed by the condensation of Acetyl CoA with oxaloacetate. Citric acid thus formed is dehydrated to cis-aconitic acid which inturn dehydrated to form isocitric acid. Isocitric acid is dehydrogenated and decarboxylated to Ketoglutaric acid, which is again decarboxylated and dehydrogenated to succinyl CoA. Then the succinyl CoA is converted to succinic acid. The oxaloacetic acid which is taken up in the first reaction is again regenerated from succinic acid through fumaric acid and malic acid. At the end of glycolysis and Krebs Cycle for every molecule of glucose 10 molecules of reduced NAD^+, 2 molecules of reduced FAD, 6 molecules of carbon dioxide, 2 molecules of ATP and 2 molecules of GTP are formed. The reduced NAD^+ and FAD contain most of the energy and this energy is released in the respiratory chain or oxidative phosphorylation.

In the respiratory chain, the electrons are passed through flavoproteins and cytochromes. The cytochromes are cyt.Q, cyt.b, cyt.c_1, cyt.c, cyt.a, & cyt.a_3. In living cells ATP molecules are generated from ADP in two ways transphosphorylation and oxidative phosphorylation. In the respiratory chain for each $NADPH+H^+$, 3 ATP molecules are formed whereas 2 ATP molecules are formed for each $FADH_2$ molecule. For every glucose molecule, a total of 38 ATP molecules are formed in glycolysis, Krebs cycle and electron transport. The total hidden energy in glucose is 690 K cals. Whereas in the oxidation of glucose to carbon dioxide and water in respiration only 456 K cals. of energy is trapped in ATP molecules. This is only 66% of the total hidden energy. ATP is required in the production of several substances essential to cellular maintenance and growth and also the storage materials. The entire process of Krebs cycle and electron transport chain takes place in the presence of oxygen and the site of these reactions is mitochondrion which is considered as the power house of the cell.

10.7. CHECK YOUR PROGRESS : MODEL ANSWERS

1. Krebs cycle is also called as citric acid cycle because citric acid happen to be one of the intermediate. As citric acid happens to be a tricarboxylic acid, this cycle is also called Tricarboxylic acid (TCA) cycle.
2. Succinyl kinase is the enzyme responsible for the conversion of succinyl CoA to succinic acid
3. Fuonarase
4. Three $NADH+H^+$ are produced in the TCA cycle starting from acetyl CoA.
5. The process of continuous electron transport system is called respiratory chain. This respiratory chain is also called as oxidative phosphorylation because phosphorylation and oxidation are coupled and ATP is produced along with oxidation.
6. Each ATP molecule contains 12 K cals of energy. One glucose molecule releases 456 K cals of energy.

10.8. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines.

1. Write an account of Krebs cycle.
2. Write an account of electron transport system.
3. What are the sights of $NADH+H^+$ and GTP production in Krebs cycle?
4. How are carbondioxide and water produced in Krebs cycle and electron transport?

II. Answer the following questions in 10 lines each.

1. Write a brief account of electron transport system.
2. What are the sites of ATP production in electron transport system?
3. What are the cytochromes involved in the electron transport?
4. How is pyruvic acid converted to Acetyl CoA?

UNIT - 11: HMP PATHWAY AND FACTORS AFFECTING RESPIRATION

Contents

11.1. OBJECTIVES

By the end of this unit you will be able to :

1. list out different reactions in the HMP pathway and recognise the different enzymes involved in the reactions, and
2. recognise the different factors, both external and internal, that affect the rate of photosynthesis.

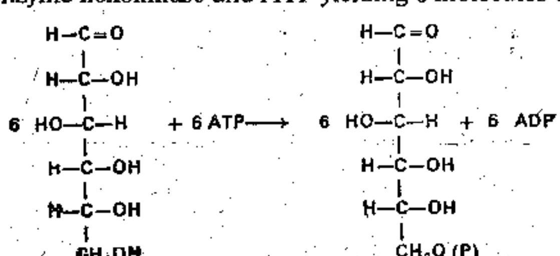
11.2. INTRODUCTION

In the earlier units you have learnt that the pathway for the break down of glucose in aerobic respiration is through glycolysis and Krebs cycle Besides this, there exists an alternative pathway in many organisms. This pathway is most commonly known as *Hexose monophosphate (HMP) pathway or Hexose monophosphate shunt or Pentose phosphate pathway or direct oxidation pathway.* Since this pathway starts with hexose monophosphate, this is called hexose monophosphate pathway. As the main intermediates of this pathway are pentose phosphates, this pathway is also called pentose phosphate pathway.

11.3. HMP PATHWAY

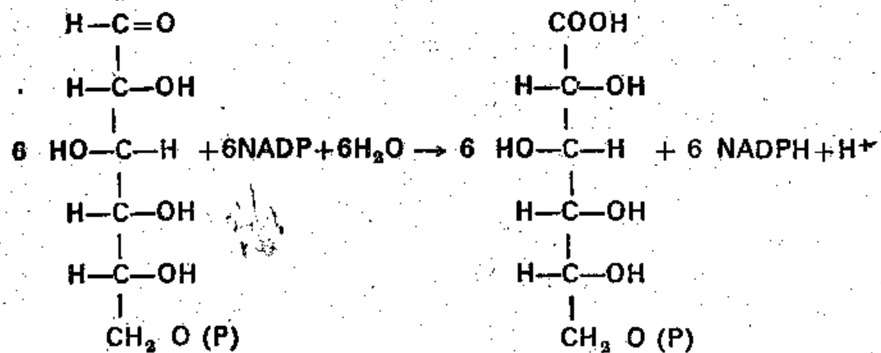
Otto Warburg and his coworkers (1930) were the first persons to indicate that glucose-6-phosphate can be oxidised in a pathway other than glycolysis. Dickens & his coworkers (1938) found that 6-phosphoglyuconic acid is formed, so, this pathway is also named as Warburg-Dickens pathway. Later, Horecker & his associates (1951) and Racker (1954) have worked out the details of this pathway. The existence of this pathway was proved beyond doubt by the studies after inhibiting the Krebs cycle by malonic acid.

1. Hexose monophosphate pathway starts with the phosphorylation of 6 molecules of glucose in the presence of the enzyme *hexokinase* and ATP yielding 6 molecules of glucose-6-phosphate.



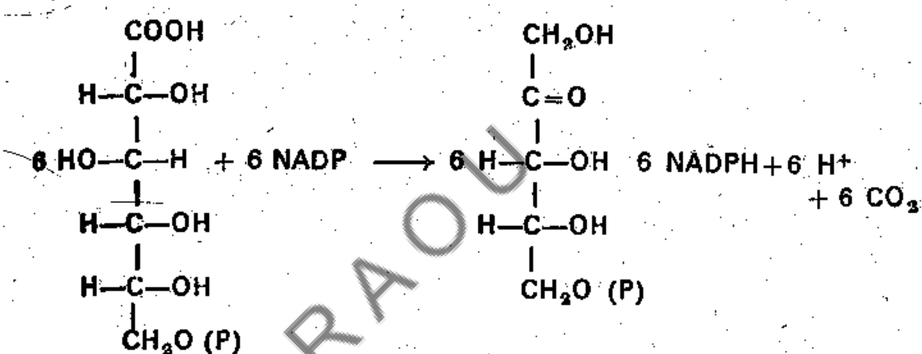
Glucose   Glucose-6-phosphate

2. Six molecules of glucose 6-phosphate are immediately oxidised to 6 molecules of phosphogluconic acid in the presence of the enzyme *glucose-6-phosphate dehydrogenase.* In this reaction 5 water molecules are used and 6 molecules of NADP are reduced to NADPH+H^+



Glucose-6-phosphate Phosphogluconic acid

3. 6 molecules of Phosphogluconic acid are dehydrogenated and decarboxylated to 6 molecules of ribulose-5-phosphate in the presence of the enzyme phosphogluconic acid dehydrogenase. In this step NADP is reduced and 6 molecules of CO_2 are formed.

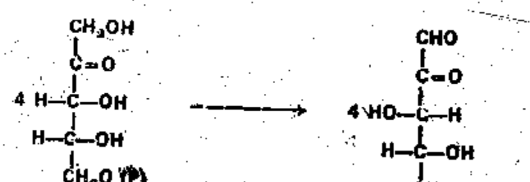


Phosphogluconic acid Ribulose-5-phosphate

4. In the fifth step out of 6 molecules of ribulose-5-phosphate 2 molecules are converted to its isomer ribose-5-phosphate in the presence of the enzyme pentosephosphate isomerase.

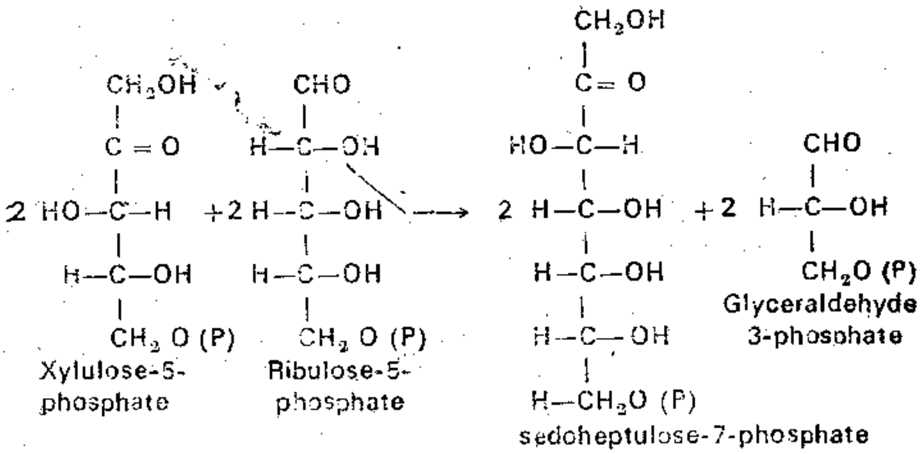
Ribulose-5-phosphate Ribose-5-phosphate

5. The remaining 4 molecules of ribulose-5-phosphate are converted to their epimer xylulose-5-phosphate in the presence of the enzyme epimerase.

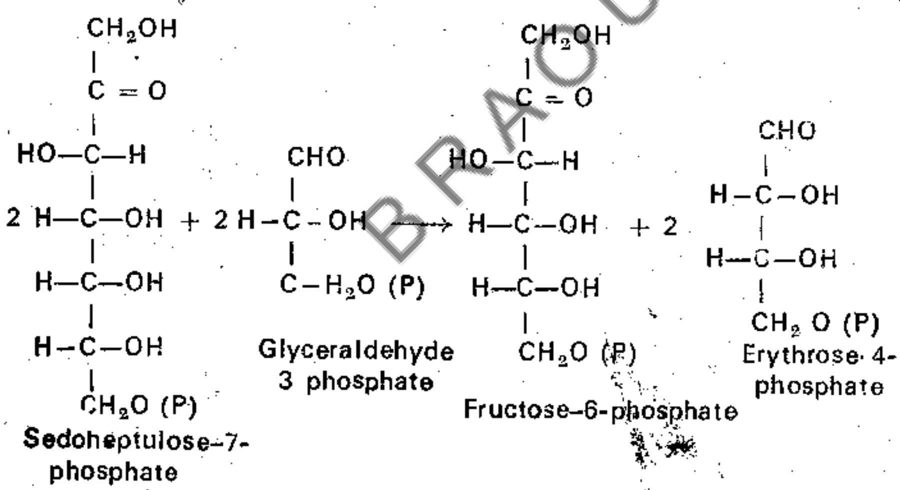


Ribulose-5-phosphate Xylulose-5-phosphate

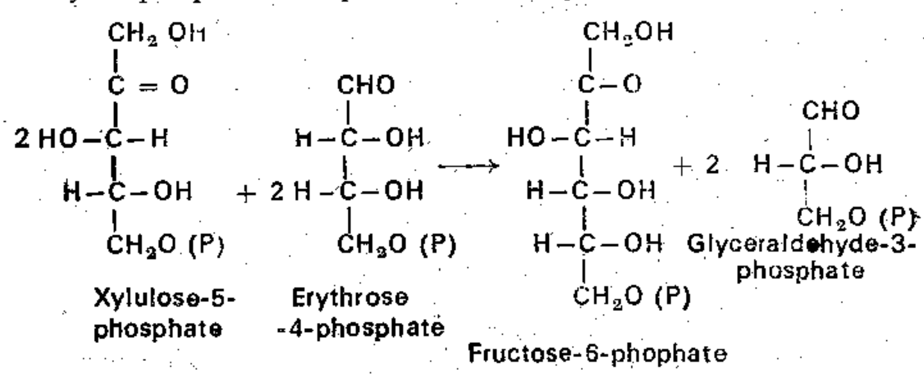
6. Now we have 2 molecules of ribose-5-phosphate and 4 molecules of xylulose-5-phosphate. In the next reaction 2 molecules of xylulose-5-phosphate and ribose-5-phosphate react to form 2 molecules of sedoheptulose-7-phosphate (a seven carbon compound) and 2 molecules of glyceraldehyde-3-phosphate (a three carbon compound) in the presence of the enzyme transketolase.



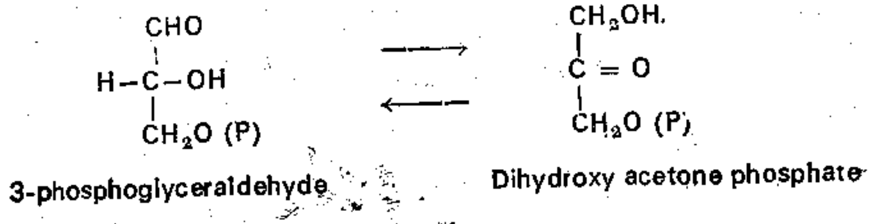
7. Next, the two molecules of sedoheptulose-7-phosphate and 2 molecules of glycerldehyde-3-phosphate react to form 2 molecules of fructose-6-phosphate & 2 molecules of erythrose-4-phosphate in the presence of the *enzyme transaldolase.*



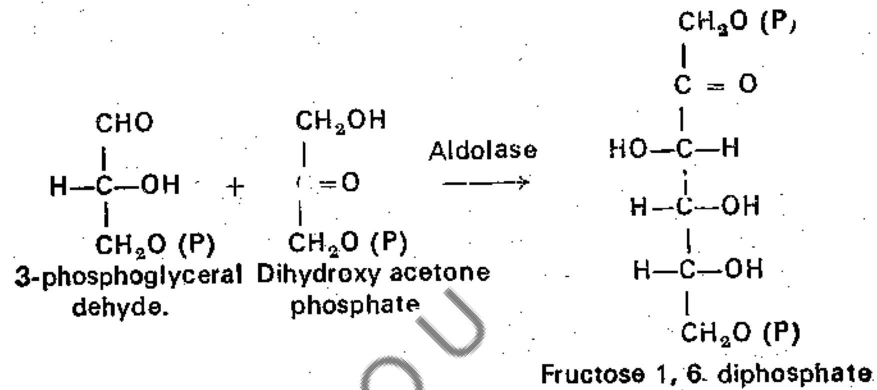
8. Two molecules of erythrose-4-phosphate then react with the remaining 2 molecules of xylulose-5-phosphate formed in step 5 and form 2 molecules of fructose-6-phosphate and 2 molecules of glycerldehyde-3-phosphate in the presence of the *enzyme transketolase.*



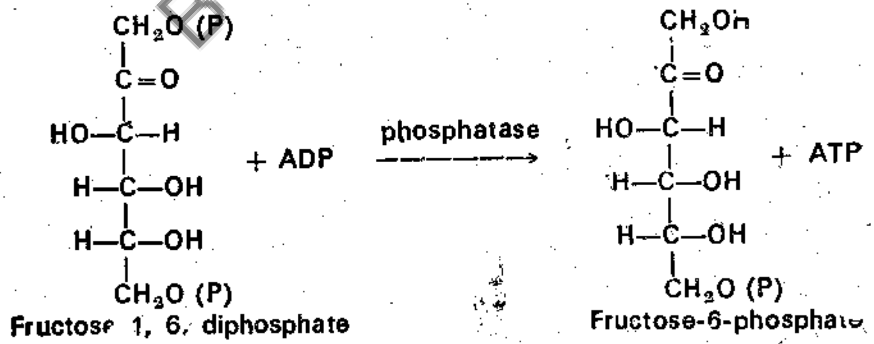
9. One of the two molecules of glyceraldehyde-3-phosphate formed above is converted to its isomer dihydroxyacetone phosphate in the presence of the enzyme *triose phosphate isomerase*.



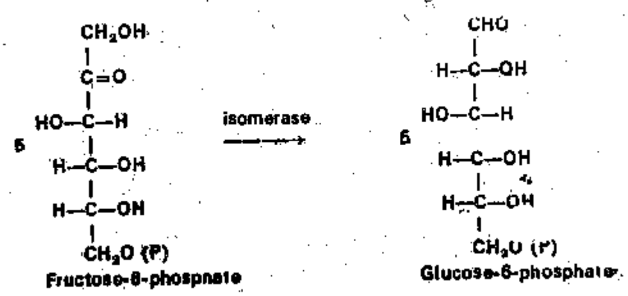
10. In the next reaction one molecule of 3-phosphoglyceraldehyde and one molecule of dihydroxy acetone phosphate react to form 1 molecule of fructose 1,6 diphosphate in the presence of the *enzyme aldolase*.



11. Next, fructose 1,6-diphosphate loses one phosphate in the presence of ADP and dephosphorylating enzymes (phosphatases) and forms fructose-6- phosphate.



12. In the last reaction all the 5 molecules of fructose-6-phosphate formed in reactions 7,8&11 are converted to glucose-6-phosphate in the presence of the *enzyme isomerase*.



The sequence of steps of hexose monophosphate pathway is given in Fig.11.1.

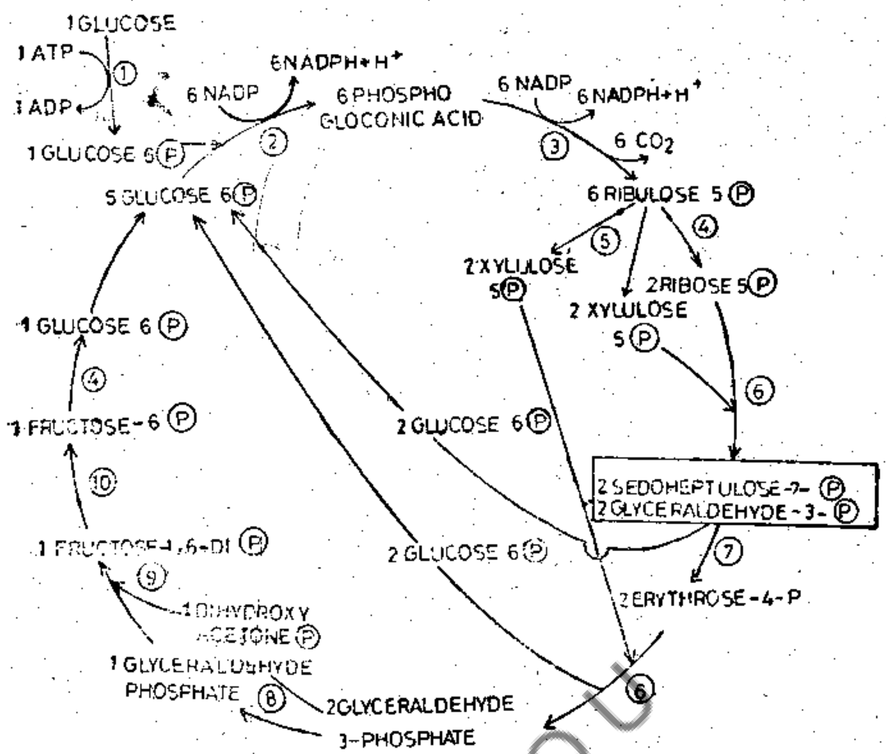


Fig.11.1. H.M.P.pathway. 1.Hexokinase 2.Glucose-6-phosphate dehydrogenase 3.Phosphogluconic acid dehydrogenase 4.Isomerase 5.Epimerase 6.Transketolase 7.Transaldolase 8.Triose phosphate isomerase 9.Aldolase 10.Phosphatase.

Thus, 6 molecules of glucose enters the pathway in the form of glucose-6- phosphate and five molecules are regenerated. That means one molecule of glucose is completely oxidised to carbon dioxide. But step 3 show that 6 molecules of carbondioxide are released from 6 molecules of phosphogluconic acid. This means from each molecule of phosphogluconic acid one molecule of carbondioxide is released. So, in this pathway glucose molecule is partially degraded. The overall reaction of this pathway can be written as:

$$6 \text{ Glucose-6-phosphate} + 12NADP + 6H_2O \longrightarrow 5\text{Glucose-6-phosphate} + 12NADPH + H^+ + 6CO_2 + 12H_2O$$

or

$$\text{Glucose-6-phosphate} + 12NADP \longrightarrow 12NADPH + H^+ + 6CO_2 + 6H_2O$$

The 12 molecules of $NADP+H_+$ thus formed are oxidised in the respiratory chain in the presence of flavoproteins and cytochromes as mentioned in the earlier unit. In the respiratory chain for each molecule of $NADP+H^+$ 3, molecules of ATP are formed. So a total of (12 x 3) 36 molecules of ATP are formed in HMP pathway.

$$12\ NADPH + H^+ + 6\ O_2 \xrightarrow[\text{Flavoproteins}]{\text{Cytochromes}} 12\ H_2O + 12\ NADP + 36\ ATP$$

Unlike the glycolytic pathway the pentose phosphate pathway degrades glucose without the involvement of pyruvic acid. The intermediates of pentose phosphate pathway serve as starting materials for the biosynthesis of a variety of cell constituents. For e.g., the ribose-5-phosphate formed as an intermediate is used in the biosynthesis of nucleic acids and Erythrose-4-phosphate is used in the biosynthesis of complex phenolic compounds. The reduced NADP is not usually oxidised by electron transport but useful in reductive biosynthetic reactions.

The important features of HMP pathway: (1) The reactions take place in cytoplasm. (2) Oxygen is required from the very beginning. (3) Without glycolytic pathway & Krebs cycle, the hexose is broken down to CO_2 & water, (4) NADP is the oxidising agent instead of NAD in glycolysis & Krebs cycle. The reduced NADP is used in the reductive biosynthetic reactions. (5) This pathway is the source of five carbon and 4 carbon compounds which are used in the synthesis of nucleic acids and complex phenolic compounds. (6) This pathway is as efficient as glycolysis-Krebs cycle pathway in the synthesis of ATP molecules.

Check Your Progress - 1, 2 & 3

1. HMP pathway is also called pentose phosphate pathway - Why?
2. Triose phosphate isomerase is the enzyme responsible for the conversion of ____________ to ____________.
3. Phosphogluconic acid is converted to Ribulose-5-phosphate in the presence of the enzyme ____________.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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11.4. FACTORS AFFECTING RESPIRATION

The rate of respiration in plant cells is governed by several factors. The factors may be external or internal. The internal factors are (1) Age and type of tissue, (2) Concentration of respiratory material (3) Water and (4) Respiratory enzymes. The external factors are (1) Temperature (2) Light, (3) Oxygen, (4) Carbondioxide, (5) Some chemical substances, (6) Injury, (7) Mechanical effects.

11.4.1. Internal Factors

1. **Age and Type of Tissue:** The rate of respiration depends more on the age and the nature of the tissue. Younger cells which have more and active protoplasm respire more rapidly than the older cells. In older cells the protoplasm is in lesser quantity due to the presence of large vacuoles and more wall materials. Tissues with high metabolic turnover respire more actively than the resting tissues. The physiological status of the tissues, the change of the substrate composition, the quality and quantity of enzymes, etc., effect the rate of respiration.

   In the overall growth of the seedlings, the rate of respiration is high during its initial period of growth and it gradually decreases with age. The respiratory rate is the lowest in the mature seedlings. In the developing leaves also, the respiratory rate is at its maximum in early stages of leaf growth and it falls down steadily. It comes to a steady state in mature leaves. During senescence, the break down of protein occurs, and some protein is released for respiration. This results in an increase in respiratory rate with the production of more carbon dioxide for a short period.

   Respiratory rate also varies with the type of tissue. Tissues with more dead material or non-metabolic components such as woody stems have low respiratory rate. Dormant organs such as buds, tubers and seeds respire very feebly. When growth is renewed with sufficient water supply, the respiratory rate increases immediately.

2. **Concentration of Respiratory Material:** The rate of respiration depend much on the concentration of respiratory material. If the conc. of respiratory substrate increases, the rate of respiration also increases. Respiratory rate in the leaves, immediately after photosynthesis, will be higher as there will be an increase in the conc., of respiratory material. The respiratory rate can also be increased by supplying the leaves with sugar (By immersing the cut ends of the stem, in sugar solution). When the leaves are kept in darkness, the rate of respiration falls off gradually. This is due to the reduction in the conc. of the respiratory substrate. Very high conc. of sugars are found to reduce the rate of respiration due to osmotic effects.

3. **Water:** The rate of respiration is not affected when water is present in sufficient quantities. But the shortage of water, reduces the rate of respiration. The percentage of bound water in the seeds is usually 16%. When the percentage of water is less than this, the respiratory rate is also very low. If the moisture content increases beyond 16% the respiratory rate also increases rapidly. The increase in respiration due to increase in moisture content is because of the following reasons: (a) Water maintains the turgidity of cells, (b) It is useful in the hydrolysis of water to soluble sugars, (c) It provides the medium for many respiratory reactions, (d) It accelerates the action of many respiratory enzymes and (e) It acts as a medium for oxygen to diffuse into the respiring protoplasm.

4. **Respiratory enzymes:** Several types of enzymes such as carboxylases, dehydrogenases, Oxidases, Phosphorylases etc. take active part in respiration. You must have already noticed that almost all the reactions in biological system occur only in the presence of enzymes. So,the entire rate of respiration is much dependent upon the presence or absence of respiratory enzymes.

11.4.2. External Factors

1. **Temperature:** The rate of respiration is much affected by temperature. The rate of respiration is very low at very low temperatures and it increases with rise in temperature. The increase in the rate of respiration due to increase in temperature follows Vant Hoff's rule. This rule states that for every $10^{o}C$ rise in temperature the rate of respiration gets doubled if any other factor is not limiting. In between $0^{o}C$ and $45^{o}C$, the rise in temperature causes an increase in the rate of respiration. As temperature rises above $45^{o}C$ the rate of respiration declines with time. This is due to the following reasons: (a) The inactivation of enzymes. (b) oxygen may not be supplied fast enough to maintain the rate of respiration (c) The removal of carbon dioxide may not be fast enough and (d) the respiratory substrate may become a limiting factor.

2. **Light:** The increase in the intensity of light increases the rate of respiration. You know very well that light is not necessary for respiration because respiration takes place during day time as well as night time. So light may have an indirect effect. In the presence of light photosynthesis occurs and in this process more respiratory material (carbohydrate) is synthesized. It is already mentioned that the increase in the respiratory substrate increases the rate of respiration. Light also increase the temperature which inturn increases the rate of respiration. Finally, light affects the opening of stomata. Because of this the exchange of gases is facilitated and this increases the rate of respiration.

3. **Oxygen:** For continuation of life many plants require oxygen. The O_2 in atmosphere is always constant (20%) and so it does not affect the rate of respiration. The presence or absence of oxygen determines the type of respiration, and also the respiratory products. In the presence of oxygen aerobic respiration takes place whereas in the absence of oxygen anaerobic respiration occurs. This is already mentioned in unit-9. The rate of respiration reaches its minimum when the oxygen conc. is reduced to 1%. Below this conc. fermentation occurs.

4. **Carbon dioxide:** Carbon dioxide concentration has an inverse relationship with the rate of respiration. That is, a high concentration of carbondioxide reduces the rate of respiration. Higher carbondioxide concs. are useful in the preservation of fruits. The atmospheric air contains 0.03% carbon dioxide and this is always constant. But in the soil the CO_2 conc.

may go up to 10% and O_2 content is reduced to minimum. Due to this, the absorption of salts, growth of the roots and germination of seeds may be drastically affected.

5. **Some chemical substances:** Many chemical substances such as cyanides, azides, carbon monoxide, chloroform, ether, formaldehyde, alkaloids, glycosides have been found to affect the rate of respiration. In minor quantities all these substances individually increase the rate of respiration. If the conc. is increased the rate of respiration is decreased drastically. These chemicals usually act as enzymatic inhibitors.

6. **Injury:** Injuries increase the rate of respiration. When a portion of the plant is injured, the conversion of starch to sugar is increased in that portion. This increase in the sugar content is responsible for the increase in the respiration in the injured portion.

7. **Mechanical effects:** Mechanical effects such as rubbing the leaves, bending the blade increase the rate of respiration.

Check Your Progress - 4 & 5

4. Mention the reasons for the decline in the rate of respiration when the temperature is raised to above 45°C.

5. Name the internal factors that affect respiration.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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11.5. SUMMARY

HMP pathway is also called pentose phosphate pathway, direct oxidation pathway, Warburg-Dickens pathway. Hexose monophosphate pathway starts with the phosphorylation of 6 molecules of glucose to glucose-6-phosphate. Six molecules of glucose-6-phosphate are converted to 6 molecules of phosphogluconic acid. Phosphogluconic acid undergoes several changes with 3,4,5,6&7 carbon compounds as intermediates and produce 5 molecules of glucose-6-phosphate. During these reactions 12 molecules of $NADPH+H^+$ and 6 molecules of carbon dioxide are produced. HMP pathway is as efficient as glycolysis-Krebs cycle pathway in producing the ATP molecules. The intermediates of HMP pathway serve as starting materials for the biosynthesis of a variety of compounds. The reduced NADP is also useful in the reductive biosynthetic reactions.

The internal factors affecting respiration are (1) Age and type of tissue, (2) Concentration of respiratory material, (3) Water and (4) Respiratory enzymes. The external factors are: (1) Temperature, (2) Light, (3) Oxygen, (4) Carbondioxide, (5) Chemical substances, (6) Injury and (7) Mechanical effects. Younger cells which have more and active protoplasm respire more rapidly than the older cells. Tissues with more dead material or non-metabolic components such as woody stems have low respiratory rate. When the conc. of respiratory substrate increases the rate of respiration also increases. When the leaves are kept in darkness the rate of respiration falls off gradually due to the reduction in the respiratory substrate. The shortage of water, reduces the rate of respiration. If the moisture content increases beyond 16% the rate of respiration also increase rapidly. The respiratory rate is also dependent on the presence or absence of enzymes. The rate of respiration is very low at low temperatures and it increases with the rise in temperature. For every 10°C rise in temperature the rate of respiration gets doubled, Temperatures above 45°C reduce the rate of respiration. Light has an indirect effect on the rate of respiration. Light increases the respiratory substrate, and also the temperature. These factors inturn increase the rate of respiration. The presence or absence of oxygen determines the rate of respiration. The rate of respiration reaches its minimum when the oxygen conc. is 1% below which fermentation occurs. Higher concentrations of carbon dioxide reduces the rate of respiration. Some chemical substances such as cyanides, azides, chloroform, carbon monoxide etc.decrease the rate of respiration. Injuries and mechanical effects such as rubbing the leaves, bending the blade increase the rate of respiration.

11.6. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The pathway is also called as pentose phosphate pathway because the main intermediates of this pathway are pentose phosphates.
2. 3-phosphoglyceraldehyde; Dihydroxy acetone phosphate,
3. Phosphogluconic acid dehydrogenase.
4. (a) The inactivation of enzymes (b) oxygen may not be supplied fast enough (c) The removal of CO_2 may not be fast enough (d) Respiratory substrate may be a limiting factor.
5. (a) Age and type of tissue (b) one of respiratory material (c) Water (d) Respiratory enzymes.

11.7. MODEL EXAMINATION QUESTIONS.

I. Answer the following in 30 lines each.

1. Write an account of HMP pathway.
2. Write an account of the factors that affect respiration.

II. Answer the following in 10 lines each.

1. Why is HMP pathway called Warburg-Dickens pathway? What are the important features of hexose monophosphate shunt?
2. How does the age and type of tissue of plant affect the respiratory rate?
3. What is the effect of temperature and light on the rate of respiration?
4. How does carbon dioxide and oxygen affect the rate of respiration?
5. Injuries and chemical substances also play a role in the rate of respiration. How?

UNIT-12: PHOTORESPIRATION

Contents

12.1. OBJECTIVES

By the end of this unit you will be able to:

1. define photorespiration in plants,
2. list out the different cell organelles in which the photorespiration takes place,
3. recognise the different reactions of the cycle that occur in chloroplasts, peroxisomes and mitochondria,
4. describe the method of control of photorespiration in plants.

12.2. INTRODUCTION

Respiration takes place in all the living cells of the plant, all the time i.e., during day in the presence of light and in the night in the absence of light. During the process of respiration, O_2 is taken up and CO_2 is liberated. This is in contrast to photosynthesis taking place only in the presence of light in chlorophyll-bearing cells. During the process of photosynthesis CO_2 is assimilated and O_2 is liberated.

About two decades ago, it was found that in many plants (including algae, bryophytes, pteridophytes, gymnosperms and many angiosperms), the amount of CO_2 liberated during day time (i.e. in the presence of light) is more than CO_2 liberated by the same plants in the absence of light (i.e. in darkness or in night). This extra CO_2 is liberated due to increased respiration in the presence of light.

The light induced respiration over and above the normal dark respiration taking place at all times, is called photorespiration. This sort of definition of photorespiration is too simple, because the metabolic processes that lead to the formation of CO_2 in respiration and photorespiration are different. Respiration normally implies metabolism that takes place in the mitochondria and leads to the formation of ATP. Photorespiration, although it may, usually does not result in storage of energy. Further, the oxidation part of photorespiration takes place mainly in organelles called peroxisomes with the exchange of metabolites from chloroplast and mitochondria. In other words, photorespiration is a metabolic process associated with 3 organelles-chloroplasts., peroxisomes and mitochondria.

Thus the process of photorespiration bears a superficial similarity to respiration in that in both the processes, CO_2 is liberat ed. In case of respiration, CO_2 is released due to the oxidation of intermediates of Krebs cycle ; the substrate for photorespiration is compound consisting of 2 carbon atoms, called glycolic acid. This is a product of photosynthesis, synthesised in chloro plast in the presence of light. Another main difference between normal (dark) respiration and photorespiration is that the former takes place in all cells in which mitochondria are present but photorespiration takes place only in cells having chloroplasts, because of its dependence on photosynthetic product as a substrate. Further the normal (dark) respiration is quite sensitive to the level of O_2. It gets saturated at as low a level of oxygen as 2%. Higher concentrations of oxygen inhibit dark respiration. On the contrary, photorespiration goes on increasing as the con centrations of oxygen go up even upto 100%. Therefore, dark respiration and photorespiration are two different processes.

As the substrate for photorespiration is a C_2 compound which is produced during photosynthesis, photorespiration is more dependent on photosynthesis. However, it should be clearly understood that photorespiration is not a kind of photosynthesis. Photosynthesis is reductive synthetic process whereas, photorespiration is an oxidative degradative process. The carbon that is assimilated in photosynthesis should normally go into synthesis of sugars, and resulting in storage of energy. However, about 1/5 to 1/4 of carbon assimilated during photosynthesis, is respirated away (wasted without production of ATP) during photorespiration; according to some scientists, even this figure is an under-estimate.

The connection between photosynthesis and photorespiration therefore is glycolic acid. If the plants do not produce glycolic acid then, there cannot be any photorespiration. As the photorespiration takes place in single membraned organelles called peroxisomes, there is little photorespiration in plants lacking peroxisomes e.g., C_4 plants. In majority of plants, photosynthesis occurs by Calvin cycle, and such plants are called C_3 plants.

There are other plants like maize,, sorghum, euphorbias, *amaranthus*, in which in addition to photosynthetic carbon reduction cycle (Calvin's pathway), carbon is assimilated by C_4 pathway also and these are called C_4 plants.

It has been found that the rate of photorespiration is quite high in case of C_3 plants and these possess peroxisomes. In case of C_4 plants, there is negligible photorespiration and the number of peroxisomes in the cells are very few or none at all. It is also known that as the oxygen concentration increases, the photosynthetic assimilation in C_3 plants gets reduced, because of increased photorespiration. On the contrary, increased carbon dioxide concentration or lowering of O_2 level will increase the amount of photosynthesis in case of C_3 plants. These differences in C_3 photosynthesis and photorespiration under contrasting conditions, is due to the sensitivity of the main carboxylating enzyme of C_3 plants to oxygen. RuBP carboxylase acts as a carboxylase and also acts as an oxygenase depending on the concentrations of oxygen present. This enzyme is therefore, now called as RuBISCO (Ribulose bisophosphate carboxylase oxygenase). If oxygen level is low, and carbon dioxide level is high, the enzyme acts as a carboxylase; the oxygenase activity is suppressed and there is less formation of glycolic acid and less of photorespiration. Under these conditions, most of the carbon assimilated will be in the form of sugars. If the oxygen level is more and carbon dioxide concentration is less, the enzyme also acts as an oxygenase and therefore, carbon which should normally go into sugars is converted into glycolic acid, a C_2 compound. As photorespiration begins with a C_2 compound the cycle of several reactions is called C_2 cycle.

Check Your Progress - 1

What is meant by photorespiration?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end of this unit.

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12.3. REACTIONS OF PHOTORESPIRATION

In the chloroplast of C_3 plants, Calvin cycle takes place. During this process ribulose 1,5-bisphosphate is formed.

In the presence of higher amounts of oxygen and limitation of the Co_2 enzyme RuBISCO acts as RuBP oxygenase. RuBP accepts O_2 and gives rise to phosphoglycolate (C_2 compound) and phosphoglyceric acid (a C_3 compound). Thus RuBP which should normally accept CO_2 and give rise to an unstable C_6 compound and subsequently yield 2 molecules of PGA, is diverting its carbon to form glycolic acid. It does not mean all the RuBP is utilised for the formation of glycolic acid but only a part of it (upto 30% in certain conditions) is used for the formation of glycolic acid.

The enzyme RuBP carboxylase also accepts carbon dioxide, and synthesize, Calvin cycle intermediates. As mentioned earlier, in the presence of higher amounts of oxygen, it is also competing for the active sites on the enzyme along with CO_2. Therefore the amount of CO_2 that is normally fixed by RuBP-carboxylase becomes less, consequently photosynthesis is inhibited in the presence of higher amounts of O_2. This oxygen inhibition of photosynthesis is called Warburg effect. Increase in temperature also increases the rate of photorespiration just as the increase in oxygen level. The interrelations of C_2 cycle, C_3 cycle and RuBP carboxylase are shown in Fig.12.1.

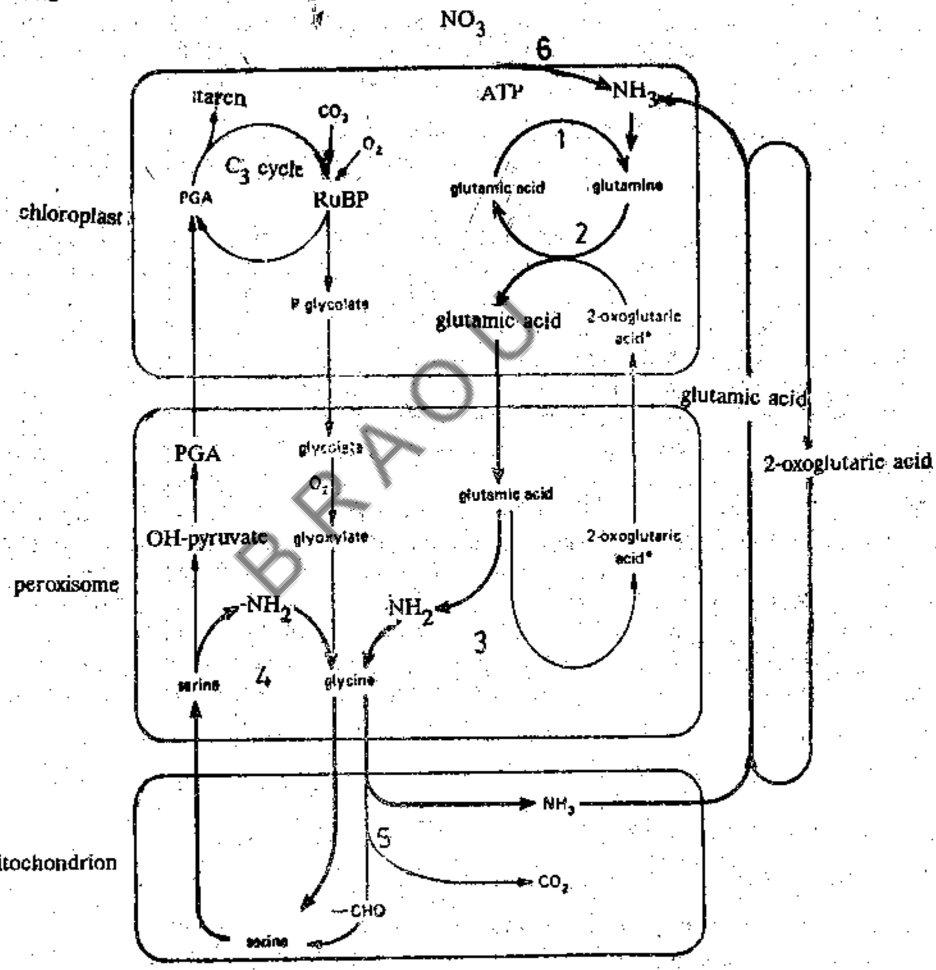

Fig.12.1. The nitrogen cycles associated with C_2 photorespiratory cycle. 1.Glutamine synthetase 2.Glutamic acid synthetase 3.Glutamic acid-glycine transminase 4.Serine-glycine transaminase 5.Glycine decarboxylase 6.Nitrate/nitrite reductase.

Besides photosynthesis, chloroplast is the site for synthesis of aminoacids. NO_3 is reduced to NO_2 and then to NH_3 in the chloroplast with the help of ATP produced during photophosphorylation. Ammonia combines with glutamic acid giving rise to glutamine (an amide) which on deamidation gives rise to glutamic acid. Amino group released from glutamine, combines with 2-oxoglutaric acid and gives rise to glutamic acid. This glutamic acid produced in chloroplast which enters peroxisomes, also takes part in photorespiration as shown later.

The glycolate cycle or the chemical steps related in photorespiration begin in chloroplast. The glycolate formed in the chloroplat enters peroxisomes which usually lie adjacent to chloroplasts in C_3 plants. The glycolate is oxidised to glyoxylate in the peroxisomes. The glyoxylate accepts amino groups (NH_2) derived from glutamic acid (transamination) and gets converted to glycine, an amino acid.

Then glycine enters mitochondria. Out of each set of two molecules of glycine gets converted to NH_3 and CO_2 (decarboxylation) and-CHO. This aldehyde combines with the other molecule of glycine and give rise to a serine (another amino acid) molecule. The serine enters back peroxisomes and gets deaminated and the amino group (-NH_2) combines with glyoxylate to form glycine. In other words the aminoacid glycine is formed in peroxisomes due to glyoxylate getting aminated by the amino group donated by glutamic acid (an amino acid synthesized in the chloroplast) and also from serine (synthesized in mitochondria). The serine after deamination, gives rise to phosphoglyceric acid (PGA) which re-enters the chloroplast and takes part in Calvin cycle.

The various reactions connected with photorespiration, therefore, take place in 3 organelles in a co-ordinated manner with exchange of metabolites between them. The series of reactions can be summarised as follows:

1. In chloroplast, glycolate is formed from RuBP; glutamic acid is also formed.

2. In peroxisomes (a) glycolate is oxidised to glyoxylate and (b) glyoxylate is converted to glycine by the NH_2 group donated by glutamic acid (or serine).

3. In mitochondria (a) glycine is converted to serine (b) a part of glycine is broken down to NH_3 and CO_2 (c) Serine enters peroxisomes, serine is deaminated and as PGA enters chloroplast and becomes a part of Calvin cycle.

It should be clearly understood that the cycle is connected to nitrogen cycle in these organelles. The various reactions taking place in the three organelles are shown in Fig.12.2.

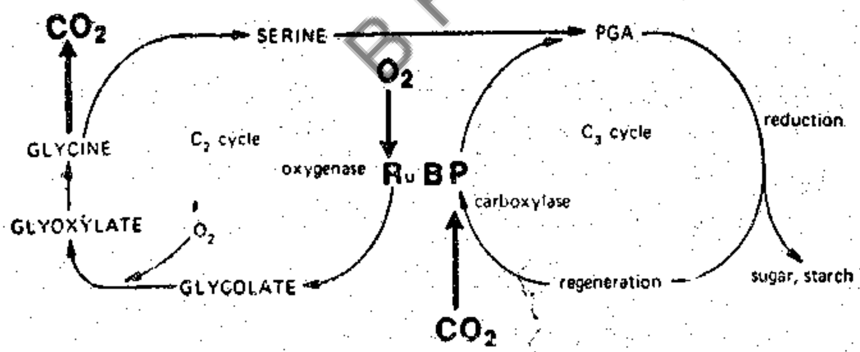

Fig.12.2. Integration of C_3 and C_2 cycles. The C_2 cycle is so called because the product of RuBP oxygenase is a C_2 compound.

Check Your Progress - 2&3

2. What is meant by Warburg effect?

3. What are the 3 cell organelles that take part in photorespiration?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end of this unit.

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12.4. CONTROL OF PHOTORESPIRATION

Because of the 'Wasteful' nature of the reaction (oxidation of photosynthetically fixed up carbon during photorespiration without producing ATP), a number of research laboratories have carried out physiological experiments to inhibit photorespiration. One of the attempts was related to inhibit the activity of the enzyme glycolic acid oxidase by a chemical inhibitor, hydroxy-2 pyridine methane sulfonic acid (HPMS). Use of this chemical reduced photorespiration to a large extent.

Another chemical that has been tried was, glycidate an analogue of glycolic acid, which inhibits the formation of the initial substrate of photorespiration i.e., glycolic acid. Here again, the suppression of glycolic acid formation has largely reduced photorespiration. But these chemicals are very costly and they tend to inhibit photosynthesis as well, in certain cases.

Another approach to reduce photorespiration was through breeding programmes with varieties having consistently low photorespiration and high photosynthesis. But these attempts were disappointing in the sense that they resulted in plants with low productivity (fruit/seed). But a number of research programmes are continued for the selection of plants with high photosynthesis and low photorespiration by resorting to modern tissue culture methods like somatic cell hybridisation. The main problem of photorespiration is the sensitivity of the most abundant protein and the main carboxylating enzyme ribulose-bis-phosphate carboxylase, to oxygen.

Attempts are also being made to alter the basic character of the enzyme (i.e., to reduce its sensitivity to oxygen) by methods of molecular genetics and genetic engineering. But so far, these experiments are of academic interest and have not yielded successful results.

Photorespiration is wasteful and inescapable in C_3 plants in view of RuBP carboxylase acting as oxygenase, in the presence of oxygen. But some physiologists argue that photorespiration would have been lost during the course of evolution of plants spanning over millions of years, had it been so entirely a useless or detrimental process.

In fact, some beneficial effects of photorespiration are suggested; one of them is that photorespiration is helpful in maintaining a steady level of CO_2 to keep RuBP carboxylase in active form also to maintain the turnover of CO_3 cycle intermediates. Some even suggest that in case of high light intensity, when stomata are closed with meagre CO_2 supply, this 'wasteful' process might serve to dissipate 'unwanted' energy. But these explanations remain mostly teleological.

12.5. SUMMARY

Light induced liberation of extra carbon dioxide due to peroxisomal respiration as against mitochondrial dark respiration, is called photorespiration. Photorespiration occurs in all groups of plants including many angiosperms called C_3 plants. Photorespiration, is however negligible in C_4 plants having Hatch-slack pathway. Dark respiration takes place in all the living cells all the time and its site of oxidation is mitochondria. This oxidation involves synthesis of ATP (Oxidative phosphorylation). On the contrary, oxidation step of photorespiration takes place in single-membraned organelles called peroxisomes and does not involve synthesis of ATP. Photorespiration is a wasteful process and diverts away 1/5 to 1/4 photosynthetically fixed carbon without forming energy rich sugars. The starting point of photorespiration is glycolic acid, a C_2 compound, which is produced in the chloroplast. Hence, the series of reactions during photorespiration is called C_2 cycle. Ribulose bisphosphate carboxylase, the main carboxylating enzyme in C_3 photosynthesis acts as an oxygenase in the presence of high levels of O_2 or low levels of CO_2. In other words, oxygen competes for active sites on the enzyme with CO_2 and the production of photosynthetic products of Calvin cycle decrease. This oxygen inhibition of photosynthesis is called Warburg effect. Besides oxygen, increase of temperature also increases photorespiration. The series of reactions in C_2 cycle take place in 3 organelles (a) Chloroplast (b) Peroxisomes and (c) Mitochondria. In chloroplast, the C_2 cycle and C_3 cycle get integrated because of the same enzyme catalyzing the reactions of those two cycles, under different and contrasting conditions. Chloroplast is also the seat of nitrogen cycle. The glutamic acid (an amino acid) so synthesized, also takes part in the reactions of photorespiration after entering peroxisomes. Glycolic acid is oxidised to glyoxylate in peroxisomes; glyoxylate accepts amino group from glutamic acid and gives rise to glycine, another amino acid. Glycine enters mitochondria and gives rise to serine another amino acid. Serine enters peroxisomes back and gets deaminated; the carbon skeleton gives rise to PGA which re-enters chloroplast and forms a part of Calvin cycle. Photorespiration can be experimentally controlled by the use of chemical inhibitors like hydroxy- pyridine methane sulfonic acid (HPMS) and glycidate. But these chemicals though cut down photorespiration drastically, inhibit photosynthesis sometimes. Breeding experiments with plants with low photorespiration and high photosynthetic rate have not yielded any significant beneficial results. Attempts with somatic cell hybridisation and genetic engineering technology to reduce photorespiration are underway.

12.6. CHECK YOUR PROGRESS: MODEL ANSWERS

1. The light induced respiration over and above the normal respiration that takes place continuously in plants is called photorespiration.
2. The inhibition of photosynthesis in plants by higher amounts of oxygen is called Warburg effect.
3. The three cell organelles that take part in photorespiration are chloroplasts, peroxisomes and mitochondria.

12.7. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Explain the formation of glycolic acid and its conversion to glyoxylic acid.
2. Photorespiration is considered to be a wasteful process - Why?
3. Explain the role of peroxisomes and mitochondria in photorespiration

II. Answer the following questions in 10 lines each.

1. How does glycolate form? What is its significance?
2. What is the role of peroxisomes in photorespiration?
3. Explain the nitrogen metabolism concerning to photorespiration.
4. Explain the link between Calvin cycle and photorespiration.
5. What are the conditions under which photorespiration is stimulated?

UNIT - 13: NITROGEN FIXATION

Contents

13.1. OBJECTIVES

By the end of this unit you will be able to:

1. describe the fixation of physical nitrogen,
2. list out the different biological organisms that are able to fix atmospheric nitrogen,
3. define symbiosis and be able to recognise the organisms, involved in this process,
4. describe the process of nitrogen fixation in leguminous plants,
5. describe the biochemical aspects of nitrogen fixation, and
6. recognise the different organisms involved and their role in nitrogen cycle.

13.2. INTRODUCTION

It was Boussingault in 1883 who established the fact that leguminous plants fixed nitrogen from the air. Later, Winogradsky in 1893 established that certain free living anaerobic bacteria, *Clostridium*, fixed nitrogen. The capacity of the nitrogen fixation by aerobic bacteria, *Azotobacter chroococcum* was demonstrated by Beijerinck. These observations led to later studies of the physiology of nitrogen fixing bacteria and the mechanism of nitrogen fixation in view of its practical application in agriculture.

Nitrogen is one of the nutrients required by plants in large amounts, and is obtained mostly from the soil. Nitrogen in the form of nitrate (NO_3) and ammonium (NH_4) is available in the soil water through the nitrate and ammonium salts. This limited amount of nitrogen is removed from the soil by intense agriculture, leaching and denitrification. The atmosphere contains 79% of molecular nitrogen gas. The higher plants are not able to utilise this and they require nitrogen in the fixed form as nitrates or ammonia ions. A number of microorganisms convert elementary nitrogen into combined forms of inorganic nitrogenous compounds. This process is known as nitrogen fixation.

Nitrogen is fixed by chemical (industrial), physical (atmospheric) and biological (microbial) means. A wide variety of fixed nitrogen chemicals are manufactured. According to an estimate of 1980, about 50 million tons of nitrogen is fixed chemically by industrial methods. The biological nitrogen fixation is very significant in soil fertility. Annually, 170 million tons of nitrogen are fixed by microorganisms in the world. About 40 million tons of nitrogen are fixed in cropping systems and 36 million tons of nitrogen are fixed in oceans.

13.3. PHYSICAL NITROGEN FIXATION

Of the 170 million tons of nitrogen fixed annually by a natural process, about 10% of this is due to physical processes such as lightning strokes and thunder storms. Lightning dissociates the water vapour into hydrogen and oxygen atoms and hydroxyl (-OH) radicals. The free atom and radical, which are extremely reactive, combine with the nitrogen present in the atmosphere and produce a fixed form of nitrogen. Gaseous nitrogen and oxygen combine to form nitric oxide. The nitric oxide (NO) is further oxidized to nitrogen peroxide (NO_2). Nitrogen peroxide combines with water during the rains and produces nitrous acid and nitric acid. Traces of nitric acid are also produced in the stratosphere by photochemical reactions between ozone and nitrogen oxides. The acids are brought down to the earth along with the rain, where it reacts with alkaline radicals and produces nitrates (NO_3^-) and nitrites (NO_2^-).

13.4. BIOLOGICAL NITROGEN FIXATION

The transformation of the free inert nitrogen gas of the atmosphere into the fixed form by soil microorganisms is known as biological nitrogen fixation. Soil microorganisms fix 80% of the and help in maintaining the nitrogen balance of the earth.

Nitrogen fixation in the soil is carried by both free living (asymbiotic) and symbiotic microorganisms. They are called diazotrophs and are widespread in their occurrence. The list of the biological agents that fix nitrogen is given in Table 13.1 and the nitrogen fixing organisms in Figure 13.1.

Table 13.1. Major Nitrogen-Fixing Biological Systems

I. Free living (asymbiotic) microorganisms	
Bacteria	
Aerobic	Several species of the genus *Azotobacter*
Anaerobic, non-photosynthetic	Several species of the genus *Clostridium;* also a few other genera.
Anaerobic, photo-synthetic	Several species of the genus *Rhodospirillum;* also a few other genera.
Blue-green algae	Several species of the genera *Nostoc* and *Anabaena.*
II. Symbiotic systems	
Host	Microorganisms
Vascular plants	
Angiosperms	
Certain laguminous plants	Several species of bacteria of the genus *Rhizobium*
Certain nonleguminous plants, e.g., *Casuarina* *Alnus* and *Myrica*	Several unidentified species of actinomycetes

Gymnosperms	
Certain cycads	Certain unidentified species of blue-green alage
Ferns	
Azolla, a genus of small water ferns	*Anabaena* sp., a blue-green algae.
Nonvascular plants	
Lichens Certain fungi	Nostoc sp., a blue-green alga

The soil harbours many groups of free living nitrogen fixing microorganisms. They include blue green algae and a number of aerobic and anaerobic bacteria.

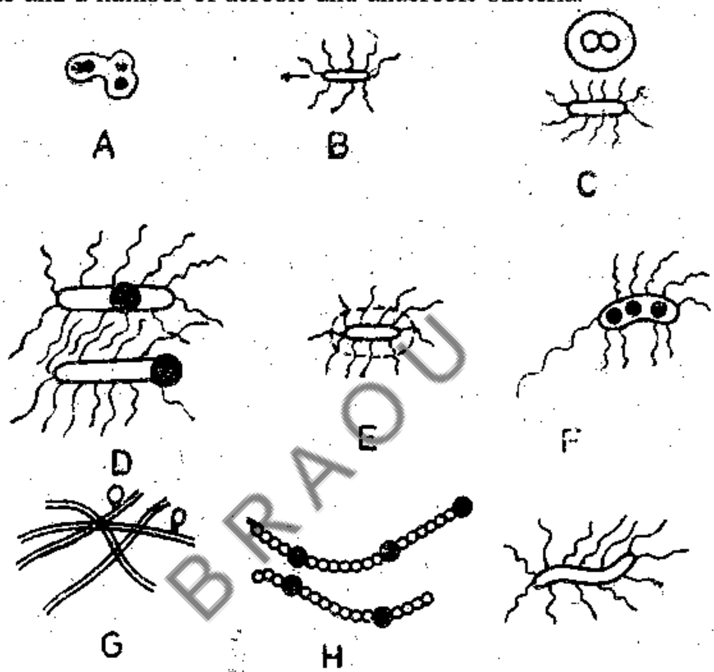

Fig.13.1. Nitrogen fixing organisms. A. *Azotobacter* B.*Rhizobium* C.*Bacteroids* D.*Bacillus polymyxa* and *Clostridium pasteurianum*. E. *Klebsiella pneumoniae*. F.*Azospirillum brasiliense*. G.*Actinomycetes frankia* H.*Anabaena* I.*Rhodospirillum rubrum*.

13.4.1. Blue-green Algae

About 40 species of blue green algae (Cynanophyceae) belonging to the families, Nostocaceae, Scytonemataceae, Rivulariaceae and Stigonemataceae are known to fix nitrogen. The blue green algae are the predominant agents, which maintain the nitrogen supply especially in the price fields in the tropics. It was thought earlier that only heterocyst bearing blue green could fix up nitrogen. Now it is established that non-heterocystous members like *Phormidium* and unicellular *Gleocapsa, Gloeotheca,* also fix up nitrogen to some extent. Some of the common genera of free living blue green filamentous heterocyst bearing ones are *Anabaena, Anabaenopsis, Nostoc, Tolypothrix, Calothrix and Scytonema*. Some like *Anabaena* and *Nostoc* form symbiotic association with some Fungi, Bryophytes, Pteridophytes, Gymnosperms or Angiosperms and fix nitrogen. The *nostoc* in the cavities of Bryophytes like *Anthoceros and Blansia* is reported to fix nitrogen. The symbiotic association of *Anabaena* with the floating fern *Azolla* fixed 312 kg nitrogen per hectare. One fifth of the Lichens which form a symbiotic association with only blue green algae fix up a considerable amount of nitrogen.

Almost all the nitrogen fixing filamentous blue green algae have heterocysts. It is differentiated cell with a thick wall, pale in colour and slightly larger than the other cells in the algal filament. Heterocyst is the site for fixing nitrogen as it offers protection for the nitrogen reducing enzyme from oxygen as the pigments performing activities of photosystem I and photosystem II which release oxygen are absent in it.

13.4.2. Asymbiotic Bacteria

Many general of free living bacteria are capable of fixing molecular nitrogen. Some of them are obligate aerobes like *Azoto bacter, Beijerinckia, Derxia, Actinobacter* and *Bacillus;* others are facultative aerobes like *Aerobacter, Klebsiella* and *Pseudomonas. Clostridium* is an anaerobic nitrogen fixing bacteria.

The aerobic vigorously respiring nitrogen fixing bacteria are wide spread in the soil. Fixation of nitrogen is a deductive process. *Azotobacter* may add to the soil 10-15 Kg. of nitrogen per hactare per year though the facultative group of bacteria growing either aerobically or anaerobically can also fix up nitrogen. *Clostridium,* an anaerobic nonphotosynthetic bacteria is known to fix nitrogen because it produces a relatively stable complex of soluble enzymes capable of fixing nitrogen.

Many photosynthetic bacteria (photoautotrophs) are capable of fixing nitrogen in the presence of sun light. The purple sulphur bacteria *Chromatium* and green sulphur bacteria *Chlorobium* are able to fix up nitrogen.

13.4.3. Symbiotic Nitrogen Fixing Agents

Symbiotic nitrogen fixing organisms contribute more nitrogen than asymbiotic ones, inspite of widespread occurrence of the latter. Nitrogen fixation is 100-200 times more in root nodules by symbiotic bacteria than by free living microorganisms. Bossingault's experience in 1883 revealed the ability of legumes to fix nitrogen. The importance of root nodules of leguminous plants and the association of symbiotic bacteria in nitrogen fixation was observed by Hellriegel and Willfarth in 1888. Beijerinck experimentally proved in 1888 that infecting *Rhizobium* cause root nodules in legumes. Although nitrogen fixation has been known over a century, the physiology and molecular biology of the process have been studied only during the last four decades.

The symbiotic bacteria *Rhizobium* is present in the root nodules of leguminous plants. A leguminous crop often fix annually 100 kg. nitrogen per hectare. According to Delwiche (1970) the legume crop fix annually $14X10^6$ metric tons of nitrogen on the earth.

Of the 13000 species of Leguminosae examined for nitrogen fixation, 90% of them fix a significant amount. A particular species of *Rhizobbium* is effective on any one species of legume and often one variety of the species. It is now known that a special type of proteins called betins which bind free glycoside radicals are involved in the selection mechanism. For convenience they are usually classified in bacterial plant groups or in cross inoculation groups. A number of economically important legume groups are presented in Table 13.2.

Table 13.2 Bacterial Plant groups.

Bacteria	Representative plants nodulated.
Rhizobium melioloti	Sweet clover, Alfalfa.
R.trifolii.	Red Clover, White Clover.
R.leguminosarum	Pea, Sweet Pea, Broad Bean.
R.phaseoli.	Bean.
R.japonicum.	Soyabean.
R.lupini.	Lupin.

Formation and Morphology of Nodules: The symbiotic bacteria present in soil as sapro phyte is stimulated by the roots of the legume plant, secreting organic substances into the soil. The rhizobia cluster around the root hair before the establishment of the infection. The root hair curl at their tips in response to the production of indole acetic acid (IAA) by the bacteria. The bacteria then enter the root hair and the plant produces an infection thread (Fig.13.2).

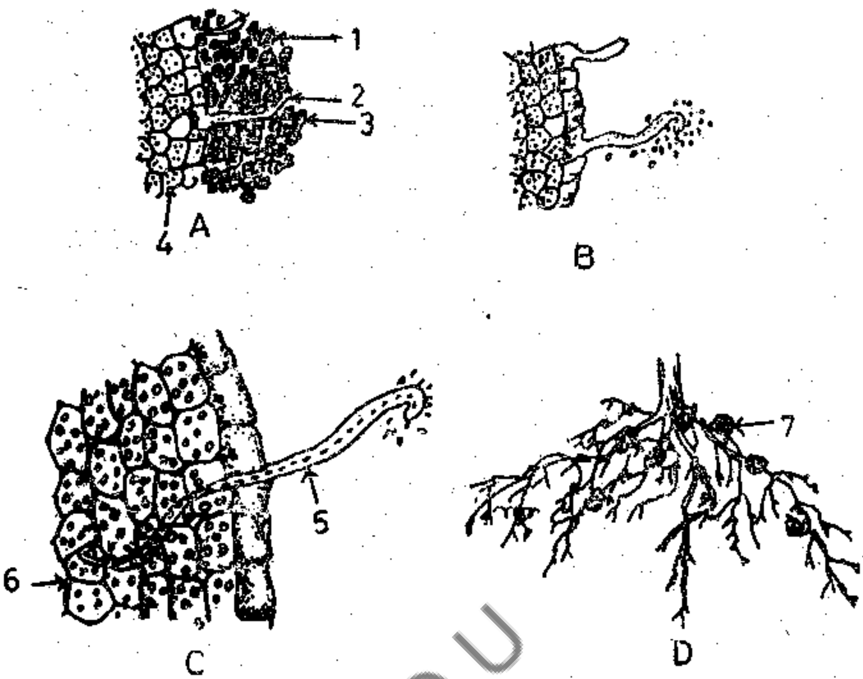

Fig.13.2. Stages in the formation of root nodules. A&B Substances are presumably released from root hairs and they stimulate *Rhizobia* to divide. C.Bacteria enters the root hair and move intro-& inter-cellularly. Bacteria stimulate tetraploid cortical cells to divide rapidly. E.Mature nodules on the roots.

The infection thread is a part of the greatly folded plasmalemma of the cell being invaded. The infection thread containing the proliferated bacteria enter the cortex and extend even upto pericycle. The association of plant and bacteria is very delicate. Many infection threads abort before a nodule is formed. Simultaneously the cells of the cortex are stimulated to divide as a consequence of the growth promoting substances produced by the host cell. Actively dividing bacterial cells with dissimilar shapes are called bacteroids. A group of both infected and uninfected cells develop into a young root nodule. The nodule is a highly organised structure. The bacteroid zone forms a central core of a mature nodule surrounded by many layers of cortical cells. The vascular tissues of the root become continuous with the newly formed cortical cells of the nodule. The bacteroid has no flagella, usually swollen branches and vacuolated. They are present in the cytoplasm in groups. Each group is surrounded by a membrane sheath. The cells of the root nodule is polyploid due to the production of the polyopid-inducing compound cytokinin by the bacteria.

A reddish pigment develops in the nodules harbouring the bacteroids. Kubo (1939) recognised that the red pigment in the nodules is haemoglobin, very much similar to the haemoglobin of red blood cells of mammals. It is named leghemoglobin which means the hemoglobin of legumes. There is no nitrogen fixation unless leghaemoglobin is formed in the nodule. The more the haemoglobin in the nodule, the greater the nitrogen fixation. The presence of leghaemoglobin in the root nodule is unique as it is not found in other higher plants. It has a high affinity for oxygen and combines rapidly with it and acts as an efficient oxygen scavenger. The enzyme (nitrogenase) in the bacteroids responsible for the nitrogen fixation is highly sensitive to oxygen and combines rapidly with it and acts as an efficient oxygen scavenger. The enzyme (nitrogenase) in the bacteroids responsible for the nitrogen fixation is highly sensitive to oxygen and its activity gets inhibited by the presence of oxygen. But the actively growing bacteria require oxygen for their respiration during which ATP is produced. Again ATP is essential for nitrogen fixation. The leghaemoglobin satisfies these contrasting conditions by regulating the concentration of the dissolved oxygen to the nitrogen fixing bacteroids just enough to enable ATP formation through oxidative phosphorylation, but insufficient to inactivate the enzyme nitrogenase. Thus leghaemoglobin ensures an adequate and stable supply of oxygen for the maintenance of nitrogen fixation.

As nitrogen fixation is an energy dependant process requiring ATP, photosynthesis or availability of carbohydrates (in case of respiratory ATP) restrict the nitrogen fixation under field conditions. In other words photosynthesis and nitrogen fixation compete in a way for ATP. Nitrogen fixation is also regulated by the available combined nitrogen (nitrate and ammonia) in the soil. High soil nitrogen depresses nitrogen fixation. However, small amounts of nitrate are required initially for nodulation.

Microbiology Division, IARI, New Delhi, supply packets of *Rhizobium* for inoculating legume seeds for better yields. Similarly blue green algae cultures are supplies in plastic packets for inoculating rice fields with nitrogen fixing *cyanobacteria*.These commercially available nitrogen fixing microorganisms are sold in the market as Bio-fertilisers.

Nitrogen fixation plays a greater role in the temperate zones. But in the tropics, *cyanobacteria* (free living) holds great promise. Experiments conducted at the Central Rice Research Institute, Cuttack; Indian Agricultural Research Institute, New Delhi; and the Botany Department, Osmania University, Hyderabad, have proved that there is an increase in the yield of rice grown in fields infected with blue green algae. *Azolla*, a water fern, harbours in its lobed aerial leaves, *Anabaena azollae*, a cyanobacterium which fixed large amounts of nitrogen. Field experiments conducted at CRRI, Cuttack, have demonstrated the potential use of *Azolla-Anabaena* system in place of commercial fertilizers in rice cultivation. To promote *Azolla* growth in rice fields phosphorus supplementation in necessary.

Azospirillum, a heterotropic, microaerophillic bacterium living in the soil and in close association with roots (rhizosphere) of grasses, fixes up nitrogen. Its potential use in increasing yield in grasses, including cereal crops, like maize and millets, is being explored.

13.4.4. Non-leguminous Plants

About 310 species of woody nonleguminous Dicotyledons belonging to 13 genera are able to fix nitrogen symbiotically. They include the members of the genera *Casuarina*, *Alnus* (alder) *Myrica* (bog myrtle) and others. *Alnus* with prominent root nodules can fix large amounts of nitrogen. The nitrogen fixation in the root is found to be due to the presence of an *Actinomycete*, *Frankia*.

Check Your Progress - 1, 2 & 3

1. What is meant by biological nitrogen fixation?
2. Name the blue green algae that are capable of fixing atmospheric nitrogen?
3. What is leghaemoglobin?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.end.

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13.5. BIOCHEMISTRY OF NITROGEN FIXATION

Early studies on the biochemistry of fixation were confined to the investigation into intact organisms. With the help of la belled nitrogen (^{15}N) it was firmly established that ammonia is the key intermediate of nitrogen fixation in the organisms (Fig.13.3)

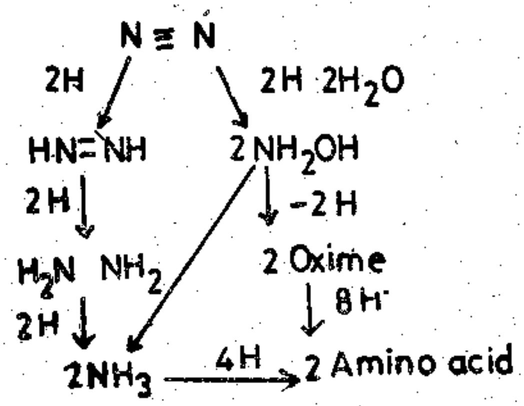


Fig.13.3 Possible pathways for reduction of nitrogen.

The important findings of the ability of the cell free extracts of aerobic and anaerobic bacteria to fix nitrogen in the same way as the intact bacterial cells have led to the isolation of the enzyme nitrogenase responsible for the absorption and reduction of nitrogen gas. Now introgenase enzyme is isolated from all nitrogen fixing organisms except from those organisms present in nonleguminous nitrogen fixing plants.

The enzyme nitrogenase catalyses the reduction of nitrogen to ammonia. The hydrogen atoms derived from the intermediary metabolism are added successively in pairs as follows:

$$N_2 \xrightarrow{+2H} \underset{\text{Diamide}}{[NH=NH]} \xrightarrow{+2H} \underset{\text{Hydrazine}}{[NH_2-NH_2]} \xrightarrow{+2H} \underset{\text{Ammonia}}{2NH_3}$$

The intermediaries are still not known and have not yet been detected. They are not released, but bound to the enzyme nitrogenase. The reduction of nitrogen to ammonia depends on a cellular supply of reduced substrate capable of donating hydrogen atoms to nitrogen. This reaction needs an electron carrier of very low potential which can link oxidation of the reduced substrate to the reduction of nitrogen. The path of electron flow is as follows.

Substrate (reduced)   **Carrier Oxidized)** NH_3
Substrate (oxidized)   **Carrier (reduced)** N_2

According to the reactions given above, different sources of reduced substrate of low potential carrier for the reaction depends on the organism. The naturally occurring electron carrier proteins ferredoxin and flavodoxin are the strong reductantss. The reducing power of reduced ferredoxin is as powerful as that of hydrogen gas itself.

In the anaerobic bacteria, *Chlostridium*, ferredoxin, the electron carrier provides the reducing power for nitrogen reduction. The reducing power in aerobic bacteria *Azotobacter* appears to be the respiratory electron transport chain itself. A low potential electron transport similar to ferredoxin has not been reported. In the bacteroids of the root nodules of legumes, the reductant appears to be the Coenzyme NADH and a low powered electron carrier is yet to be established. Nitrogen fixation is an energy intensive process; to convert one molecule of nitrogen 2molecules of ammonia, 12 ATP and 6 electrons are required. Each pair of electrons transferred to the respiratory chain would yield 3 ATP molecules; thus each ammonia molecule synthesized would cost an investment of about 10 molecules of ATP. Besides this process of nitrogen fixation, the growth of root nodule and bacteria require energy. Taking all this into consideration, it is estimated that an equivalent of about 25 molecules of ATP are required for the reduction of one molecule of nitrogen. The energy required for the reduction of nitrogen by nitrogenase is provided by ATP which is generated in an aerobes in glycolysis and in aerobes through oxidative phosphorylation. Studies on the anaerobic bacterium *Clostridium pasteurianum* have revealed that the ATp needed is derived by the oxidation of pyruvate by the bacterium and the electrons transferred to ferredoxin. The pyruvate is converted into acetylphosphate, carbondioxide and hydrogen. The acetylphosphate yields the necessary reduced form of ferredoxin. From ferredoxin, electrons are added to the nitrogen reducing enzyme nitrogenase and then to nitrogen, finally producing ammonium ions (Fig.13.4)

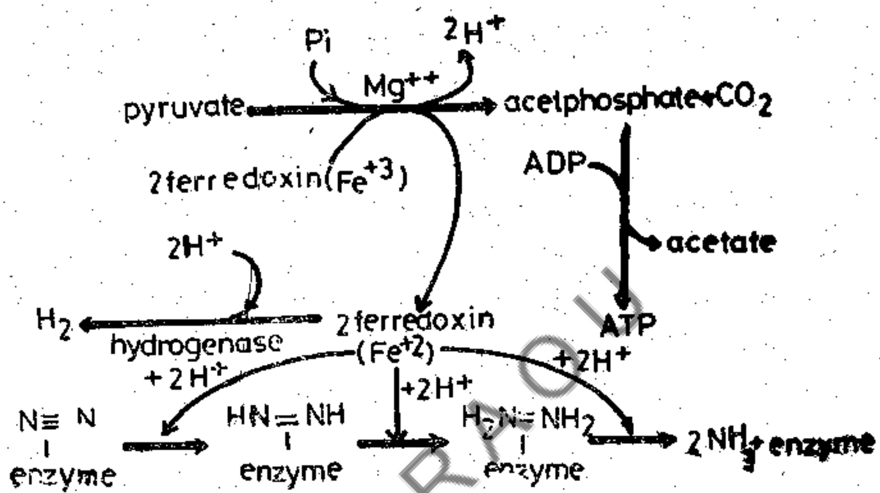


Fig.13.4. A postulated sequence for electron transfer during nitrogen fixation in *Clostridium pasteurianum*.

Most of the organisms which contain nitrogenase also contain the enzyme hydrogenase. The enzyme hydrogenase control the absorption of hydrogen and its donation to acceptor substances and regenerates the reducing mechanism. The hydrogen in the presence of hydrogenase reduces ferredoxin even in the absence of light.

Gest and Kamen discovered that purple bacteria evolve hydrogen in the presence of light and an oxidizable organic substance. Evolution of hydrogen in the presence of light and thiosulphate (oxidizable organic substance) has been demonstrated. Basically a similar thing happens in the light-activated nitrogen fixation in certain photosynthetic organisms like the purple and green bacteria and also in blue green algae. In the cells containing the necessary nitrogenase system electrons expelled from chlorophyll react with nitrogen and hydrogen ions to yield ammonia. In this type of photofixation of nitrogen the hydrogen ions necessary for reduction come from water like those for the photoreduction of carbondioxide. Photoreduced ferredoxin is the electron donor. In *Rhizospirillum rubrum* the presence of moleculear nitrogen acts as a repressor of hydrogen production. Kamen and Gest assume that the nitrogenase system competes effectively with the hydrogenase.

The nitrogenase enzyme complex consists of two proteins fractions Molybdenum and iron protein (Mol.<— Wt.220,000-270,000), containing 30 iron and 2 molybdenum and the other containing iron protein (Mol.Wt.55000-66000) having 4 iron. Both the components are necessary for their activity. With a reductant and ATP it will catalyze a variety of reactions. The enzyme is highly sensitive to oxygen, especially the iron protein part of it. It is possible to reconstitute active nitrogenase by the addition of molybdenum plus iron, and iron proteins of different microorganisms. In most of the nitrogen fixing microorganism even in aerobic organisms like *Azotobacter*,there is an undefined respiratory system which prevents oxygen to inactivate it. This phenomenon is referred to as metabolic compartmentation, meaning separation of incompatible metabolic activities inside the cells.

Two important steps are involved in nitrogenase reaction-electron activation by a suitable donor or DP and substrate reduction. The two reactions take place at two different sites on the nitrogenase molecule, but are interdependent.

From the foregoing observations, we can summarise the basic prerequisites for nitrogen fixation. They are (1) presence of hydrogenase and nitrogenase enzymes. (2) a mechanism for the protection of the nitrogenase enzyme against oxygen, (3) An electron carrier ferredoxin (4) Pyruvate for the supply of ATP and donation of electrons to ferredoxin (5) the presence of cofactors e.g. thiamine pyrophosphate coenzyme-A of inorganic phosphate and magnesium (6) the presence of cobalt and molybdenum and (7) a carbon compound for combining with the ammonia formed.

13.6. NITROGEN CYCLE

Fixed nitrogen returns to the terrestrial cycle, by biological nitrogen fixing agents, to replace the nitrogen lost by sewage, leaching, erosion and denitrification. The amount of molecular nitrogen removed from the atmosphere by microorganisms by biological nitrogen fixation is returned to the atmosphere by microorganisms that convert organic nitrogen to a gaseous state.

The transformation of nitrogen whether organic, inorganic and volatile compound may be viewed in terms of a cycle in which nitrogen is shuttled back and forth by the microflora, known as the nitrogen cycle. Many steps are involved in it.

Plants assimilate the ammonia produced in biological nitrogen fixation into amino acids, proteins and other nitrogenous products. The bulk of the nitrogenous material added to the soil in the form of organic nitrogen is not available to the higher plants, unless it is recycled. The decomposition of the organic nitrogen compounds into simpler ones is brought about by a group of soil bacteria and certain fungi. The conversion of organic nitrogen to an inorganic state is known as mineralization, which results in the generation of ammonia and nitrate. These two are distinct microbial processes. The mineralization resulting in the formation of ammonia is known as ammonification and the oxidation of ammonia to nitrate is referred to as nitrification. In ammonification soil microorganisms convert the amino nitrogen to ammonia. The ammonia reacts with the carbon dioxide and water present in the soil form ammonium salts. In nitrification the chemoautotrophic bacteria *Nitrosomonas* obtains its energy by oxidizing ammonia of the ammonium salts to nitrite (NO_2^-).

The bacteria Nitrobacter further oxidizes the nitrite to nitrate (NO_3^-). The higher plants utilise nitrate more readily than the other forms of nitrogen.

In the denitrification process the fixed nitrogen is converted to gaseous nitrogen by a group of soil bacteria known as denitrifying bacteria. The nitrite and nitrate are reduced in this process and molecular nitrogen escapes to the atmosphere. The volatilization of nitrogen depletes the soil of the vital element needed for crop growth. The denitrifying bacteria having the ability to use the oxygen from the air as hydrogen acceptor aerobically. In addition to this they can also grow in the presence of nitrate and nitrite. The conversion of nitrate into nitrogen is known as nitrate respiration. The nitrogen loss by denitrification is 830 million tons.

The predominant denitrifying bacteria in the soil are *Pseudomonas, Achromobacter, Bacillus* and *Micrococcus.*

$$2NO_3^- + 10H \longrightarrow N_2 + 4H_2O + 2OH^-$$
$$2NO_2^- + 6H \longrightarrow N_2 + 2H_2O + 2OH^-$$
$$NO_2 + 2H \longrightarrow N_2 + H_2O$$

Thiobacillus denitrificans, a chemoautotrophic bacteria present in the soils is known to oxidize sulphur also reduces nitrate to nitrogen, deriving the energy by sulphur or thiosulphate. The biochemical reaction can be summed up as:

$$5S + 6KNO_3 + 2H_2O \longrightarrow 3N_2 + K_2SO4 + 4KHSO_4$$
$$5K_2S_2O3 + 8KNO_3 + H_2O \longrightarrow 4N_2 + 9K_2SO_4 + H_2SO4$$

The biological nitrogen cycle involves the fixation of atmospheric nitrogen and assimilation of the fixed nitrogen by the plant which forms a part of the food of animals, and the removal of nitrogen from soil by denitrifying bacteria is represented in Fig.13.5.

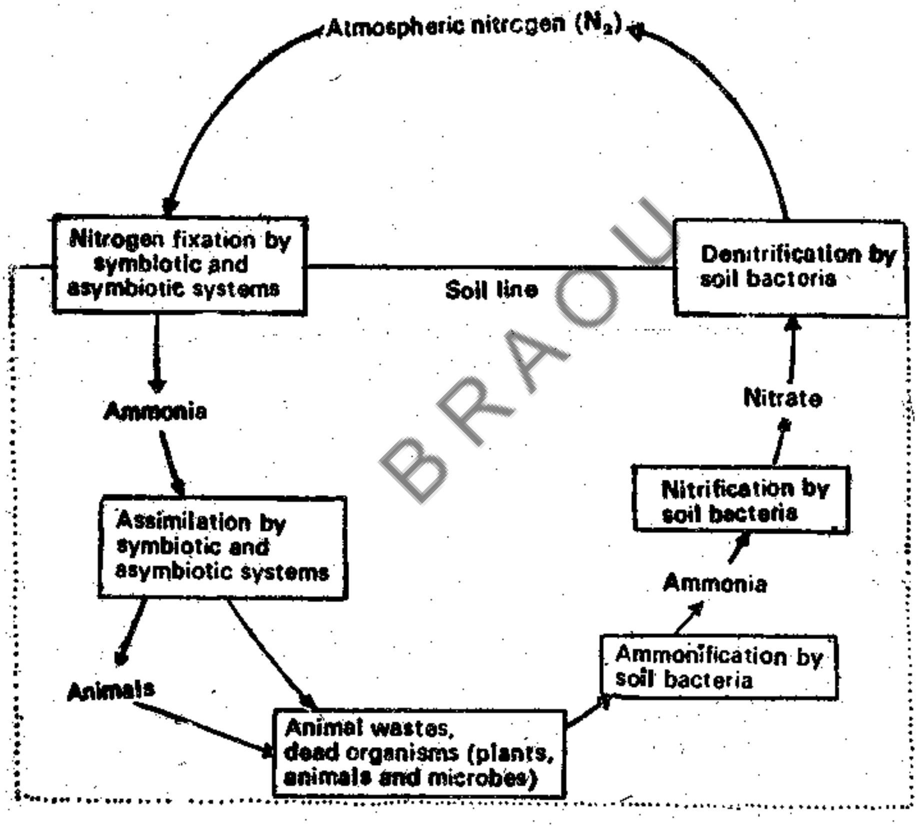


Fig.13.5. The biological nitrogen cycle.

Earlier the rate of nitrogen fixation was estimated by employing a heavy isotope of nitrogen (^{15}N) by a mass spectrometer. Nowadays nitrogenase activity in the laboratory and field is measured by acetylenethylene assay by gas chromatogrphy because the nitrogenase is able to catalyse the reduction of the molecules of triple bonded analogues of molecular nitrogen.

$$\underset{\text{Acetylene}}{(HC{\equiv}CH)} \longrightarrow \underset{\text{ethylene}}{(H_2C{=}CH_2)}$$

Biological nitrogen fixation is important in the nitrogen economy. The low levels of nitrogen present in cultivated land cannot be increased by fertilizers only. Besides, fertilizers are also expensive. Part of it must be met by biological nitrogen fixation. Production of fertilizers is energy consuming, dependent on fossil fuels. Further excessive use of fertilizers add to the problem of pollution of waters. Only less than 53% of nitrogen added as nitrogenous fertilizer is used by the plants. Urea is largely decomposed by the soil bacteria.

Scientists have transferred the 'nif' gene of *Rhizobium* to the non nitrogen fixing Bacteria., *Escherichia coli*. Attempts are being made to transfer the 'nif' gene of the nitrogen fixing system of the bacteria to cereal plants (nonlegume crop plants) which lack such a system, but they have not been successful so far.

Check Your Progress - 4

What is the difference between nitrification and denitrification?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

13.7. SUMMARY

Nitrogen is one of the soil nutrients required by plants in large amounts. Intensive agriculture depletes the limited terrestrial supply of nitrogen. Higher plants are unable to use the gaseous molecular nitrogen in the atmosphere but can assimilate it in the fixed form as nitrate and ammonia. The conversion of atmospheric nitrogen into the fixed form by living organisms is called biological nitrogen fixation and it maintains the positive nitrogen balance on earth. In the nitrogen cycle transformation of nitrogen, organic, inorganic and volatile compounds are shuttled back and forth by the microflora. The microflora involved in nitrogen fixation include the blue green algae, the free living bacteria (anaerobic and aerobic) and symbiotic bacteria in plant roots. Reduction of nitrogen to ammonia is catalysed by an enzyme nitrogenase. The nitrogenase enzyme consisting of two proteins (molybdenum + iron protein and only iron protein) is highly sensitive to oxygen, especially the iron protein part. The nitrogenase enzyme can reduce nitrogen to ammonia in the absence of oxygen. The microorganisms, capable of producing the enzyme nitrogenase, protect the enzyme from oxygen even in aerobic cells. The overall equation of nitrogen fixation can be written as $N_2 + 8H + 6$ electrons $\longrightarrow 2NH_4$. Conversion of nitrogen to ammonium is an energy dependent process and utilises ATP. Ammonium is the first stable product of nitrogen fixation and it is rapidly converted to other organic compounds.

13.8. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The fixation of the atmospheric inert nitrogen gas into nitrates and ammonia by the soil microorganisms is called biological nitrogen fixation.

2. *Anabaena, Anabaenopsis, Nostoc, Tolypothrix, Calothrix, Scytonema* etc.

3. The haemoglobin which is similar to the haemoglobin of red blood cells of mammals present in the leguminous plants is called leghaemoglobin.

4. The oxidation of ammonia to nitrate is called nitrification and in the denitrification the fixed nitrogen is converted to gaseous nitrogen.

13.9. MODEL EXAMINATION QUESTIONS

I. Answer the following question in 30 lines:

1. Give a brief account of the asymbiotic nitrogen fixation.
2. Write an account on the symbiotic nitrogen fixation.
3. Discuss the role of the enzyme nitrogenase in the reduction of nitrogen.
4. Trace the sequences and changes in the leguminous root during root nodulation.
5. What is the significance of biological nitrogen fixation in nitrogen economy?
6. Which are the different groups of microorganisms involved in nitrogen fixation?
7. Write an account on the nitrogen cycle and its importance.

II. Answer the following questions in 10 lines.

1. Write a brief account on nitrogen fixing algae.
2. Write briefly about symbiotic nitrogen fixing bacteria.
3. Give a brief account of free living nitrogen fixing bacteria.
4. What is meant by nitrification and denitrification?
5. How is nitrogen fixed by physical means?
6. Trace the steps in the reduction of nitrogen by nitrogenase.

UNIT-14: NITROGEN METABOLISM

Contents

14.1. OBJECTIVES

By the end of this unit you will be able to:

1. describe the process of metabolic reduction of nitrogen in plants,
2. recognise the enzymes that are involved in the reduction process,
3. note the relationship between the reduction of nitrate to respiration and photosynthesis,
4. describe the three major pathways of conversion of ammonia into organic nitrogenous compounds, and
5. define reductive amination and transmination.

14.2. INTRODUCTION

Nitrogen is one of the important elements present in all the plants. Plant parts, other than seeds, contain about 1-2% nitrogen on a dry weight basis; leaves contain 2-5% nitrogen. Leguminous seeds (pulses) contain 15-25% nitrogen. Amongst the enzymes which contain nitrogen, RuBP Carboxylase - the enzyme concerned with the fixation of carbon dioxide, is a major source of protein and nitrogen in the leaves. Apart from enzymes, nitrogen is a constituent of all aminoacids which occur free in cytoplasm and also function as building blocks of all proteins. As proteins are part of membrane structure, nitrogen is also found in membranes. It is found in the nucleotides other than carbohydrates. Thus nitrogen is found in varied metabolic compounds and its need and importance is next to carbon.

It was thought earlier that nitrogen assimilation resulted only in proteins. A large part of the total nitrogen in plants is present in the free form as amino acids and amides, or in an alcohol soluble state. Many of the fleshy storage organs of plants (potato tuber and carrot) contain more soluble nitrogen than protein nitrogen. In plants, nitrogen is present mainly as proteins and amino acids. They also exist in other compounds like nucleic acids, chlorophylls, cytochromes, alkaloids, vitamins and phytohormones.

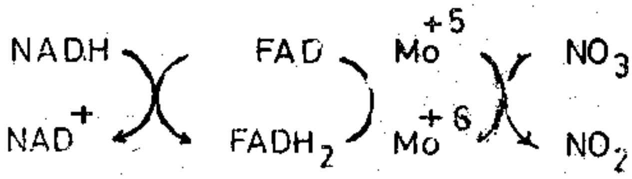
14.3. METABOLIC REDUCTION OF NITROGEN

Plants can utilize nitrogen only in the inorganic form as nitrate nitrogen (NO_3^-) or ammonium nitrogen (NH_4^+). Nitrogen is present in soil water mostly as NO_3^- and NH_4^+ ions, as a result of the decomposition of organic remains of plants and animals or directly from the inorganic salts. Of these two forms of nitrogen in soils, nitrate is the principal source of nitrogen to plants under normal field conditions. Nitrate as a nitrogen source is preferred by most plants to ammonia. Specific soil bacteria transform ammonia into nitric acid (HNO_3) rapidly by the process known as nitrification. The nitric acid is excreted into the soil by the nitrifying bacteria, where it dissociates into hydrogen (H_2^+) and nitrate (NO_3^-) ions in well drained aerated soil. Plants growing in cool and cloudy weather as encountered in the winter season of the temperate regions, prefer nitrate nitrogen. Excess of ammonium nitrogen depletes these plants of the carbohydrate reserves and the plants become thin walled, soft and succulent, sometimes resulting in ammonium toxicity. Plants growing in warm sunny weather with high photosynthetic rate, can tolerate large amounts of ammonium nitrogen, as the carbohydrate content in these plants is high.

Nitrate which is an oxidized form of nitrogen is reduced to ammonia (reduced form) in the root tips and also in the leaves. A considerable amount of nitrogen absorbed by the roots of the vigorously growing plants, is transported to the leaves by the transpiration stream. No matter where the nitrate is assimilated, either in the roots or the leaves, it is first reduced to ammonia. The reduction of nitrate to ammonia is enzymic. In higher plants, reduction of nitrate is released as water, in the reaction. Later further reduction of nitrite results in the formation of ammonia, with the release of two more atoms of oxygen as water. The reaction involved can be summed up as:

$$\underset{\text{Nitrate}}{NO_3^-} \xrightarrow[\downarrow H_2O]{} \underset{\text{Nitrite}}{NO_2^-} \xrightarrow[\downarrow 2H_2O]{} \underset{\text{Ammonium}}{NH_4^+}$$

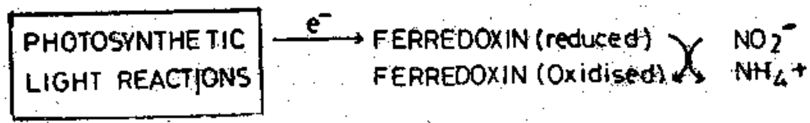
The reduction of nitrate to nitrite is catalyzed by the enzyme nitrate reductase. This enzyme is a cytoplasmic enzyme and is an induced one, synthesis stimulated only if the substrate (i.e., nitrate) is provided. This enzyme is classified as a molybdoflavoprotein, as it contains both molybdenum and flavin co-enzyme FAD and each one acts as an electron carrier in the reaction catalysed by nitrate reductase. For the reduction of nitrate the hydrogen (reducing power) is donated by NADH. Nitrate reductase reacts with NADH and transfer the hydrogen to flavin co-enzyme FAD, which is bound tightly to the prosthetic group. FAD is thus converted to $FADH_2$. The oxidation of $FADH_2$ may be by the transfer of electrons to molybdenum ion which is bound to the enzyme. Finally the electrons are transported from molybdenum to nitrate as shown below:



This reaction is catalysed by the enzyme nitrate reductase as follows:

$$NO_3^- + NADH + H^+ \longrightarrow NO_2^- + NAD + H_2O.$$

The reduction of nitrate to ammonia is the second phase of nitrate assimilation in plants The nitrite ions are reduced to ammonia by the enzyme, nitrite reductase present in the chloroplast. The direct source os electron for nitrite reduction in leaves is ferredoxin (an iron containing non-heme protein of low molecular weight). In green plants the reducing power for nitrite reduction is obtained from a portion of the reducing power derived during photosynthesis. The photosynthetic light reaction reduces ferredoxin in the chloroplast. The reduced ferredoxin reduces $NADP^+$ to NADPH, the co-enzyme participating in the pentose pathway in photosynthesis. Ferredoxin is a powerrful reducing agent having as much reducing power as hydrogen itself. Besides reducing $NADP^+$ to NADPH, ferredoxin reduces nitrite to ammonia directly. The pathway in these reactions is presented below:



The reduction of nitrite to ammonia catalysed by the enzyme nitrite reductase, is given below:

$$NO_2^- + 6e^- + 8H \longrightarrow NH_4^+ + 2H_2O$$

It is evident from the above reaction, that 6 equivalent of electrons yielded by reduced ferredoxin and 8 equivalents of hydrogen ions are required to reduce one molecule of nitrite to ammonia. Nitrite reduction, though mostly occurs in chloroplasts, it also takes place in non-green tissue like roots. In non-green tissues, ferredoxin is not involved in nitrite reduction, but an unidentified electron carrier similar to that of ferredoxin acts as an electron donor. The respiratory metabolism and not the photosynthetic light reaction provides the reducing power for nitrite reduction in roots.

Check Your Progress - 1 & 2

1. What is the enzyme responsible for the conversion of NO_3 to NO_2?
2. Nitrite reductase is present in the chloroplast whereas the nitrate reductase is present in the ________________.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

14.4 RELATION TO RESPIRATION AND PHOTOSYNTHESIS

In roots and non-green parts of the plant, the reduction of nitrite to ammonia is obtained by the reducing powerr of NADH derived during respiration, whereas, green parts reduction of nitrate by nitrite reductase is by $FADH_2$ or MADPH in chlorophyll dependent on photosynthesis, because light causes the formation of $FADH_2$, NADPH and reduced ferredoxin from photolysis of water. It is clear that there is a dependence of nitrate reductase upon photosynthesis and respiration.

Assimilation of Ammonia: Ammonium nitrogen is the inorganic form of nitrogen. They are the ammonium ion (NH_4^-) and ammonia (NH_3). They are inconvertible in water solution.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

Ammonia nitrogen is produced in different ways in plant cells. It may be produced by the reduction of nitrate to nitrate and finally to ammonium ion (NH_4^+), or it may be formed as a result of metabolic breakdown of reserve proteins during germination of some seeds. The plant may often absorb nitrogen from urea (NH_2CONH_2) used as a fertilizer which decomposes to ammonia and carbondioxide in plant cells by the enzyme urease present in them.

$$NH_2CONH_2 + H_2O \longrightarrow 2NH_3 + CO_2$$

Plant cells capable of fixing elementary nitrogen also contribute ammonia when plants are supplied with ammonium salts or anhydrous ammonia as fertilizers to soil. They absorb ammonium nitrogen directly from the soil. Generally, ammonia absorbed by any source is rapidly assimilated in plant cells. Traces of ammonium nitrogen are not harmful to plants but in high concentrations ammonia is toxic to most of them. It inhibits the production of ATP in the mitochondrial and photosynthetic electron transport system. Only a few plants *(Begonia)* with acidic vascular contents can store large amounts of ammonium ion without injury.

14.5. ASSIMILATION OF NITROGEN

1. The maintenance of cellular nitrogen balance is due to the control of both uptake and reduction. Ammonia is converted into organic nitrogenous compounds through three major pathways.

1. Synthesis of aminoacids
2. Synthesis of amides
3. Synthesis of carbamyl phosphate

14.5.1. Synthesis of Amino Acids

Ammonium nitrogen is incorporated into one or the other of the amino acids or amides. The metabolic pathways in their assimilation are presented in Fig.14.1. In living systems there are many reactions involving direct assimilation of ammonia into organic compounds. The ammonia nitrogen is converted into amino nitrogen. Amino acids contain both the amino (NH_2) and carboxylic acid (COOH) groups. The general formula of an amino acid is:

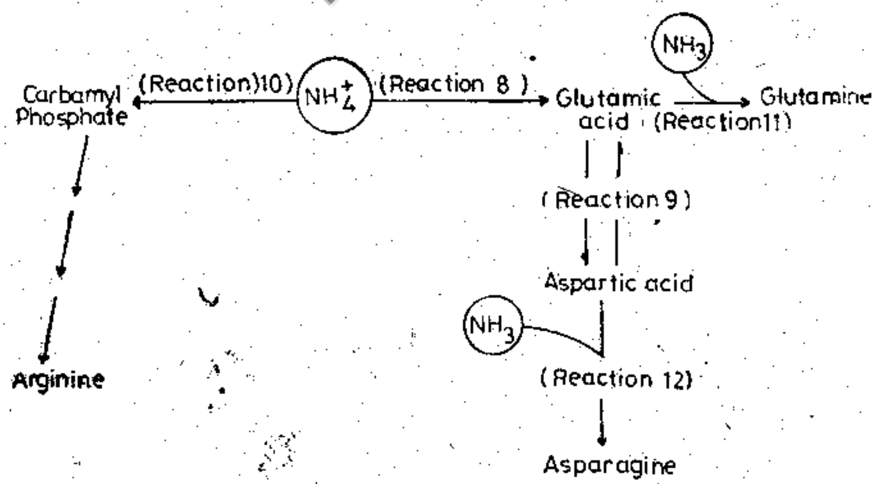
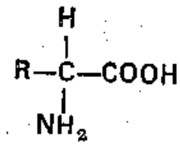


Fig.14.1. Pathway of ammonia nitrogen assimilation. Incorporation is shown into 3 aminoacids namely glutamic acid, aspartic acid and arginine and into 2 amides namely glutamine and asparagine.

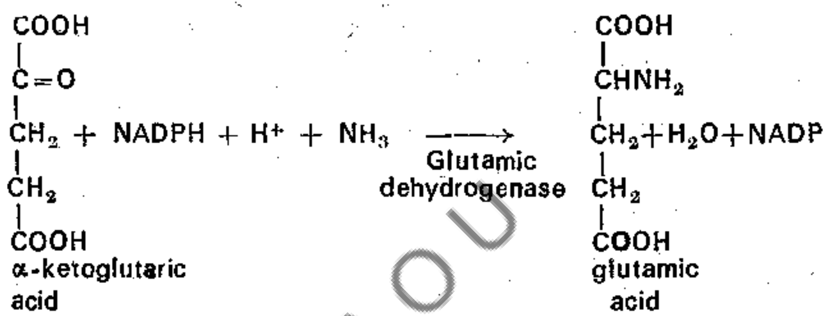
involving direct assimilation of ammonia into organic compounds. The ammonia nitrogen is converted into amino nitrogen. Amino acids contain bath the amino (NH_2 and carboxylic acid (COOH) groups. The general formula of an amino acid is:



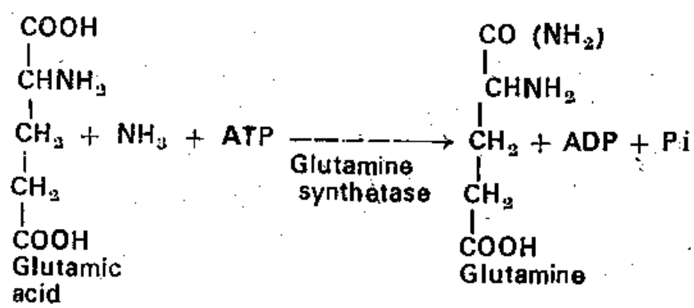
Where R is usually a carbon chain or ring. Amino acids are synthesized by 2 ways;

1. Reductive amination
2. Transmination

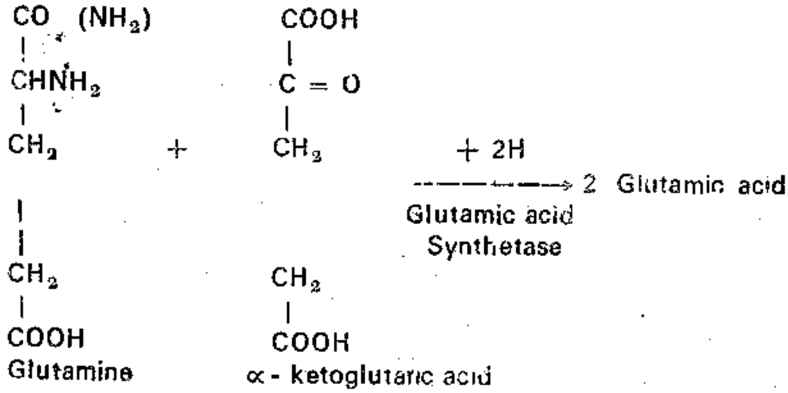
Reductive Amination: Ammonia nitrogen is incorporated into ∝-keto glutaric acid, an intermediate compound in Krebs cycle.



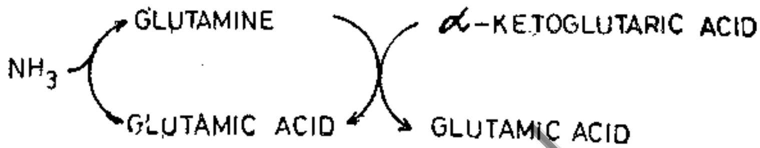
This is called reductive amination and the reducing power is derived from NADPH. The enzyme glutamic dehydrogenase catalyse this reaction. This pathway operates in bacteria, fungi and green plants. But recent studies in green plants using radioactive $^{15}NH_3$ have revealed that glutamine gets labelled first and later glutamic acid. In other words, glutamic acid and not ∝-ketoglutaric acid is the initial acceptor of ammonia. Small amounts ot glutamic acid (catalytic amount) are required as a substrate for the synthesis of glutamine. The enzyme glutamine synthetase and ATP are required as shown in the following reaction:



The glutamine formed, reacts with∝ketoglutaric acid of Krebs cycle to form glutamic acid.



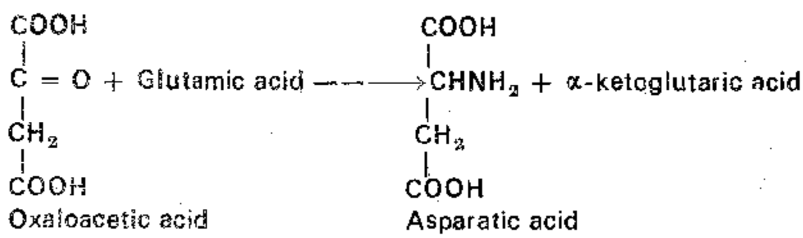
This reaction takes place in chloroplasts. The reducing agent supplying hydrogen in green leaves is reduced ferredoxin formed during light reaction of photosynthesis. These two reactions may be summed up as:



Transmination: This reaction involving the transfer from one organic compound containing amine (NH_2) i.e., glutamic acid in this case to another organic compounds initially not containing organic nitrogen(α-ketoglutaric acid) is called transmination. Other amino acids like asparatic acid can be synthesized by incorporating ammonia to oxaloacetate. Pyruvate incorporating ammonia gives rise to another amino acid called alanine.

The glutamic acid formed by reductive amination can produce other amino acids by the transfer of its amino groups to other ketoacids. These reactions are catalysed by enzymes known as amino transferases.

As many as 17 different amino acids can be produced from glutamic acid. The best example of transamination occurs between glutamic acid and oxaloacetic acid which produces asparatic acid and α ketoglutaric acid.



Several other amino acids also transfer their amino group to other α-keto-acids. This transamination is reversible. The transaminase enzymes need pyridoxal phosphate as a co-enzyme. It is a derivative of vitamin B_6, one form of which is pyridoxal itself. Glutamic acid is decarboxylated to amino butyric acid in plants by pyridoxal phosphate. This non-proteinaceous amino acid is of common occurrence in plants. There are several amino acids in plants and about twenty kinds of amino acids are incorporated into proteins

14.5.2. Synthesis of Amides

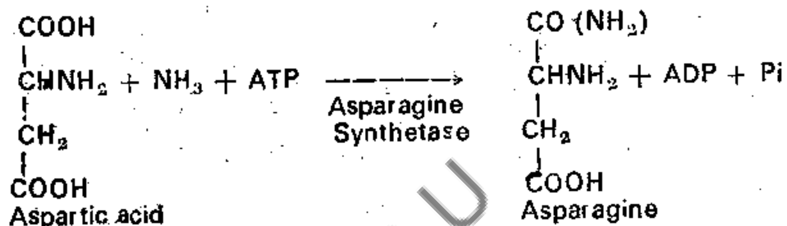
The amides are additional products of amino acids and ammonia. The assimilation of ammonia nitrogen involves synthesis of amides. The reaction is dependent on ATP. In plant tissues the glutamine and asparagine are the two amides which are most frequently present.

Glutamic acid and ammonia ions combine to form glutamine. The reaction requires ATP and an enzyme glutamine synthetase which catalyses the reaction. This is a reversible reaction and allows some ATP synthesis from ADP and iP.

$$\text{Glutamic acid} + NH_3 + ATP \xrightarrow[\text{Glutamine Synthetase}]{} \text{Glutamine} + ADP + iP$$

Glutamine is needed for the synthesis of a number of cellular metabolites including several amino acids. The amide nitrogen is incorporated into histidine, tryptophan, arginine by way of carbamyl phosphate [$NH_2COO(P)$] and also is constituted to citrulline.

Aspartic acid combines with ammonia and produces asparagine in the presence of the enzyme, asparagin synthetase and ATP.



Both the amides, glutamine and arginine, are accumulated in the tissues of healthy plants when plants are provided with ammonia nitrogen fertilizers. The amides control the toxic levels by storing excess of ammonia nitrogen. Glutamine and aspargine can be used through transfer reactions for synthesising purines and amino group of cytosine.

Check Your Progress - 3 & 4

3. Define transamination

4. The enzyme responsible for the conversion of glutamic acid to glutamine is ______________.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

14.5.3. Synthesis of Carbamyl Phosphate

The assimilation of ammonia nitrogen in higher plants may synthesise carbamyl phosphate [$NH_2COO(P)$] as a result of ammonia reacting with carbon dioxide as indicated below:

$$NH_3 + CO_2 + ATP \longrightarrow NH_2COO(P) + ADP$$

Carbamyl phosphate is essential for the formation of amino acid, arginine. The first step in the pyrimidine (Cytosine, Uracil and Thymine) formation is the synthesis of carbamyl phosphate.

14.6. SUMMARY

Nitrogen is one of the important elements present in plants as different compounds. Plants can utilise nitrogen in the inorganic form. Nitrate (NO_3) is the preferred combined nitrogen source for the plants. The nitrate is assimilated in root tips and leaves in the reduced form, ammonium (NH_4^+). Reduction of nitrite to ammonium is enzymic. The enzymes involved in nitrate and nitrite reduction are nitrate reductase and nitrite reductase respectively. The assimilation of nitrogen by plants is by three major pathways by reductive amination or transamination. The pathways are by synthesis of amino acids or amides or carbamyl phosphate.

14.7. CHECK YOUR PROGRESS: MODEL ANSWERS

1. Nitrate reductase
2. Cytoplasm
3. The transfer of amine group from one organic compound to another organic compound which is devoid of it is called transamination.
4. Glutamine synthetase

14.8. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Give a brief account on nitrogen metabolism in higher plants.
2. Describe in brief the steps involved in metabolic nitrogen reduction in green plants.
3. Give the steps involved in reductive amination.
4. What is meant by transamination.
5. What are amides? How are they synthesized?
6. Give an account of the importance of nitrogen.

II. Answer the following questions in 10 lines each.

1. Give a brief account of the role of the enzymes in nitrate and nitrite reduction.
2. Describe briefly the dependance of nitrate reduction upon photosynthesis and respiration.
3. What is the difference between reductive amination and transamination.
4. Write a note on glutamic acid.
5. Give a brief account on the reduction of nitrate.

UNIT-15: PLANT GROWTH & GROWTH REGULATORS

Contents

15.1. OBJECTIVES

By the end of this unit you will be able to:

1. define growth in plants,
2. recognise the growth points in plants and also the different phases of growth,
3. list out different definitions given to growth,
4. recognise the main groups of phytohormones that are involved in the promotion of growth,
5. locate the distribution and movement of auxins in plants, and
6. describe the importance of auxims, gibberellins and cytokinins in plants.

15.2. INTRODUCTION

Growth is defined as a gradual increase in size, development or attainment of full size or maturity. To a layman it means "increase in size", but to a biologist it is a complicated and the most obvious phenomenon of life. "'Size' is the most obvious character which is expressed in terms of length or volume, mass or weight (either fresh or dry weight). However there may not be a close relationship among these parameters in a growing organism, because growth is not a continuous process and often occurs at regular intervals at unrelated rates in different cells and organs. As such, no single definite criterion can be prescribed for the study of all aspects of growth.

15.3. GROWTH

Generally, the study of growth may begin with the seed, though it is not the starting point. A seed germinates and grows into a seedling which finally develops into a mature plant. By measuring weight or length against time, we find that initially the rate of growth is slow, but later it increases rapidly, finally slows down and becomes steady. If a graph is plotted between growth and time, a characteristic 'S' shaped curve called "sigmoid curve" is obtained.

In plants, growth is generally restricted to certain regions of the plant body known as meristems. According to their position these meristems may be apical, intercalary or lateral. The meristems show three phases of growth (1) Phase of cell division (2) The cell elongation phase and (3) Phase of cell maturation. Of all the phases, growth is more during the cell enlargement phase. The characteristic growth curve (Fig.15.1) shows that initially the rate is slow which represents the phase of cell division or lag phase, then rapid elongation represents the phase of cell elongation or log phase (or exponential phase) and finally the decrease and stationary phase or steady growth phase represents the cell maturation phase. The exponential period of growth which represents maximum velocity of growth is the so-called "Sach's Grand Period of Growth"

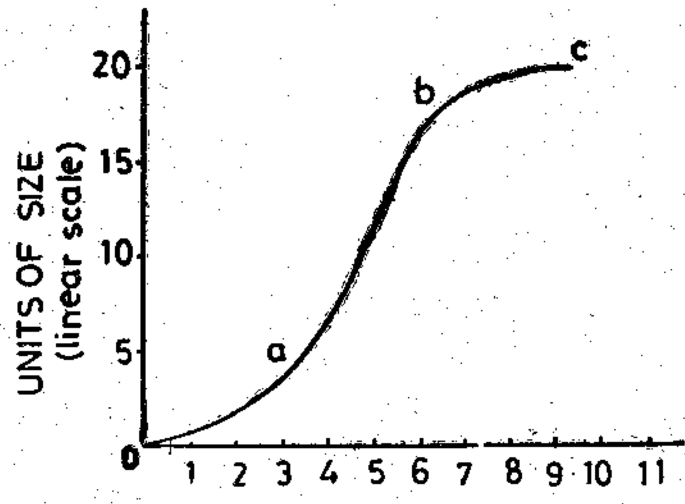


Fig.15.1. An idealised growth curve which is typically 'S' shaped. o-a=lag phase, a-b=log phase, c=stationary phase.

In general, growth is defined in different ways and each definition indicates a certain aspect of growth and has its own limitations. For e.g., (1) an increase in biomass dry weight-this cannot be taken as a criterion as during the dark seed germination which grows into a seedling there is loss of dry weight: (2) protoplasm duplication - it is extremely difficult to measure functional protoplasm; (3) cell multiplication - in some instances this does not indicate growth; (4) a permanent increase in volume - this may be considered as growth since it is permanent and irreversible increase in volume. On the other hand, development is usually defined as a change towards a more complex state of an organism. Development may take place without growth and growth without development, but the two are often combined as a single process. Development is usually implied to changes which are abrupt or gradual. For example, some processes as germination or flowering are caused due to sudden change in the growth patterns of the plant.

Thus, growth is a quantitative concept which is measurable, whereas differentiation is a qualitative concept which involves visible morphological changes in the nature of the growth in an organism.

Check Your Progress - 1 & 2

1. What is lag phase?

2. What is meant by Sach's Grand Period of Growth?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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15.4. GROWTH REGULATORS

The external environmental factors may directly act on certain genes which become active or by regulating endogenous factors. In plants organized growth and development is due to internal stimuli generated within the organ. Development may also be affected or controlled by hormones that are synthesized at one location and translocated to another. They regulate growth, development or metabolism at very low concentrations. Several classes of hormones are known which are growth promoting and growth inhibiting. The growth promoting hormones are known as phytohormones. The main groups of phytohormones are (1) auxins (2) gibberellins and (3) cytokinins.

15.4.1. Auxins

The term auxin now includes all those chemicals, which have certain biological activities. It is derived from the Greek word *Auxin* i.e to increase. The first group of plant hormones to be discovered are auxin. They generally promote growth of stem or coleoptile sections and decapitated coleoptiles, but in the same concentrations are almost totally without effect on intact plants. They bring about cell elongation in shoots and may also affect other processes like rooting, apical dominance etc.

Julius von Sachs was the first person to show that "organ forming substances" are produced in leaves and translocated downwards. In 1910-13 Boysen-Jensen from various experiments demonstrated the existence of such substances. Charles Darwin observed that young seedlings of canary grass elongated and curved towards the source of light. By using a light proof cap he was able to show that the light sensitive region was the apical tip of the seedling and not the region further back which actually underwent bending (Fig.15.2).

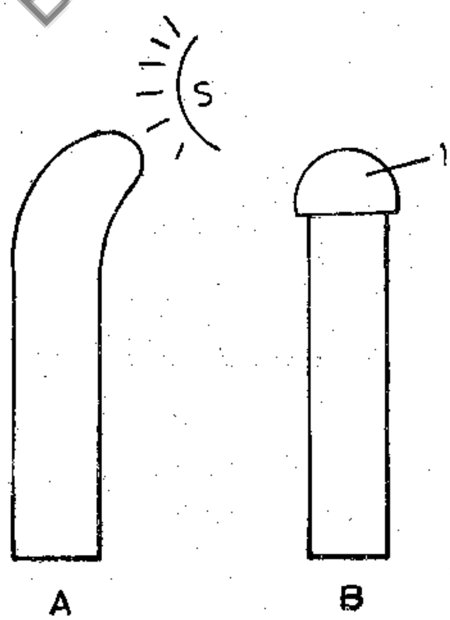

Fig.15.2. Diagram showing the light sensitive region. 1. Light proof cap.

Various experiments with at coleoptiles (*Avena sativa)* were performed by Boysen-Jensen. On germination the oat seed produces a hollow cylindrical structure with a conical tip which encloses the shoot apex and the first foliage leaf (Fig.15.3). This structure is the coleoptile. The substance is present at the tip of the coleoptile. When the tip is removed, there is no curvature, but when placed back on the decapitated stump, with a layer of gelatin in between the cut surfaces, curvature is brought about (Fig.15.4).

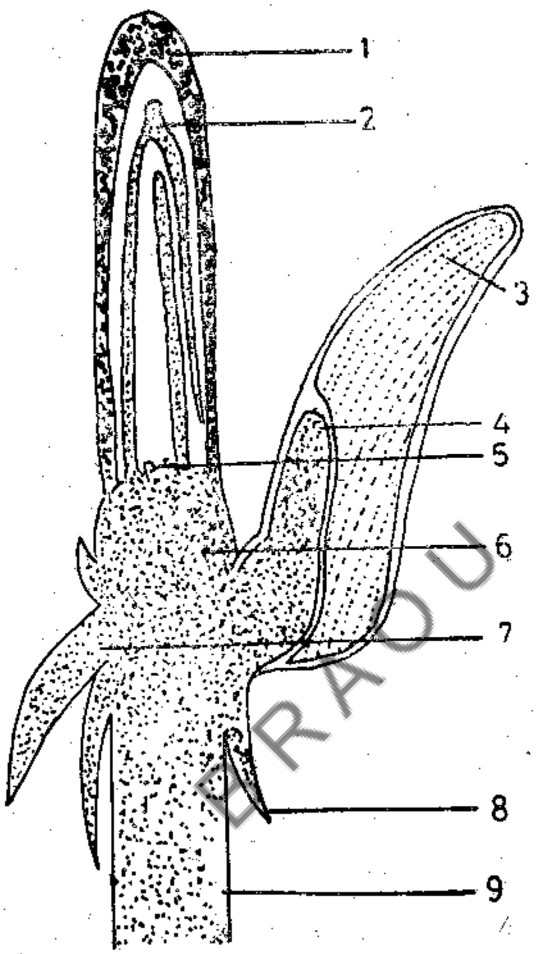

Fig.15.3. Longitudinal section of *Avena coleoptile*. 1. Coleoptile 2. Primary leaf 3. Endosperm 4. Scutellum 5. Shoot apex 6. Mesocotyl 7. Secondary root 8. Coleorhiza 9. Primary root.

This shows that the substance diffuses from the tip and that it is water soluble, thus moving through the gelatin to the region of curvature. When the tip is cut off in the dark and when the cut tip is placed along one side of the decapitated stump, the side opposite shows curvature, thus showing that curving is brought about due to uneven distribution of the growth promoting substance; the side of the seedling receiving more grows faster than the opposite side, resulting in curvature. (Fig.15.5).

The substance responsible for curvature was isolated in 1928 by a Dutch botanist F.W.Went. He cut off the coleoptiles and placed them on an agar block for one hour. When this agar block was replaced on the stump, curvature occurred, thus showing that the substance from the tip flows downwards into the agar block (Fig.15.6).

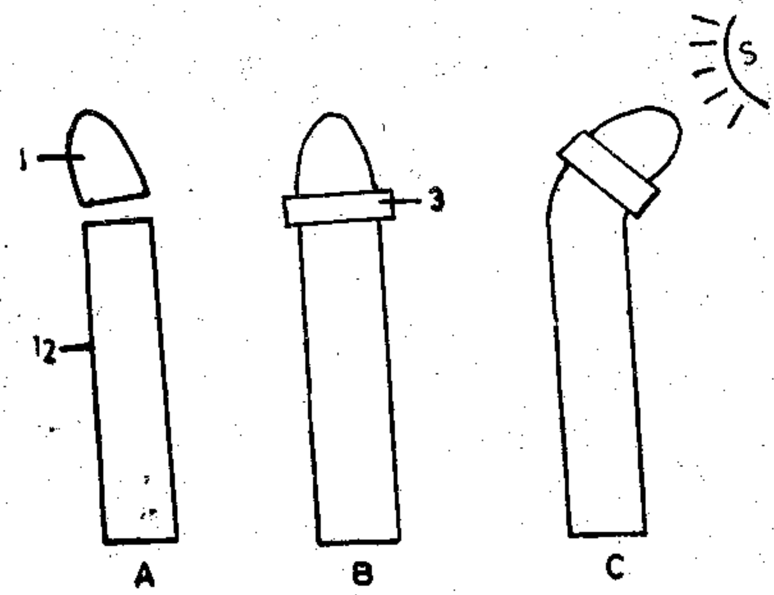

Fig.15.4. Auxin diffusion 1. Tip 2. Decapitated stump 3. Gelatin

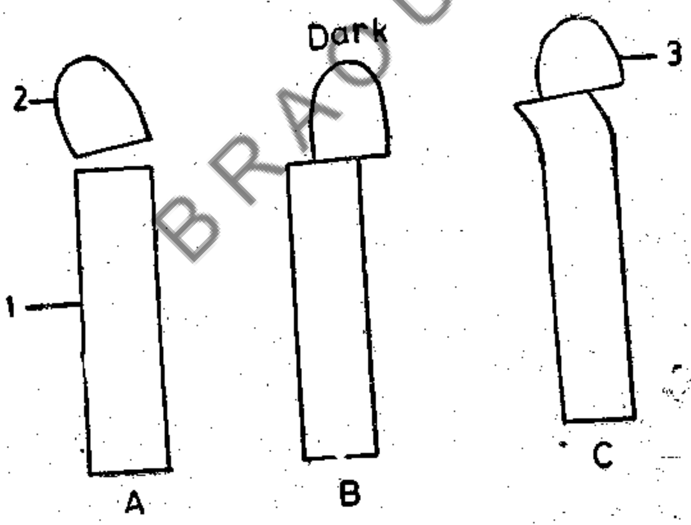

Fig.15.5. Uneven distribution of auxin. 1.Decapitated stump 2.Tip 3.Tip placed on one side (opposite side showing curvature)

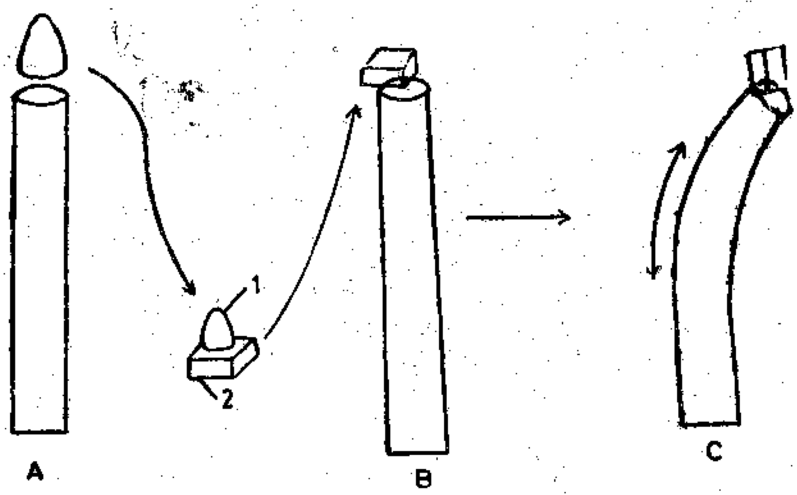

Fig.15.6. Pictorial representation of an experiment by Went demonstrating the presence of auxin that could diffuse from the coleoptile tip into an agar block and cause growth and curvature of coleoptile.

This technique of Went was employed for extraction and estimation of hormones. The chemical nature of the hormone was identified and named as Indole Acetic Acid (IAA), which is also called as heteroauxin. As the auxins are soluble in water, alcohol, chloroform, ether etc. their extraction is done by solvent method.

IAA is universally found in plants and is synthesized from its precursor molecule tryptophan found in leaves and other parts of plants. Tryptophan is converted to indole pyruvic acid or tryptoamine, which further gives rise to indole acetaldehyde. This is further oxidized to form IAA.

Indole acetonitrile (IAN) is a derivative of indole and was first extracted from cabbage leaves in 1952. In many plants this may be converted to IAA. IAA and IAN can also be derived in some plants (Cruciferae) from hydrolysis of glycosides called ascorbigen and glucobassicin.

IAA appears to be the only naturally occurring auxin in plants. The others are synthetic auxins. Two other auxins are naphthalene acetic acid and 2,4 dichlorophenoxy acetic acid (2,4-D).

Check Your Progress - 3, 4 & 5

3. What is coleoptile?
4. Write the full names of IAA & IAN.
5. What are the intermediate compounds in the conversion of tryptophan to IAA?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Distribution of auxin in Higher Plants: A survey of the auxins in etiolated oat seedlings has shown that greatest quantities occur in the root and shoot apices and lowest in the non-growing regions of the plant. The concentrations are of the order of 10g/Kilogram of plant fresh weight. In plants auxins exist in three different states i.e., (1) extractable (2) diffusable and (3) bound auxins. Auxin content can be measured by the amount of auxin diffusing out of a cut tissue into an agar jelly or by extracting with organic solvents.

Movement of Auxin in Plants: Auxins act at a distance from their source. They move in a basipetal direction i.e., towards the morphological base and exhibit a distinct polarity. That basipetal movement continues against a concentration gradient was shown by experiments performed by Went (1928). Transport rate of auxin through living cells was calculated and found to be about 10mm per hour. Polarity plays a very important role in the developmental effects of auxins (Fig.15.7).

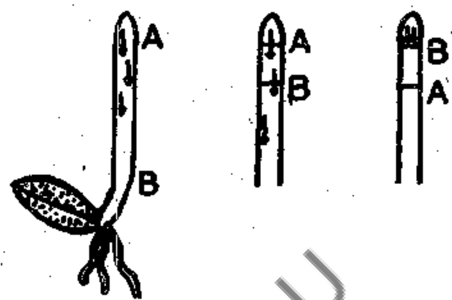

Fig.15.7. Polarity of auxin movement. Auxin moves from the apical end (A) to basal end (B). This movement stops when a coleoptile section is removed and inserted with the basal end (B) upper- most.

The amount of auxin in the tissues of the plant must be regulated for the proper functioning as a growth-controlling substance and this is brought about in various ways i.e., (1) auxin may be bound to a protein and thus inactivated, (2) formation of inactive derivatives by reacting with other chemicals and (3) can be oxidized by enzymes called IAA oxidases. These enzymes are found in many tissues and are thought to be important in the regulation of auxin levels.

Check Your Progress - 6

What is the transport rate of auxin per 1/2 an hour.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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Bioassy of Auxins: The term bioassy is used when the efficacy or strength of a substance is tested with the help of a living material. Oat coleoptile curvature test is one of the methods used for bioassay of auxins. Auxins can be separated by chromatogrphy and detected either by bioassays of extracts from pieces of the chromatogram by chemical reactions. The chromatographic procedures are much favoured because bioassays are frequently nonspecific and do not distinguish between IAA and other substances that have auxin or auxin-like activity.

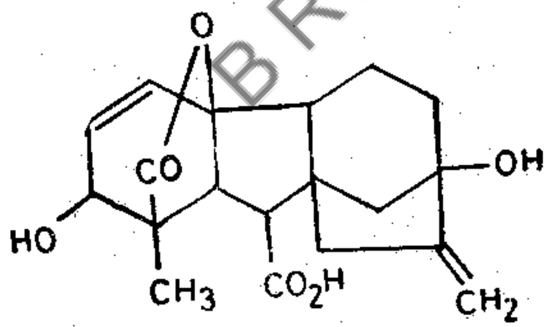
The hormonal effect on the growth of coleoptile and root differs. This was shown by Cholodny. He found that when the root tips were cut off, the root continued to grow and when the cut tip was placed, it retarded growth. The same cut root tip when placed on a decapitated coleoptile brought about curvature. This shows that the same hormone bring about curvature of the coleptile whereas it retards the growth of the root. The differential behaviour of stem and root can be explained by assuming that the optimum concentration of hormone for root is much less i.e., about 0.001 mg/lt of IAA, whereas ffor shoot growth the requirement is **1.0 mg/lt** of IAA.

Gradient of IAA found in plants exert a variety of developmental effects, suppression of lateral buds or shoots to stimulation of shoot or root elongation in different parts of the plant. Effects of auxins are many and diverse.

15.4.2. Gibberellins

Gibberellins strongly promote plant growth, especially of stems. They also have striking effects on flowering and dormancy and were first discovered by Japanese scientists. The bakanae (mad plant) disease of rice caused by a fungus *Gibberella fujukuroi* leading to excessive elongation of internodes led to the discovery of gibberellins. The plants infected by this fungus grew gigantic and had a drawn-out and yellowish appearance and were worn-out due to abnormally rapid growth rate. It was later found that these symptoms were due to the excretion of certain substance by the fungus and when this substance was applied to uninfected plants the same symptoms developed leading to understand that the substance was diffusable. The active material was purified, crystalized, and called gibberellin A after the genus of the fungus.

Many gibberellins are known that are named as GA_1, GA_2, GA_3 and so on (nearly about 50). All have the same basic structure (Fig.15.8) as gibberellic acid but differ in the nature of various side chains.



GA_3

Fig.15.8. Gibberellic Acid. Other gibberellins have various side chains on the same nucleus.

Biosynthesis of such complex molecules is a complex process and the synthetic pathway is linked with those for the production of steroids and terpenoids. Active gibberellin synthesis occurs during germination of certain seeds.

Unlike auxins they move freely within the plant and do not exhibit any polarity. Their effect is mostly on the increase of growth of intact plant. Genetically dwarf plants of maize and soyabeen grow taller into normal plants. The "bolting" in rosette plants before flowering is due to gibberellic acid. GA stimulates the denovo synthesis of amylase during germination. GA does not have a role in apical dominance.

Check Your Progress - 7

What is the causal organism of backanae disease of rice? What is its main symptom?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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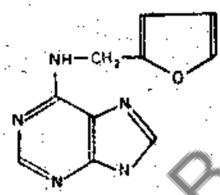
15.4.3. Cytokinins

They are adenosine derivative substances which primarily trigger cell division. They were discovered in early sixties by F.Skoog and his coworkers at Wisconsin University and named as kinetin. Kinetins are degradation products of purines and nucleic acids. The compound 6-furfuryl aminopurine was isolated as a degradation product of animal DNA and named kinetin because of its activity in inducing cytokinesis. Other adenine derivatives as benzyladenine and urea derivatives behave as kinetins in bringing about cell division and chemicals having the same biological action as kinetin are called as cytokinins. Though many naturally occurring cytokinins exist only a few have been isolated and identified. One of them is zeatin, extracted from corn endosperm which appears to be widely distributed among plants (Fig.15.9).



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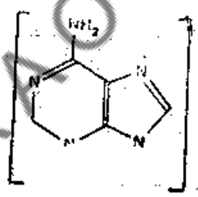

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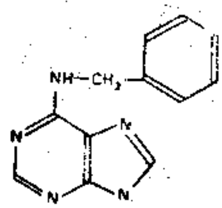

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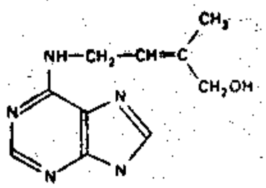

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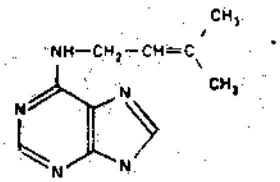

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Fig.15.9. Some cytokinins. They are all adenine derivatives. 1. Kinetin (6-furfurylaminopurine) 2. Adenine 3. 6-benzy laminozurine 4. leatin 5. Isopenteny adenine.

Besides stimulating cell division (occurs with concentrations as low as 5X10-11M) they mediate a wide range of responses. They are not as mobile as auxins or gibberellins in plants. They can move in the xylem from the roots to stems and probably also to leaves and fruits. Kinetin can bring about root, shoot organisation in un-organized callus growth in cultured tobacco pith tissue in the presence of auxins. They act to release buds from apical dominance. They can also prevent the onset of senescence in aging tissues and cause protein synthesis. Many of the bioassays that have been developed make use of this aspect of their activity. In case of guard cells, cytokinins allow more K^+ get into them from the neighbouring epidermal cells. They also antogonise the action of abscissic acid.

15.4.4. Inhibitory Chemicals

In addition to auxins, gibberellins and cytokinins there are numerous chemicals that have inhibitory actions which are particularly important in the developmental processes such as dormancy, as well as in general growth. Some of them are phenolic acids. Lactones and flavonoids (e.g.caffeic acid, coumarin, naringenin etc). Recently discovered and important inhibitor is abscisic acid (alternative names are abscisin II and dormin) which can be synthesized in leaves and then translocated in the phloem to stem apices. It is also found in seeds and stems. They cause dormancy in seeds and buds. They also cause the fall (abscission) of leaves. They inhibit protein synthesis and cause senescence. Inhibitor B is widespread and is a mixture of substances including phenolic and abscissic acids.

Check Your Progress - 8

What is the substance that is responsible for the abscission of leaves?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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15.5. SUMMARY

Growth is typically a biological problem and one of the most basic attributes of living matter. The three important manifestations of growth are (1) increase in size (2) increase in number of cells and (3) development and differentiation. A typical 'S' shaped curve known as sigmoid curve is obtained when a graph is plotted between growth and time. In plants growth is generally restricted to the meristems. Organized growth and development is due to internal stimuli generated within the organ of the plant. The substances that are synthesized at one location and translocated to another to bring about various growth manifestations are termed phytohormones. Phytohormones include auxins, gibberellins and cytokinins. Auxins promote growth of stem or coleoptile bring about cells elongation in shoots, bring about rooting and suppression of lateral buds or shoots (apical dominance) etc. Effects of auxins are many and diverse concerned with movements in plant organs. Gibberellins strongly promote plant growth. Though initially discovered in a fungus *Gibberella fujikuroi*, they are now extracted from many plants. They are concerned with breaking up of dormancy and cause flowering in biennial plants. Cytokinins are adenine derivative substances which primarily trigger cell division. They can also prevent the onset of senescence.

15.6. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The growth curve shows that initially the rate of growth is slow and it represents the phase of cell division or lag phase.
2. Sach's grand period of growth is the exponential phase of growth which represents the maximum velocity of growth.

3. On germination the seed produces a hollow cylindrical structure with a conical top. It encloses the shoot apex and first foliage leaf. This structure is called coleoptile.
4. Indole Acetic Acid; Indole Acetonitrile.
5. Indolepyruvic acid and Indole Acetaldehyde.
6. The transport rate of auxin per 1/2 hour is 5mm.
7. The bakanae disease of rice is cause by a fungus - *Gibberella fujikuroi.* The symptom of the disease is the excessive elongation of intermodes.
8. Abscisic Acid.

15.7. MODEL EXAMINATION QUESTIONS

I. Answer the following in 30 lines each.

1. What are growth curves? Explain the use of them.
2. Explain the different parts of sigmoid growth curve.
3. How is the growth rate in maize plant determined in terms of fresh weight and height?
4. What are auxins? How do they control growth in plants?

II. Answer the following in 10 lines each.

1. Explain the kinetics of growth.
2. How do you differentiate between growth and differentiation?
3. Explain apical dominance and its significance?
4. What is the role of gibberellins in growth of the plants?
5. What is a bioassay? What is it used for? Explain it in relation to auxins?
6. What is the role of cytokinins in plants?

UNIT-16: ROLE OF GROWTH REGULATORS

Contents

16.1. OBJECTIVES

By the end of this unit you will be able to :

1. describe the role of natural and synthetic auxins in the growth of plants,
2. list out and describe the practical uses of auxins,
3. recognize the important compounds which retard the growth of plants,
4. describe the role of growth hormones on dormancy of seeds and senescence of leaves, and
5. describe the effect of hormones on geotropism and phototropism.

16.2. INTRODUCTION

Growth regulators are organic substances which in minute quantities either promote, inhibit or modify growth. Hormones are regulators which are produced in minute quantities as a result of metabolism and migrate from the place of production to the place of action and regulate the physiological process. Growth hormones are of various kinds, generally referred to under the term phytohormones. The action of these phytohormones in plant growth and development are varied and are listed in Table - 1.

The control of the growth process cannot be attributed to the activity of a single hormone but is a result of the interaction of various phytohormones.

Table-1 The actions of phytohormones

Process affected	Cellular basis	Auxins	Gibberellins	Cytokinins	Inhibitors (e.g.Abscisic acid)
Stem growth	Division at apex	No action	Active	Possibly active Active in lateral buds	Active (dormancy)
	Cell enlargement	Active	Active (but requires auxin)	Sometimes active	Active
Coleoptile growth	Mainly enlargement	Active	Active at some stage	Active at some stage	Active (stops auxin action)
Fruit Growth	Division and enlargement	Active	Active	Active	—
Root Growth	Division and enlargement	Active	Generally inactive	Generally inactive	–
Leaf growth	Mainly enlargement	No action	Active	Active	—
Promotion of flowering	Differentiation	No action	Active (e.g.in L.D.P)	Generally inactive	Sometimes active
Sex expression	Differentiation	Favours femaleness	Favours maleness	No action	—
Root initiation	Division,enlargement differentiation	Active	Inhibits	No action	—
Bud formation(e.g. on calluses or cuttings)	Division,enlargement differentiation	Sometimes active	Sometimes active	Active	—
Breaking of dormancy in seeds and buds	Division,enlargement	No action	Active	Sometimes active	—
Lateral bud inhibition	Division and enlargement	Active	Enhances auxin action	Antagonises auxin	—
Cambial division	Division and differentiation	Active (with gibbs)	Active (with auxins)	?	—
Protein synthesis		Active	Active	Active	Sometimes active
Leaf abscission	Differentiation	Active	No action	No action	Sometimes active
Delay of senescene		Active	Active	Active	—

This table summarises the effects of hormones and, necessarily, includes some generalisations. Different species do not respond equally nor do the various growth substances invariably act as shown.

Gibberellins are here 'mimicking' the favour of light temperature.

16.3. ROLE OF HORMONES IN AGRICULTURE

Numerous developmental responses of plants are under auxin control. Some of these responses can also be produced by the use of auxin preparations which may be applied from the outside as growth regulators. These are the synthetic auxins. Though synthetic auxins do not possess a wide range of activity as IAA they do show their effects on growth and development of a plant similar to natural auxins. Many do not show polar transport and differ from the natural auxins. They also differ in being resistant to enzymic attack and may persist for several weeks after application. This character of synthetic auxins is of value in agriculture and horticulture. They are regarded as second in importance, as agricultural chemicals, only to the fertilizers.

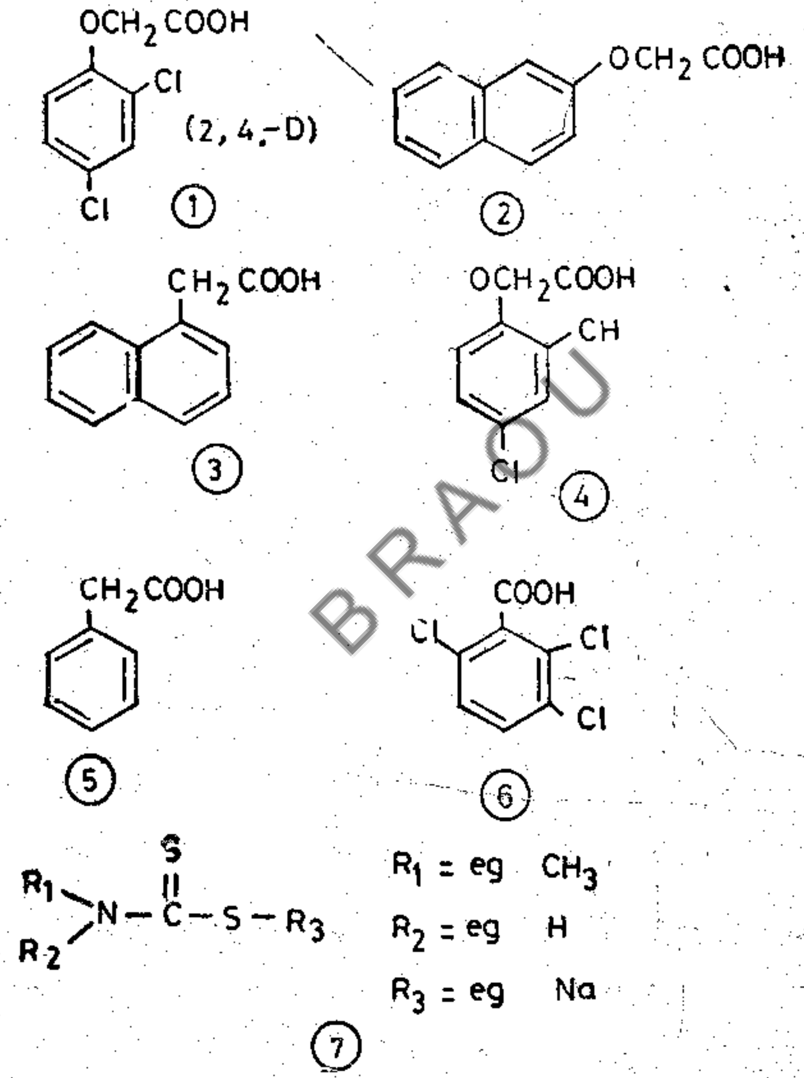

Fig.16.1 Synthetic chemicals with auxin activity. 1. 2,4-D. 2. 2- napthotracetic acid 3. NAA 4. MCPA. 5. phenylacetic acid. 6. 2,3,6-trichlorobenzoic acid. 7. Dithio carbamates (they have O instead of S)

Some of the synthetic auxins recognized after the identification of natural auxins IAA are the substituted indoles - indolepropionic acid and indole butyric acid; 1-naphthalene acetic acid (NAA), 2-naphthooxyacetic acid, phenylacetic acid and various chlorinated phenoxyacetic and benzoic acids. Certain dithiocarbamates also have auxin-like properties.

Synthetic auxins are most commonly applied in weed control as selective herbicides. The selective and toxic action of these chemicals was first noted in the case of naphthalene acetic acid and later in the methyl-chlorophenoxyacetic acid (MCPA) and 2,4- dichlorophenoxyacetic acid (2,4-D). These chemicals have lethal effect on the dicotyledonous weeds, while the monocotyledonous cereal crops are unaffected. Thus, the benefits deriving from the use of these herbicides in agriculture are substantial (increasing crop yield).

The synthetic auxins have many other important practical uses. For e.g. in orchards, NAA and 2,4-D are employed in increase fruit set and fruit size. They are also used to prevent pre-harvest drop (in apples and pears), to thin out surplus fruit by inducing fruit drop, so that fewer and sturdier fruits are obtained, and also as defoliants in agriculture. Rooting is also promoted by certain synthetic auxins. The following are the practical uses of hormones.

16.3.1. Rooting

stem cuttings when applied at the base with auxins, promote root formation. generally IAA is used for root initiation. It has commercial application in the propagation of economically important plants.

16.3.2. Weedicides

2, 4-D and related compounds are used as selective weed killers. It is of very great value to the farmers, wherein they can eradicate the weeds growing amidst their crop by spraying with 2, 4-D.

16.3.3 Apical Dominance

In many kinds of plants the terminal bud is the dominant, inhibiting the growth of lateral buds. This phenomenon called bud inhibition is demonstrated strikingly in the potato tuber. If the whole potato is planted, only the terminal cluster of buds develop into sprouts, whereas if the tuber is cut into several pieces, all of them develop into sprouts. This phenomenon is known as apical dominance and is due to the influence of auxin which is produced in the apical bud and transported downwards. In the aerial stems, the development of branches is related to this apical dominance. So long as the terminal bud is present the lateral buds remain inactive, but when the terminal bud is removed the lateral buds grow rapidly. This is the principle involved in getting the bushy growth in hedge plants on 'pruning' by the gardener.

16.3.4 Breaking of Dormancy

By spraying gibberellins the dormancy of buds is broken and allowed to sprout.

16.3.5 Interaction Between Vegetative and Reproductive Activities

In many plants, appearance of flowers and development of fruits exert an inhibiting effect on the vegetative growth of the plant. If flowers are picked as soon as they open, this inhibiting effect does not occur. According to some physiologists, the development of the reproductive structures initiates the production of inhibitory hormones which retard vegetative growth.

16.3.6 Flower and Fruit Formation

The flowering of plants appears to be related to hormone activity. Information concerning this is meagre, since it has not been possible to isolate chemically a flower inducing hormone.

In crops where flowers are not needed, the formation can be checked by spraying chlorophenoxypropionic acid. By spraying 2,4-D the early fall of fruits can be checked. This can also be induced by spraying auxins to get a larger size of fruits.

16.3.7. Abscission

Abscission of leaves, flowers and fruits was in the past, controlled by auxins. The abscission of leaves or fruits is due to the decrease of auxin supply in them, then compared to the stem. When IAA concentration in a leaf or a fruit is higher than in the stem, abscission normally does not occur, and this is so when they are growing. But, when matured, the IAA concentration decreases and abscission occurs. This decrease in the auxin level in these cases is usually accompanied by an increase in the levels of abscissic acid.

Fruit growers make practical use of these relationships. In spring they spray auxins on stems which increase the hormone concentration of stems thus resulting in premature abscission of fruits and a consequent thinking of the fruits. The fewer remaining fruits become larger in size. Later in the year, auxins are sprayed on fruits to delay abscission and permit longer tree-ripening of the fruits.

16.3.8. Parthenocarpy

The development of fruits from the ovaries of flowers and possibly, also the ripening of ovules into mature seeds after fertilization are controlled, in part, by auxins. This was first shown in 1910 when the water extract of orchid pollen grains were applied to the orchid ovaries, they developed into fruits which had no seeds, because there was no fertilization of the ovules. The ovary thus developed into a parthenocarpic fruit. Further studies have shown that auxins and related growthsubstanceswhen applied to the ovaries of flowers, cause the development of seedless fruits. This technique is of commercial value for the large-scale production of seedless fruits, for e.g. seedless grapes, lemons oranges etc.

16.3.9. Seed germination

Cytokinins and gibberellic acid help in the germination of tobacco and lettuce seeds. They also check the apical dominance and activate the development of lateral buds.

Thus, auxins which were of an academic curiosity for Darwin, have now become an established practice in applied botany.

Check Your Progress - 1, 2 and 3

1. What is apical dominance? What is the substance responsible for it?

2. The dormancy of seeds can be broken by spraying ______________

3. The root formation is promoted from stem cutting by______________

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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16.4. ROLE OF GROWTH RETARDANTS

Chemicals which impede the action of auxins are antiauxins for e.g. maleic hydrazide. It induces branching or reduces growth. It prevents bud growth in potatoes.

Many antiauxins are chemically similar to auxins but are deficient in the correct ring structure and side chain for e.g. 2, 6-dichlorophenoxyacetic acid, 2, 4-dichloroanisole etc.

Chemically ethylene is unlike the auxin, and has many important effects on plants. This includes the promotion of leaf abscission and breaking of bud and seed dormancy in the form of ethylene chlorohydrin. It is a natural product produced in leaves inducing senescence and in fruits affect the ripening process unlike the other antiauxins.

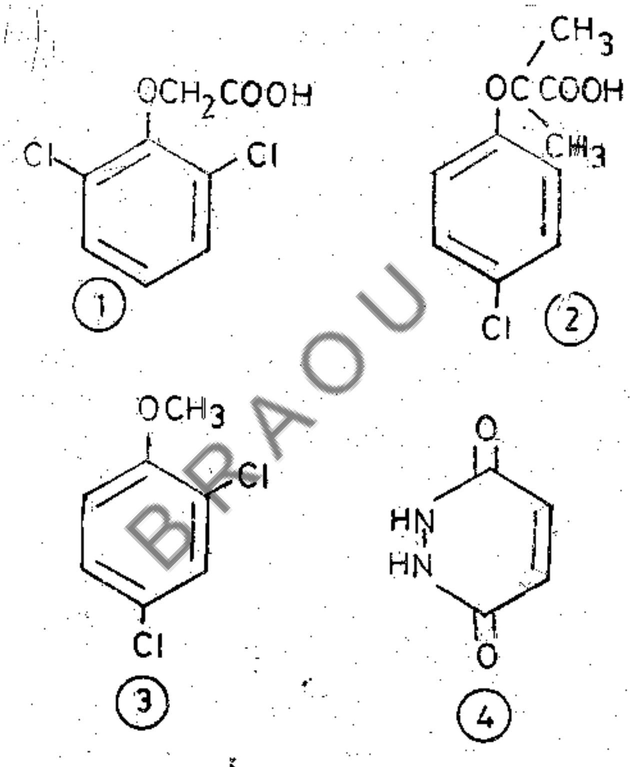

Fig.16.2 Antiauxins. 1. 2, 6-dichlorophenoxyaceticacid. 2. 4- chlorophenoxy isobutyric acid, 3. 2,4-dichloroanisole. 4. Maleic hydrazide.

Certain chemicals such as Phosphon, AMO and CCC (chloro choline chloride chloride) are used in the horticultural industry as growth retardants. They act as antigibberellins when applied to plants, and interfere with the biosynthesis of gibberellins. Their action is prevented by the addition of gibberellins. Embryos of barley and wild oats fail to synthesize gibberellins in the presence of CCC. Otherwise, they grow and metabolise normally. In recent years, these substances are used to study the roles of endogenous gibberellins.

All the above mentioned substances are frequently used in both the practical aspects of growth control and for investigations into the mechanisms of plant growth.

Check Your Progress - 4

Define antiauxins with one example.

Note: (a) Write the answer in the space given below.

(b) Compare your answer with the one given at the end.

16.5. ROLE OF HOROMONES IN DORMANCY

Dormancy is extremely important to the plant as it provides a means of survival and is a defense mechanism against winter frost or summer drought. In all plants there is a period of dormancy and this phase is passed through as seeds. In most cases the growth of 'dormant' organs is resumed upon reabsorption of water at a suitable physiological temperature, and with adequate air supply. But some seeds and buds fail to grow. This state of the tissue or organ (which will not resume growth at normal conditions) is defined as dormancy. This shows that some special 'trigger' is required to release the organ from its dormancy.

What causes a shoot apex to stop growing and form a dormant bud, or a seed to develop has to be understood. In many cases, environmental factors such as the temperature and the day length (photo-period), might be responsible for causing dormancy. However, it may be said that dormancy is maintained by some internal control mechanism. The maintenance and breaking of dormancy depend on an interaction between the environment and the plant. The best known internal mechanism for maintaining dormancy are inhibitors.Inhibitors are found in many dormant tissues such as potato buds, ash buds, variety of seeds etc. Some of these inhibitors found in many seeds are ammonia, cyanide, coumarin, abscisic acid and many unidentified chemicals.

The most important factor in inducing dormancy appears to be photoperiod. Though the photoperiod is perceived by leaves, the apex and the buds are the responding parts. This shows that the leaves are induced to make an inhibitory substance that is translocated to the buds.

The inhibitory substances have been separated chromatographically from the leaves of the trees such as *Acer* (maple) that were exposed to short days. These substances inhibit the growth of test plants. This inhibition can be overcome by the plant through exposure to long days, or application of gibberellic acid.

P.F.Wareing and his colleagues found that the extracts of leaves of *Betula pubescence* kept on short days inhibited the elongation of *Avena* coleoptiles and in 1963 were able to isolate a substance having physiological properties of the dormancy- inducing inhibitor, which they called dormin. At the same time F.T.Addicott and his workers isolated a senescence- inducing substance, abscisin II. The two compounds were found to have the same structure and are now called abscisic acid (ABA).

ABA is known to occur in many parts of the plants of varying types. It is present in dormant tissue or in tissues entering dormancy. Its concentration decreases in tissues treated for the breaking of the dormancy. ABA application induces various symptoms of dormancy and senescence. The effects of ABA are:

1) Induction of dormancy
2) Maintenance of dormancy
3) Inhibition of germination
4) Inhibition of gibberellic acid - induced enzyme synthesis in seeds
5) Inhibition of flowering
6) Abortion of buds
7) Abortion of fruits
8) Senescence of leaves
9) Acceleration of abscission
10) Formation of terminal buds on shoots
11) Formation of buds scales
12) Reduction of cell division
13) Induction of biochemical charges leading to senescence and leaf abscission.

In addition ABA appears to be a general inhibitor of growth.

For many seeds, a low temperature treatment is essential for germination. This requirement is artificially done by the process of stratification. In this process the seeds are layered in trays in cold moist air for a period of up to several weeks or months. Temperature between 0 and 10^{o}C are most effective. The low temperature treatment appears to be necessary for the breakdown of ABA present in the seeds. It is also necessary for the activation of gibberellin synthesis.

In some seeds, seed coat is involved in dormancy and removal of this seed coat helps seed germination.

Light also plays an important role in the breaking of dormancy and stimulating seeds to germinate. This is shown with respect to tobacco and lettuce seeds. Red light is most effective and the period of daily exposure of seeds to light varies for breaking seed dormancy.

In both seeds and buds, light is essential not only for breaking dormancy along with low temperature, but also for the onset of dormancy. Thus the mechanism of dormancy in these organs appears to be similar.

Check Your Progress - 5

Define dormancy.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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16.6. ROLE OF HORMONES IN SENESCENCE

It is easy to follow senescence in leaves which results in the destruction of chlorophyll. Prior to the destruction, yellowing occurs followed by other symptoms such as wilting, and shrivelling. Biochemical analysis of the leaves reaching the end of mature life show that proteins breakdown in aminoacids accompanied by nucleic acid degradation. Metabolic processes such as respiration and photosynthesis decline.

Leaf abscission is due to senescence. Application of hormones to plant tissues markedly affect senescence and thus hormones may be, likely, involved in senescence. Abscisic acid which can promote abscision has been isolated from plant tissues. Leaves treated with kinetin lose their chlorophyll and proteins at a much slower rate and thus the leaves remain green for long-periods. This property has been used commercially to prolong the storage life of green vegetables.

Application of gibberellins and auxins to leaves also inhibits their senescence in some species which may be due to the ability of these chemicals to stimulate synthesis of certain proteins.

Like any other growth phenomenon, senescence is caused and controled by the complex interaction of many factors, both external and internal.

16.7. GROWTH MOVEMENT

Plants may react by growth movement to directional stimuli, particularly light and gravity. These movements are irreversible changes resulting from growth and are related to the direction of stimulus. Such movements are termed tropic movements. Tropic movements of plant organs are always directive i.e., the direction of movements determined by the direction from which the stimulus is applied and organs move other towards the source of stimulus or away from it. They include geotropism (response to gravity), phototropism(response to light), thigmotropism(response to touch) and hydrotropism(response to water). The best understood are phototropism and geotropism and these movements are under the control of auxins.

16.7.1. Geotropism

The movement of plant organs in response to the force of gravity is called geotropism. The primary roots grow into the soil along the line of gravity and they are positively geotropic, whereas stems grow away from the centre of gravity and are known as negatively geotropic. The secondary roots, branches and the leaves of the stem are placed horizontally or obliquely and are described as plageotropic or diageotropic respectively, according to the direction. The lateral roots are not sensitive to gravity and they are ageotropic. Gravity is not perceived throughout the whole plant and the perceptive region appears to be the root cap. This is so because if the root caps are removed there is no geotropic response. Geotropism can be demonstrated experimentally by using a Klinostat.

Response to gravity is inductive and can be separated in time. This shows that two separate processes are involved i.e., perception and reaction. According to certain physiologists statoliths (small bodies of high specific gravity) perceive the gravity.

As we have seen earlier (unit-15), the unilateral illumination of a growing stem reduces the auxin effect on the side facing the light source. So, this makes the cells on the opposite side to elongate more extensively and then the stem curves towards the light. Thus the result is positive phototropism of stems. How can the negative phototropism of roots be explained?

It has been experimentally found by F.Went and N. Cholodny that IAA concentration required for stem growth is very much higher when compared to the concentration required for root growth. IAA concentration which stimulate root growth is so low that it does not promote stem growth. For e.g. if a stem-root system(in water) is illuminated from one side, the stem tip will curve towards light, whereas any auxin diffusing into the root on the side away from light will be sufficiently concentrated to inhibit root-elongation on that side. So, the side facing the light will grow faster and the root tip curve away from the light stimulus, hence the negative phototropism of roots.

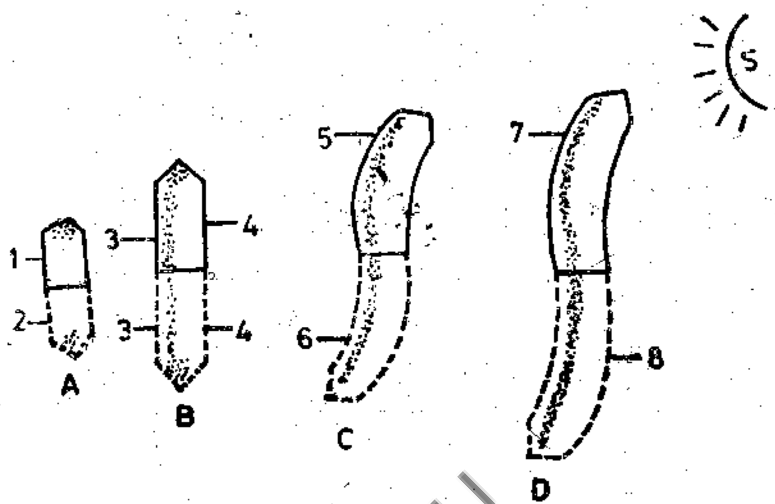

Fig. 16.3. Diagrams showing the response of stem and root to sunlight. 1. Stem (Auxin diffuses down) 2. Root (Auxin diffuses up). 3. Auxin active on this side. 4. Auxin inhibited on this side. 5. Auxin stimulates on this side. 6. Auxin inhibits on this side. 7. Stem growth faster on this side. 8. Root growth faster on this side.

The negative geotropism of stems and the positive geotropism of roots can be explained by migration of auxin under the influence of gravity. If a plant is placed horizontally the stem tip grows upward, whereas the root tip grows downward. This response takes place even in the dark and thus shows that it is gravity - dependent and not light-dependent. In a horizontal plant 2/3rd of the auxin in a shoot tip is present on the lower side of this tip. As the auxin moves back to cells which are capable of elongation, most of it therefore reaches the cells located on the lower side of the stem and thus these cells elongate more than the cells on the upper side and the stem tip curves upward as a result. The lower side of the root cells which receive the auxin, inhibits the growth of those cells on that side and thus the cells on the upper side elongate more rapidly. Therefore the root tip curves downward in the direction of the centre of the earth.

Evidence supports this mechanism for shoots. In roots, root caps have been found to be the source of growth-inhibiting substance i.e., abscisic acid. It is believed that geotropic growth stimulus is mediated by the assymmetric production of ABA.

Leopold and his colleagues have demonstrated that auxins are transported laterally under the influence of a gravitational field in corn coleoptiles. According to Juniper statoliths may be responsible for lateral redistribution of hormones.

Check Your Progress - 6

What is meant by geotropism?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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16.7.2. Phototropism

The understanding of the mechanism of phototropic reaction goes back to Went's experiments leading to the discovery of auxin (Unit-19). The movement of plant organs as determined by the direction of incidence of rays is called "Phototropism". The stems grow towards the source of light and are known as positively phototrophic. The leaves and stem bend towards the light and are known as tranversely phototropic or diaphototrophic. Phototropism can be easily demonstrated by using a phototrophic chamber.

It is suggested that the phototrophic response of leafy shoots is due to unequal synthesis and expo.. of auxin.

The work of Leopold suggests that cotyledons are necessary for phototrophic response. Recent experiments in Holland by J. Bruisma show that the cotyledons produce an inhibitory substance.

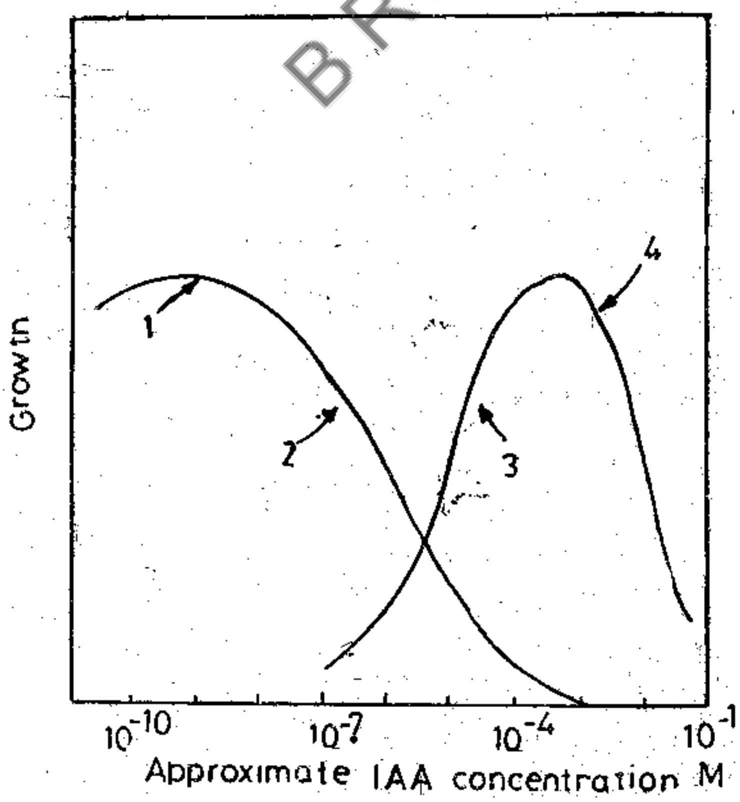

Fig. 16.4 Effect of different concentrations of IAA on root growth and shoot growth.

Though most of the tropisms exhibited by plants is due to changes in auxin distribution and differential effects of auxin concentrations on different plant parts, in most cases, however it is still not quite clear how the external stimulus actually elicits particular auxin activation.

Unlike growth movements, turgor movements are initiated largely by external stimuli. They are rapid and are transient and repeatable. Here again auxins appear to be the chief controlling agents. Turgor movements include, guard cell movements, contact movements and sleep movements. These different movements come under the general name nastic movements.

Growth may not be a continuous and simple but involves a series of complex discrete steps.

Check Your Progress - 7

Define phototropism.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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16.8. SUMMARY

Growth regulators are organic substances. They are produced in minute quantities and migrate from the place of production to the place of action. Regulators which promote, inhibit, or modify growth are growth regulators or growth hormones. These growth hormones are of various kinds (auxins, gibberellins, cytokinins) and are generally termed phytohormones. Phytohormones show varied effects on growth and development of plants. Activity of synthetic auxins are similar to natural auxins. However, they differ from natural auxins in not showing polar transport, being resistant to enzymes and in their persistence. As such, synthetic auxins are of value in agriculture and horticulture. Examples of synthetic auxins are indole propionic acid, indolebutyric acid, 1-napthalene acetic acid (NAA), 2-napthooxyacetic acid, phenylacetic acid and various chlorinated phenoxyacetic and benzoic acids. Mostly they are used as selective herbicides in weed control[2, 4-Dichloro phenoxy acetic acid (2, 4-D); methylchlora phenoxyacetic acid(MCPA)]. In addition they are also used for fruit setting, fruit drop, to promote rooting, as defoliants, in breaking dormancy in seed germination and also in obtaining seedless fruits. There are other chemicals which impede the action of auxins. These are referred to as antiauxins e.g. Maleic hydride. In addition to phytohormones, inhibitory substances are also produced within plants. For e.g. the dormancy-inducing inhibitor dormin; the senescence-inducing substance-abscisin II (They are now renamed as Abscisic acid -ABA). Auxins are also responsible for the various growth movements. These growth movements are irreversible changes brought about by growth in relation to the directional stimuli and are known as tropic movements. They particularly, include phototropism(directional stimulus-light) and geotropism (directional stimulus-gravity). Other movements which come under the general name , nastic movements, include guard cell movements,contact movements and sleep movements.

16.9. CHECK YOUR PROGRESS : MODEL ANSWERS

1. In several plants the terminal bud is dominant and it inhibits the growth of other lateral buds as long as it is present. This phenomenon is called apical dominance. The substance responsible for apical dominance is auxin.
2. Gibberellins
3. Indole Acetic Acid (IAA)
4. Antiauxins are the chemicals that impede the action of auxins.
5. Most of the seeds and buds germinate after absorbing water at a suitable physiological temperature. But some cannot develop. This state of tissue or seeds is called dormancy.
6. The movement of the plant organs to gravity is called geotropism.
7. The movement of plant organs in response to sunlight is called phototropism.

16.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each

1. What are tropic movements? Explain the role of auxins in phototropism and geotropism.
2. Explain geotropism and phototropism. Describe their underlying mechanism.
3. What is seed dormancy ? What are its causes and what methods are used to break it ?
4. Define dormancy? What are the methods used to break seed dormancy..
5. Describe the physiological significance of abscission ?

II. Answer the following questions in about 10 lines each.

1. Give a brief account of phototropism.
2. Briefly describe the role of hormones in senescence.
3. Give an account of synthetic auxins.
4. Write a brief account of practical uses of horomones.

UNIT-17: PHYSIOLOGY OF FLOWERING

Contents

17.1. OBJECTIVES

After going through this unit you will be able to:

1. define photoperiodism,
2. recognise the different groups of plants classified on the basis of the length of photoperiod requirements,
3. describe and differentiate between long day plants, short day plants, day neutral plants, plants promoted by long days and short days, long-short day plants, and short-long day plants,
4. recognise the number of photoinductive cycles necessary for the flower initiation in plants,
5. recognise the site of photoinductive perception in plants,
6. describe the process of translocation of stimulus,
7. describe the role of different hormones in flower initiation,
8. recognise the two different states of photochrome and also describe its role in flower initiation, and
9. define vernalisation and also describe the effect of vernalisation on plants.

17.2. INTRODUCTION

The initiation of floral primordia is a major event in the life cycle of a plant. It is because it involves a transition from vegetative phase to reproductive phase. The plant passes through several phases of development before the flower primordia are produced. The plant must complete a period of vegetative growth or attain certain minimal leaf number before the flower primordia are initiated. This condition of the plant is said to be ripe-to-flower condition. This condition of the plant is generally achieved after producing several leaves. The fruiting trees require several years to attain this condition whereas the annual herbs flower in a few months only. Some cereal grasses have to develop seven leaves before they attain the ripe-to-flower condition. The Japanese morning glory (*Pharbitis nil*) attain the ripeness to flower with a day after the cotyledons have emerged. Some of the processes involved in the flower primordia initiation are given below.

Vegetative growth
↓
Ripeness to flower
↓
Perception of environmental factors
↓
Altered metabolism
↓
Formation of flower stimulus
↓
Translocation of flower stimulus to shoot apex
↓
Transformation of vegetative shoot apex to reproductive shoot apex
↓
Initiation of flower primordia
↓
Development of flower buds and flowers

It is found that two environmental factors are responsible for flowering. They are (1) Periodicity of light (photoperiodism) and (2) Temperature (vernalisation).

17.3. PHOTOPERIODISM

Photoperiodism is defined as the response of a plant to the relative lengths of light and dark periods. The significance of light in flowering was recognised only after the work of Garner and Allard (1915-1920). They observed that the common widely grown variety of tobacco- Maryland Narrowleaf, flowered during the summer. A mutant developed from these plants had grown much taller and produced very large leaves. This mutant was named as Maryland Mammooth. This mutant did not flower in the summer along with Maryland Narrowleaf. It flowered only in winter. This observation made them to study the flowering behaviour of Maryland Mammoth and other plants. After thorough investigation, they found that the length of the light and dark period are responsible for the flowering of plants. These two scientists have recognised 3 groups of plants on the basis of the length of the photoperiod requirements of the plants. They are: 1) Long day plants, 2) Short day plants and 3) Day-Neutral plants

The later workers have added few more groups to the original 3 groups of Garner and Allard. They are: (4) Plants promoted by long days, (5) Plants promoted by short days, (6) Long-short day plants and (7) Short-long-day plants.

of perception to the site of flowering. It is found experimentally that the stimulus moves through phloem both upwards and downwards independent of photosynthates.

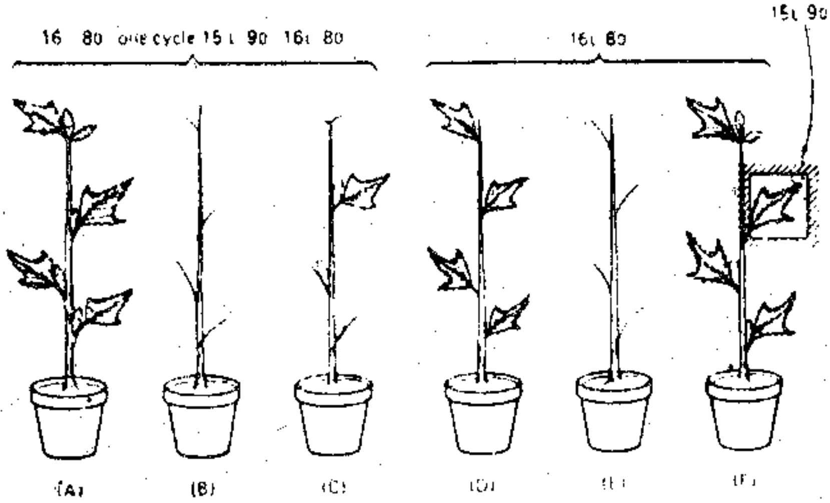

Fig.17.1. Response of *Xanthium* to photoperiod (After Naylor, 1952)

The experiments showed that the stimulus travels at the rate of 20mm/h in *Pharbitis nil*, a short day plant. It is also observed that the stimulus moves at the rate of 500 mm/h in long day plants. These rates are comparatively slower than the velocity of photosynthates which travel at the rate of 1500 mm/hour.

17.4. ROLE OF HORMONES IN FLOWER INITIATION

The translocation of stimulus from the leaves to the site of flower initiation indicates that it is in the form of chemical. This flower inducing chemical or hormone is named as florigen by Russian Scientist, Chailkahyan(1937). This fact is also supported by grafting experiments with Maryland Mammoth a short day plant and *Hyoscyamus niger*, a long day plant. When the grafted plants are exposed to either long day or short day conditions both the partners flowered. This indicates that the flowering stimulus is translocated from one plant to the other if there is a graft union. If the graft union is not formed the flowering hormone is not translocated from one plant to the other. This also shows that the flowering hormone is similar in all photoperiodic classes of plants.

After the experiments of Chailkahyan (1937) several investigators have showed that indole acetic acid (IAA) induced flowering in some plants. Some scientists have also indicated that the other growth substances-gibberellins, cytokinins, abscisic acid, ethylene also participated in the flower initiation under certain conditions.

Among the above mentioned hormones, the gibberellins have spectacular effect on flower initiation in long day plants under short day conditions. But they have no role on short day plants under long day conditions. This shows that the gibberellins are not florigen because it is mentioned earlier that the flowering hormone is similar in all photoperiodic classes of plants.

Chailkahyan has modified his theory slightly to explain the effect of gibberellins on long day plants. He proposed that florigen consists of two hormones - gibberellin and anthesin. He suggested that long day plants produce anthesin under any condition. So when gibberellin is supplied flowering occurs. But short day plants produce gibberellin under any day length and anthesin only under short day conditions. As anthesin is not formed in short day plants can not be induced to flower under long day conditions. He also added that the day neutral plants produce both the hormones under any day lengths. This explanation is based on the existence of a hypothetical substance called anthesin. No such compound is isolated so far.

Gifford and Jenson discovered that the florigen alters the kinds of mRNA synthesized in the plants. This alteration changes the enzyme complex of meristems and leads to the formation of floral primordia.

Check Your Progress - 3

What is florigen?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

17.5. ROLE OF PHYTOCHROME IN FLOWER INITIATION

It is still assumed that the flowering stimulus, florigen, is responsible for the initiation of floral primordia. It is supposed that the release of this florigen is dependent upon a blue photoreceptor pigment called phytochrome. The phytochrome is proteinaceous in nature. Till now the phytochrome is reported from red algae, desmids, bryophytes, gymnosperms and angiosperms. In angiosperms, the phytochrome is detected in roots, coleoptiles, hypocotyls, stems, cotyledons, petioles, leaf blades, vegetative buds, floral tissues, seeds etc. in low concentrations. Borthwick and Hendricks suggested that the phytochrome occurs in two forms:

1. The red absorbing P_{660}(Pr) and
2. The far-red absorbing P_{775}(Pfr)

660 and 735 are the wave lengths (660 nm and 735 nm) of red and far red respectively. The two forms are photochemically interconvertible as follows:

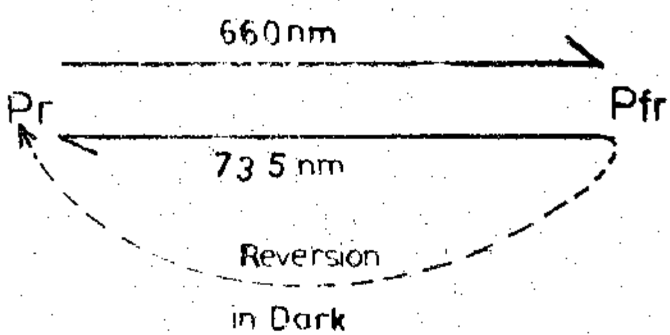

Fig. 17.2 Interconversion of phytochrome photochemically.

This shows that P_{fr} is rapidly converted to P_r by far red light and P_r is rapidly converted to P_{fr} in red light. They also suggested that the P_{fr} form is also converted to P_r form in darkness.

Recent studies have suggested that there are several intermediates in the conversion of P_r to P_{fr} or P_{fr} to P_r and the biologically active P_{fr} forms a complex with another compound (X) to form P_{fr}.X complex. This compound is responsible for the physiological response of the plaht. The X may be ATP, NAD or some other metabolite. The effect of phytochrome on the flowering of short day and long day plants is imagines as given below.

Short day plants: In short day plants, the dominance of P_r promotes flowering. During long day conditions the concentration of P_{fr} is high and the ratio of P_{fr} to P_r is such that the formation of flower stimulus is prevented. During the short day conditions (long dark periods) the concentration of P_{fr} is high and the ratio of P_{fr} and P_r is such that the flower stimulus is induced. If the dark period is interrupted by red light Pr is converted P_{fr} and the ratio of these two is such that the formation of flower stimulus is prevented.

Long day plants: In long day plants the dominance of P_{fr} promotes flowering. During long day conditions the concentration of P_{fr} is high and the ratio of P_{fr} to Pr is such that the flower stimulus is induced. During short day conditions, the concentration of P_{fr} to P_r concentration is such that the flower initiation is prevented.

Check Your Progress - 4

What are the two states of phytochrome that plays a vital role in the release of florigen?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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17.6. VERNALISATON

Before flowering many plants require a period of cold treatment. Many cereals require this cold treatment and wheat is a very good example. Some of the varieties of wheat are called spring wheat. These are usually planted in the spring and they flower in the same season.

Some varieties which are known as winter wheat should be planted only in the fall. These plants flower and fruit after winter. The cold temperatures of winter are essential for winter wheat. Without this cold treatment these plants had not flowered or the flowering was reduced. The Russian geneticist T.D. Lysenko has found that artificial cold treatment of winter wheat seeds would permit them to behave like spring wheat when planted in the spring. This process of the conversion of winter varieties to the summer varieties by cold treatment is called vernalisation.

In several plants such as *Secale cereale*,the exposure of seeds to cold treatment will be sufficient. *Hyoscyamus niger* has to be vernalised when the plant is 10 days old. In some plants vernalisation can be effected only after some vegetative growth has taken place.

It has been observed that mainly the stem apex is the region which perceives the effect of vernalisation. There are some exceptions to this. For example Wellensick (1961-62) has succeeded in vernalising the leaves and roots of *Lunaria biennis*. In some plants such as *Hyoscyamus niger* the vernalisation stimulus can be translocated via a graft union.

The mechanism of vernalisation is a complex process and it involves several processes. German physiologist Melchers (1966) postulated that a substance called vernalin is responsible for transmitting the vernalisation stimulus. This is only a hypothetical substance and it has not been isolated till now. Lang of United States has found that the application of gibberellin would replace the vernalisation in many cold requiring plants. However, gibberellin is not vernalin because the response of the plant to gibberellin is different from cold treatment.

Chailakhyan (1968) has explained the mechanism in the long day plants in the following way. He suggested that in these plants under low temperatures vernalin is produced. This, along with another substance called anthesin which is present in the long day plants cause flowering in long days. In short days, the vernalin is not converted to gibberellin and so flowering does not occur. Chailakhyan's suggestion is illustrated in the following figure (Fig. 17.3)

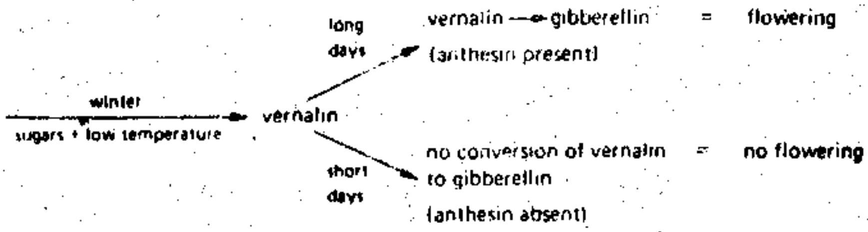

Fig. 17.3. Conversion of vernalin to gibberellin.

However, still there is no firm evidence either for vernalin or anthesin. It is more likely that some harmones and growth substances are involved in flower induction. How they are related to the process of vernalisation is not known.

Check Your Progress - 5 & 6

5. What is meant by vernalisation?

6. According to Chailakhyan what are responsible for the cause of flowering in long day plants?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

17.7. SUMMARY

The plant must complete a period of vegetative growth for the initiation of flower primordia. Light and temperature are the two environmental factors which are responsible for flower initiation. Photoperiodism is defined as the response of a plant to the relative lengths of light and dark periods. Garner and Allard were the first to recognise the effect of light on the floral initiation in a mutant of tobacco called Maryland Mammoth. On the basis of the length of the photoperiod requirements the plants are broadly classified into 3 groups - (a) Long day plants, (b) Short day plants and (c) Day neutral plants. Long day plants require longer than the critical periods of light and short day plants require shorter than the critical period of light. The day neutral plants are not affected by the length of the day. Plants promoted by long days, plants promoted by short days, long-short day plants and short long day plants are the other four groups of plants added to the first 3 major groups. The number of photoperiodic cycles required varies from plant to plant. The number ranges from one to several days. Experimental evidence proved that the site of photoinductive perception is the leaves. The stimulus induced in the leaves moves through phloem to the place of flower initiation. The flower inducing chemical or hormone is named as florigen by Chailakhyan, a Russian Scientist. It is also reported that other hormones (IAA, gibberellins, abscisic acid, cytokinins, ethylene) also participate in the flower initiation. Among them gibberellins have a spectacular role. Phytochrome triggers the initiation of florigen. Phytochrome occurs in two forms – (a) red absorbing form (P660) and (b) far red absorbing form (P735). The dominance of red absorbing form promotes flowering in short day plants and the dominance of farred absorbing form promotes flowering in long day plants.

17.8. CHECK YOUR PROGRESS : MODEL ANSWERS

1. The response of a plant to the relative lengths of light and dark period is called photoperiodism.

   Secale cereale and *Iberis durandii* are the examples for short long day plants.

2. The long day plants required long periods of light whereas the short day plants require short period of light.
3. The chemical substance or hormone that induce flowering is called Florigen.
4. The two states of phytochrome that plays a vital role in the release of florigen are: (1) the red absorbing P660(Pr) and far- red absorbing P_{735} (P_{fr}).
5. The conversion of winter varieties to summer varieties by giving cold treatment is called vernalisation.
6. Vernalin and anthesin are responsible for the flowering in long day plants.

17.9. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Write briefly about photoperiodism.
2. Write an essay on vernalisation.

II. Answer the following questions in 10 lines each.

1. What are long day and short day plants? Explain.
2. Write briefly about the site of photoinductive perception.
3. Write briefly about the role of hormones in flower initiation.
4. Write breifly about the role of phytochrome in flower initiation.
5. How does the artificial cold treatment of winter wheat seeds permit them to behave like spring wheat varieties?

17.10: GLOSSARY

1. Aerobic respiration	:	Respiration in the presence of oxygen.
2. Anaerobic respiration	:	Respiration in the absence of oxygen.
3. Cristae	:	Finger-like projections of the inner membranes into the matrix of mitochondria.
4. Fermentation	:	Chemical change followed by effervescence.
5. Grana	:	Lamellae stacked on top of each other inside the chloroplast.
6. Hydroponics	:	The technique of cultivating plants in water or nutrient solution.
7. Metal ion activators	:	Single inorganic ions such as K^+, Fe^{++}, Zn^{++}, Mn^{++}, etc. which are loosely or firmly bound to the enzymes protein.
8. Nucleolus	:	One to four roughly sphericle or prominent structures inside the nucleus of cells.
9. Prosthetic group	:	The coenzymes which are tightly bound to the enzyme molecules.
10. Red drop	:	The decrease in quantum yield in photosynthesis beyond 680 nm (red area of spectrum).
11. Quantasomes	:	Small spheres present in the thylakoid membranes of chloroplasts. Each quantasome contain about 250 chlorophyll molecules.

17.11. SUGGESTED READING

1. Bidwel, R.G.S. Plant Physiology.
2. Devlin, R.M. Plant Physiology.
3. Malik, C.P. Plant Physiology.
4. Malik, C.P and A.K. Srivatsava.Text book of Plant Physiology.
5. Noggle, G and G.F. Fritz Introductory Plant Physiology

BLOCK - II
GENETICS

UNIT-18: MENDALISM

Contents

18.1. OBJECTIVES

After going through this unit you will be able to:

1. describe the Mendelian laws of inheritance viz., - "Law of Segregation" and "Law of independent assortment",
2. define various terms of the genetical terminology,
3. describe monohybrid and dihybrid crosses,
4. list out the ratios for given number of segregating gene pairs and
5. describe incomplete dominance, codominance and lethal genes.

18.2. INTRODUCTION

The modern science of genetics orginated when Gregor Johan Mendel discovered that hereditary characteristics are determined by elementary units transmitted between generations in a uniform fashion. Until the twentieth century, little was known about the transmitted substances and characters.

Before the 17th century it was believed that life was created spontaneously e.g. maggots (tiny insect eggs) appearing on raw meat. According to Linnaeus (1707-1778), the founder of systematics there was a "fixity of species" so that organisms of one species could only give rise to organisms of the same type. The idea of spontaneous generation was finally put to rest by Pasteur (1822-1895) and by Tyndall (1820-1893), who showed that the new organisms arise only through the continuity of life.

With the discovery of eggs and sperms, the biologists of the seventeenth and the eighteenth centuries started to speculate that the new individuals were completely preformed in miniature form in the gamete. According to them such a miniature preformed embryo was considered to occur either in the egg or sperm. Later, the idea of preformationism was replaced by epigenesis. The epigenetic view proposed that many new factors, such as tissues and organs, appeared during the development of an organism which were not present in its original form. Wolff (1738-1794) opined that these organs arose completely *de novo* through mysterious vital forces.

Charles Darwin (1809-1882) the founder of modern evolutionary theory, put forward a hypothesis called pangenesis. According to this hypothesis, each organ of an individual produce many minute particles called pangenes or gemmules. These are transported by the bloodstream to the sex organs and they assemble into the gametes. This doctrine provides the explanation how heritable changes lead to the origin of a new species. Lamarck (1774-1829), the foremost popularizer and exponent of the theory of inheritance of acquired characters tried to explain the extraordinary ability of these small hereditary agents to respond directly to the environment.

By the early twentieth century, most of the morphological features of the cell had been observed, apart from the general features involved in the cell division processes of mitosis and meiosis. The work by cytologists such as Boveri (1862-1915), Henking (ca. 1891). Montgomery (1873-1912) and others had shown that such cell divisions led to an accurate partitioning and separation of the nuclear chromosomes. The constancy of chromosomes in a species and their precise partitioning in inheritance has become evident from the findings of the Gregor Johann Mendel (1822-1884), who was a monk in the monastery of Brunn, Austria. In his monastery garden he carried out the famous experiments on garden peas *(Pisum sativum)*from 1857 to 1865. The results of his experiments were published in the annual proceedings of the National History Society of Brunn in 1866, but received very little attention and thus his work was over looked for a number of years.

In 1900, the principles of heredity which Mendel had discovered were independently rediscovered by three biologists. Hugo de vries in Holland, Carl Correns in Germany and Erie Von Tschermak-Seysenegg. From that time onwards, the science of heredity or genetics has made rapid progress.

18.3. MENDEL'S EXPERIMENTS

Mendel had chosen the pure lines of garden peas in which seven pairs of contrasting traits were used for his experiments. The characters are diagramatically represented in Fig. 18.1. He crossed the tall and dwarf varieties of garden peas. All the offspring in the first filial generation, F_1 were tall indicating that the character-tallness-is dominant over dwarfness. The tall F_1 hybrid plants when self fertilized, produced 3/4 tall and 1/4 dwarf plants in F_2 generation. Thus the phenotypic ratio becomes 3:1 and the genotypic ratio 1:2:1. When F_1 hybrid forms gametes, each gamete receives only one of the factors of an allelomorphic pair. For example, gamete may carry the factor for tallness, or dwarfness but not both. From the observations Mendel proposed the "law of segregation". Thus the law of segregation states that "when a pair of alleles are brought together in the hybrid, the members of allelic-pair remain together without mixing and separate from each other when the hybrid forms gametes".

Similar results were obtained by Mendel for the inheritance of other characters. For example when violet and white flowered varieties were crosses, the resulting hybrid is violet in colour indicating it's dominance. The F_1 hybrids on self-pollination gave rise to a mixed F_2 in an appropriate phenotypic ratio of 3:1 (violet:white).

Mendel's law of segregation is the fundamental principle in heredity and is also termed the 'law of purity of gametes'. Mendel's experimental results calculated on the basis of the law of segregation have been confirmed by many scientists in a variety of plants and animals.

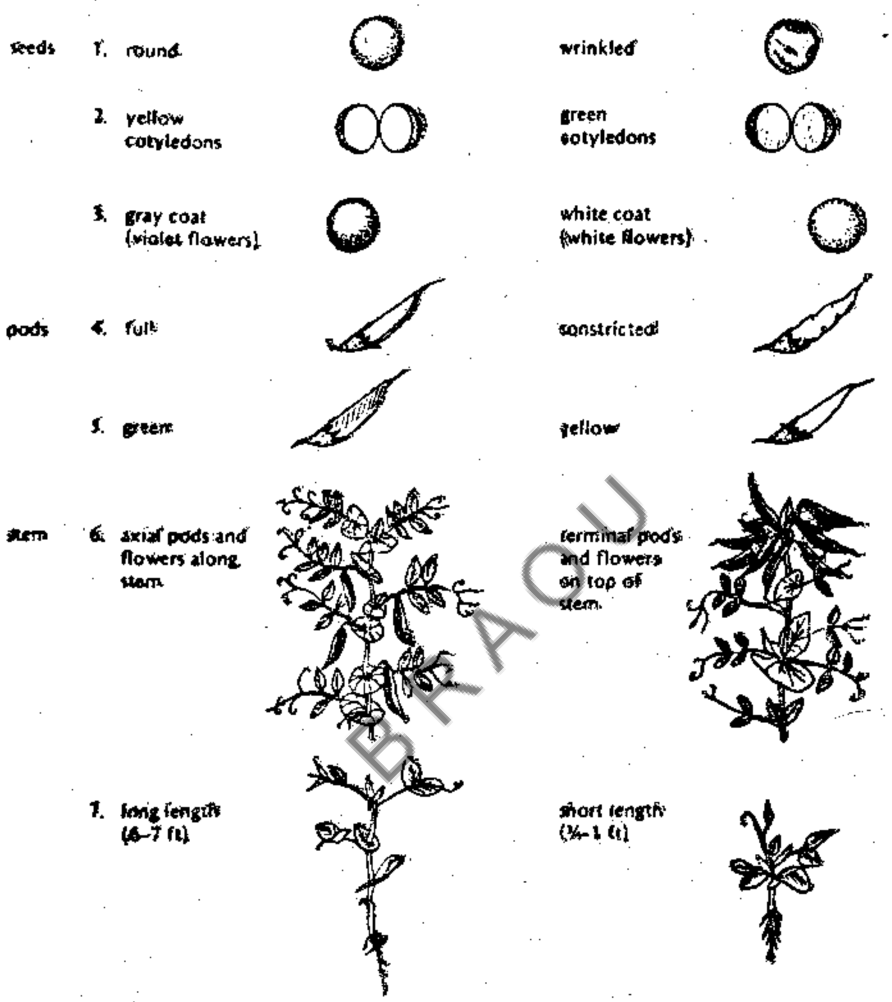

Fig. 18.1 Seven characters in peas that were observed and scored by Mendel in his published experiments.

	Tall	X	Dwarf
Parents	T T		t t
Gametes	T		t
		Tall	
F_1		T t	
	Tall	X	Tall
F_1 X F_1	T t		Tt
Gametes	T , t		T , t

F_2

♂ \ ♀	T	t
T	T T	T t
T	T t	tt

Phenotypes	Genotypes	Genetypic ratio	Phenotypic ratio
Tall	TT	1	3
	Tt	2	
Dwarf	tt	1	1

Fig.18.2. Mendel's cross between tall and dwarf garden peas and summary of phenotypic and genothypic results.

18.4. TERMINOLOGY

The inherited factor that determines a biological characteristic of an organism is called a **gene**. In diploid organisms, genes exist in pairs. The two individual genes in a particular gene **pair** are known as alleles, e.g., a wrinkled plant carries two identical alleles **s** and **s**. Though the terms genes, and alleles are interchangeable, the allele refers only to the genes of a particular gene pair. The gene for smooth "S is an allele for wrinkled '**s**', it is not an allele of the gene for yellow seed colour '**Y**'. When an organism contains two identical alleles e.g., '**S**' and '**S**', the organism is called homozygous for that character. If the two alleles are different, for example '**S**' and '**s**', it is termed **heterozygous.**

To distinguish the external appearance of an organism from its genetic make up (genome) the terms phenotype and genotype are used. The phenotype refers to all the manifold biological appearances, including chemical, structural and behavioural attributes of an organism. The genotype refers to the content of genetic material that an organism inherits from its parents.

18.5 MONOHYBRID CROSSES

Monohybrid crosses are basic to Mendelian genetics. The monohybrid results from a cross between two genetically unlike individuals (e.g., AA X aa --> Aa). Thus a monohybrid is heterozygous for one pair of alleles. The crosses involving parents that differ for one pair of alleles and are called monohybrid crosses and those will always result in 3:1 phenotypic ratio. Mendel crossed the tall and dwarf varieties of garden peas to study the single gene diffeences in monohybrid cross. (Fig 18.2 This led to the discovery of the principle of segregation.

Mendel did not stop his experiments but went on to consider the products of individual differences in two pairs of genes or dihybrid crosses.

18.6. DIHYBRID CROSSES

Mendel used for this purpose a plant with Smooth and yellow seeds (SS YY) crossed to a plant with wrinkled and green seeds (ss yy). The F_1 plants were grown and self-fertilized, that gave rise to 556 F_2 seeds in the following ratio 315 smooth yellow, 108 smooth green, 101 wrinkled yellow, and 32 green. The above ratio is very close to 9:3:3:1 ratio. To analyse them individually with respect to each gene pair is as follows:

P_1	Smooth X wrinkled	Yellow X green
	↓	↓
F_1	Smooth	Yellow
F_2	423 Smooth : 133 Wrinkled	416 Yellow : 140 green
	3/4 Smooth: 1/4 wrinkled	3/4 Yellow: 1/4 green

Here each gene pair acts independent of the other. That is the chances for a plant to be smooth or wrinkled is independent of its chances to be yellow or green. Mathematically speaking the relationship is expressed by multiplying the individual probabilities of the respective events. For example if a seed has 3/4 chance of being smooth and 3/4 chance of being yellow, its chance to be both smooth and yellow at the same time would be 3/4 x 3/4 = 9/16. Therefore, the respective probabilties of the individual phenotypes are as follows.

3/4 smooth X 3/4 yellow = 9/16 smooth, yellow

3/4 smooth X 1/4 green = 3/16 smooth, green

1/4 wrinkled X 3/4 yellow = 3/16 wrinkled, yellow

1/4 wrinkled x 1/4 green = 1/16 wrinkled, green

So from 9:3:3:1 ratio for two pairs of traits in F_2 generation, it is clear that the characters are independent of one another. Not only members of each pair of alleles segregate but genes themselves behave independently with respect to each other. Mendel therefore drew another conclusion: members of different pairs of alleles assort independently into gametes that form the second principle i.e., law of independent assortment of genes.

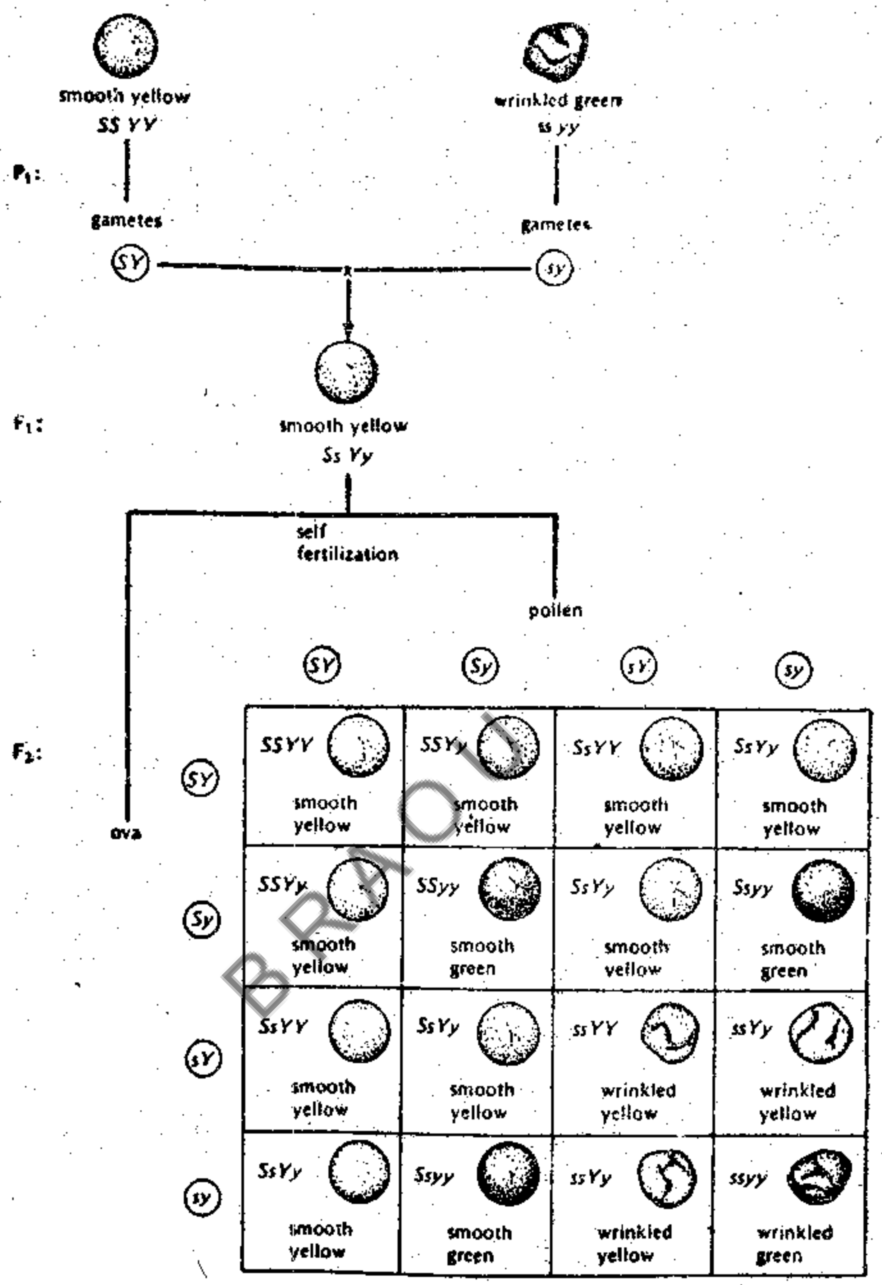

Fig.18.3. Explanation of Mendels results for the segregation and assortment of seed texture and colour.

Check Your Progress - 1 & 2

1. Name the laws of heredity proposed by Mendel.
2. Define monohybrid and dihybrid crosses.

Note: (a) Write the answer in the space is given below.

(b) Compare your answers with the one given at the end.

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18.7. TEST CROSS

The test cross is a useful way of determining homozygosity or heterozygosity of a dominant phenotype and can be equally valuable in determining genotypes in situations with two or more gene pairs. The test cross is a cross of an individual (generally dominant phenotype) with one having a recessive phenotype. In a two pair cross; in which each pair of genes are on a different chromosome pair, the resulting progeny ratio is the product of two one-pair ratios. Thus, a monohybrid test cross ratio is 1:1 and a dihybrid test cross ratio is 1:1:1:1, when one parent is heterozygous for both gene pairs. The test cross is a very useful technique in mapping gene locations on chromosomes.

Testing Dihybrid Genotypes

A test cross is a means of determining the genotypes of the offspring in a dihybrid cross. Mendel made a test cross of the dihybrid (Ss Yy x ss yy) and got the following numbers of offspring.

55 Smooth yellow (Ss Yy)

51 Smooth green (Ss yy)

49 Wrinkled yellow (ss Yy)

52 Wrinkled green (ss yy)

These numbers when reduced to a ratio approximates to 1:1:1:1. From this ratio Mendel could confirm that the dihybrid formed four classes of gametes in equal numbers and this in turn meant that the two pairs of alleles underwent independent assortment in the hybrid.

18.8. RATIOS FOR MORE THAN TWO PAIRS OF GENES

When more than two pairs of genes are involved a cross, the checkerboard system becomes very difficult. So a shorter and simpler method to consider the expected phenotypes and genotypes of a cross can be derived by 'branching method 'when there is no linkage.

As the individual probabilities are associated with each gene pair, the resultant genotypes and phenotypes are due to multiplying their respective probabilities. The same method can be used for more than two gene differences.

Ss Yy X Ss Yy
Genotypes

Ss X Ss   Yy X Yy

1 SS —→1 YY = 1 SS YY
→2 Yy = 2 SS Yy
→1 yy = 1 SS yy

2 Ss —→1 YY = 1 Ss YY
→2 Yy = 2 Ss Yy
→1 yy = 1 Ss yy

1 ss —→1 YY = 1 ss YY
→2 Yy = 2 ss Yy
→1 yy = 1 ss yy

Ss Yy x Ss Yy
Phenotypes

Ss X Ss   Yy X Yy

3 Smooth —→3 yellow =9 Smooth yellow
→1 green = 3 Smooth green

1 Wrinkled—→3 yellow = 3 Wrinkled yellow
→1 green = 1 Wrinkled green

Mating Aa X Aa, Bb X Bb, Cc X Cc

3A —→3B —→3C = 27 ABC
→1c = 9 ABc
→1b —→3C = 9 AbC
→1c = 3 Abc

1a —→3B —→3C = 9 aBC
→1c = 3 aBc
→1b —→3C = 3 abC
→1c = 1 abc

The kinds of gametes, genotypes and phenotypes that are the result of many gene differences are indicated in Table 18.1.

Table 18.1. The effect of segregation and independent assortment in crosses between individuals heterozygous for a given number of gene pairs.

Number of gene pairs in which differences occur	Different kinds of gametes produced by F_1 heterozygotes	Different kinds of genotypes in F_2	Different kinds of phenotypes in F_2
n	2n	3n	2n
1	2	3	2
2	4	9	4
3	8	27	8
4	16	81	16
5	32	243	32
10	1024	59,049	1,024

18.9. COMPLETE DOMINANCE

The common house plant, variegated coleus *(Coleus blumei)*is a good example for a simple genetic experiment. It has shallow and deep lobed leaves. The plants being homozygous lines, when two such homozygous individuals are crossed, the F_1 shows deep lobed offspring. Thus the character that expresses in all the F_1 shows deep lobed offspring. Thus the character that expresses in all the F_1 offspring has termed dominant and the trait that fails to express has been termed recessive and it is an example of complete dominance. The results in this example may be summarized as follows.

Parental generation (p)	Deep x Shallow
First filian generation (F_1)	All deep
Second filial generation(F_2)	3/4 Deep: 1/4 Shállow

18.10. MODIFICATIONS OF 3:1 PHENOTYPIC RATIO

Complete dominance was indicated in all seven allelic pairs which Mendel reported. Dominance has now been shown to be influenced by factors in the external, internal and genetic environments. Therefore, Mendel's view of dominance as a fundamental inheritant property of the gene is no longer valid for all cases. This is also evident from the examples discussed below.

18.10.1 Incomplete Dominance

When the case of complete dominance operates the phenotypic and genotypic ratios are 3:1 and 1:2:1 respectively, but in incomplete dominance the heterozygotes are intermediate between the two parental homozygotes. This is due to the relationship between genes and enzymes at physiological level resulting in 1:2:1 ratio. The ratios of both genotypes and phenotypes are the same i.e., 1:2:1 For example a case of incomplete dominance in snapdragon *(Antirrhinum majus)* is given below.

A true-breeding snapdragon plant with white flowers is crossed with a true-breeding plant with red flowers, the offspring (F_1) produce pink flowers. The pink hybrid is intermediate the red and white parents and F_2 segregates in a ratio of 1 white : 2 pink : 1 red. Examples of incomplete dominance at the macroscopic level occur in both plants and animals, for example the shape of radish.

18.10.2. Codominance

In the phenomenon of codominance, both dominant and recessive alleles lack their dominant and recessive relationships and both have the capability to express themselves phenotypically, in the heterozygous condition. The dominant and recessive traits occur side by side in a heterozyote. The F_1 heterozygote produces F_2 progeny in the phenotypic and genotypic ratios of 1:2:1 like the incomplete dominance. The best example of codominance is found in cattle. The white coat cattle (WW) when crossed with red coat (ww), the F_1 heterozygotes (Ww) are found to have phenotype, reddish gray or roan color.

Several illustrations of codominance are found in human beings. One of these involves a pair of codominant alleles, M and N, responsible for production of antigenic substances M, and N respectively, on the surface of red blood cells.

18.11. LETHAL GENES

A second major modification of the classic monohybrid ratios is produced by lethal genes.

Table 18.2. Lethal Genes.

Organism	Phenotype	Dominance		F_1 Phenotypic ratio of heterozygote X heterozygote		Age at Death
		Phenotype	Lethality	Before lethality occurs	After lethality occurs	
Human	Sickel-ceil	Codom	Recessive	1 normal : 2 sickel cell trait : 1 sickel cell anaemia	1 normal : 2 sickel cell trait	Adolescence
Corn	Albinism	Recessive	Recessive	3 green : 1 albino	All green	10-14 days
Mouse	Yellow	Inc.dom.	Recessive	Unknown	2 yellow : 1 black	Post zygote
Fowl	Creeper	Inc.dom.	Recessive	Unknown	2 Creeper : 1 normal	Early embryo
Human	Huntington's chorea	Dominant	Dominant	3 Chorecic: 1 normal	All normal	Middle age, some what variable

The phenotypic manifestation of some genes result in the death of the individual either in the prenatal or postnatal-prior to maturity; such factors are called lethal genes. Lethal genes may be dominant, partially dominant or recessive. Individuals with a dominant lethal genes die before they can leave progeny. Recessive lethals can cause death of the organisms only when they are homozygous.

In plants, several genes affect the chlorophyll production. In corn *(Zea mays)*, one such gene designated G, for normal chlorophyll production, is completely dominant to its allele g. As a result G-plants contain chlorophyll, whereas gg plants produce no chlorophyll and are thus yellowish white. When the two heterozogous green plants are crossed the following results are obtained.

P green X green

Gg Gg

F_1 1/4 green : 2/4 green : 1/4 non–green

GG Gg gg

It is clear from the above cross that the gene gg has exerted its lethal effect, so the classical phenotypic ratio of 3:1 becomes 3:0, since non-green seedling dies after two weeks.

The other examples of lethal genes are summarized in the table 18.2

18.12. SUMMARY

Various basic concepts have been established by Mendel in the field of Genetics. From his experiments on garden peas, Mendel proposed the following two laws viz., "Law of segregation" or "Law of purity of gamets" and ; Law of independent assortment of genes'. In the genetical terminology the terms phenotype and genotype refer to the appearance or decernible character of an individual and the actual genetic constitution respectively. Alleles or allelomorphs indicate alternative forms of the same gene. An individual organism carrying identical genes of a given allele pair (i.e DD or dd) is termed homozygous and one with unlike genes (i.e Dd) is termed heterozygous. An offspring of two homozygous parents differing from one another by alleles at one locus is called Monohybrid. An individual heterozygous for two pairs of alleles is called dihybrid. Incomplete dominance results when the dominance of the gene over its recessive allelle is not complete. If a heterozygote shows a mixture of phenotypic characters of both homozygotes it is called co-dominance. A gene whose phenotypic effect is sufficiently drastic to kill the bearer is termed lethal gene and it may be dominant, incompletely dominant or recessive.

18.13. CHECK YOUR PROGRESS : MODEL ANSWERS

1. Mendel proposed the following two laws viz: a) "Law of segregation" or "Law of purity of gamets" and b) "Law of independent assortment of genes"
2. The crosses involving parents that differ for one pair of alleles are called monohybrid crosses and those crosses which involve parents that differ for two pairs of alleles are called dihybrid crosses.

18.14. MODEL EXAMINATION QUESTIONS

I. Answer the following in about 30 lines .

1. Write briefly about the Mendelian laws of inheritance

II. Answer the following in about 10 lines each.

1. Write briefly about monohybrid cross.
2. Write briefly about dihybrid cross.
3. What are lethal genes? Name some of them.
4. Give reasons for modifications of 3:1 phenotypic ratio and explain them.

UNIT-19: MODIFIED MENDELIAN RATIOS AND POLYGENES

Contents

19.1 OBJECTIVES

After going through this unit you will be able to:

1. describe the interactions of gene expression,
2. define epistasis and describe various modified Mendelian ratios,
3. distinguish between modifiers, pleiotropism and atavism and
4. describe the nature of polygenes and their inheritance.

19.2 INTRODUCTION

Soon after the rediscovery of Mendel's laws, geneticists began naming each hereditary factor according to the character, i.e., tall, green etc. This helps in identifying genes by symbols other than a letter or number. At this juncture, Bateson and Punnet discovered that the genes are not separate elements producing individual effects, but they could interact to produce novel phenotypes. The occurrence of such interaction means that the phenotype we observe is not present in the genes themselves but arise from a complicated developmental processes. The genotypic ratios are easily predictable when the two gene pairs (e.g.As.Bb) segregate independently which results in a classical Mendelian ratio of 9:3:3:1 in F_2.

19.3. EACH GENE PAIR AFFECTING A DIFFERENT CHARACTER

When two different characters are affected by each of two gene pairs with complete dominance, the phenotypic ratio in F_2 becomes a classical mendelian ratio of 9:3 3:1.

e.g.:- A cross round yellow plants with wrinkled green (See unit-18).

19.4. EACH GENE PAIR AFFECTING THE SAME CHARACTER

When one character is affected by two or more gene pairs, a variety of phenotypic ratios may appear, depending upon the type and degree of interaction between the gene pairs. This is evident from the work on the comb shape in poultry by Bateson and Punnet. For example, the factors for both rose (A-bb) and pea (aaB-) combs are dominant to single combs (aa bb), however a combination of the dominant factors for rose and peas results in a different comb type, walnut (A-B-). As worked out by Bateson and Punnet, two different gene pairs interact to to give walnut combs. When both gene pairs are homozygous recessive, they result in single combs. Thus, four phenotypes are obtained from a cross between heterozygotes for both gene pairs resulting in 9:3:3:1 ratio.

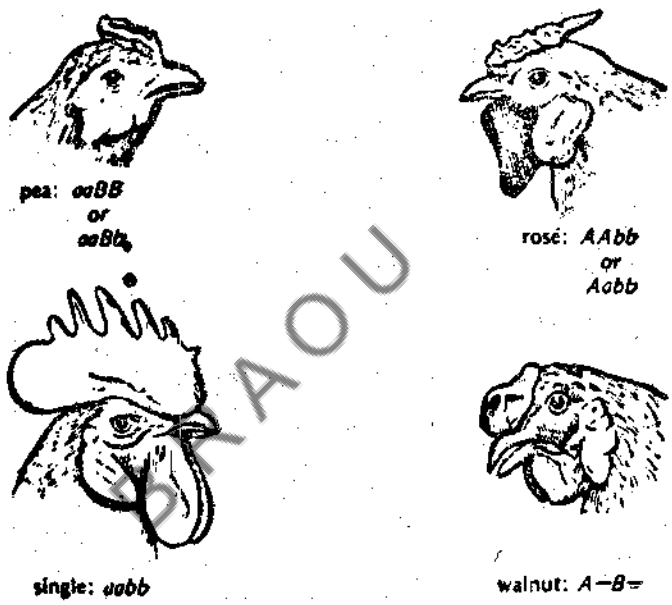

Fig.19.1. The four phenotypes

Epistasis: The formation of new phenotypes is one example of interaction between gene pairs. Other examples may include instances in which a novel phenotype does not appear but one gene pair hides the effect of the other. This latter type of interaction is called epistasis. Epistasis may be caused by the presence of homozygous recessive of one gene pair or by the presence of a dominant allele at a given gene pair.

The epistatic effects may be in only one direction from one particular gene pair to another or in both directions (A hides effect at B or *vice versa)* when each gene pair is mutually epistatic to the other.

The appearance of colour in mouse coat may be agouti (A-B), black (A-b) or albino (aa-B). In developmental terms it is clear that the appearance of colour is due to two processes, the first is controlled by gene A and the other by gene B. When the first process is inhibited by aa the second cannot occur.

$$\text{Original substance} \xrightarrow[\text{1st Process}]{\text{A gene}} \text{Intermediate substance} \xrightarrow[\text{2nd Process}]{\text{B gene}} \text{Colour}$$

Therefore, the animal appears as an albino (when A gene is absent and even though colour genes may be present at the gene pair). The presence of homozygous recessive at the colour gene(i.e., aa in genotypes 11,12,15 and 16) hides the effect of B gene pair. Thus 'a' gene pair may be called epistatic to B gene pair. Complete dominance at both gene pairs - but one gene being homozygous recessive, is epistatic to the other - is called recessive epistasis. Such a gene, which marks the effect of one or both members of different pair of genes is said to be eipstatic. The masked gene may be termed hypostatic.

Six types of gene interactions are described here. The genotypes and phenotypes with F_2 ratios are represented in the following table.

AaBb x Aa Bb

gametes	AB	Ab	aB	ab
AB	AABB (1)	AABb (2)	AaBB (3)	AaBb (4)
Ab	AABb (5)	AAbb (6)	AaBb (7)	Aabb (8)
aB	AaBB. (9)	AaBb (10)	aaBB (11)	aaBb (12)
ab	AaBb (13)	Aabb (14)	aaBb (15)	aabb (16)

For all the examples described here the genotypic and phenotypic ratios can be referred from the table.

Check Your progress - 1

Differentiate between epistatic and hypostatic genes.

Note:(a) Write the answer in the space given below.

(b) Compare your answer with the one given at the end.

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1. Dominant Epistasis (12:3:1)

Here the dominant allele (e.g. A) of one gene mask the activity of alleles of another gene(e.g. B) and expresses itself phenotypically. Then A gene locus is said to be epistatic to the B gene locus. Because, the dominant allele A can express itself only in the presence of either 'B' or 'b' allele, therefore such type of epistasis is called *dominant epistasis.*

For example the fruit colour in summer squash is white, yellow or green. The gene 'A' hides the effect of 'B' gene resulting in the white colour. In the absence of A, 'B' gene expresses a yellow colour and in the absence of B gene, there is a green colour. Here the classical 9:3:3:1 ratio becomes modified into a 12:3:1 ratio.

2. Recessive Epistasis (9:3:4)

If the recessive genotype of one locus (e.g.aa) suppresses the expression of alleles at the B-locus, the 'A' locus is said to exhibit recessive epistasis over the B locus. Only if the dominant allele is present at the A locus, the alleles of the hypostatic B locus can be expressed. Therefore the 9:3:3:1 ratio becomes 9:3:4.

Example:- Coat colour in mice.

3. Duplicate Genes with Cumulative effect (9:6:1)

If the dominant condition (either homozygous or heterozygous) at either locus produces the same phenotype, the F_2 ratio becomes 9:6:1. For example, where the epistatic genes are involved in producing various amounts of a substance, such as pigment, the dominant genotypes of each locus may be considered to produce one unit of pigment independently. Thus genotypes A-bb and aa B- produce one unit of pigment each and therefore have the same phenotype. The genotype aa bb produces no pigment, but in the genotype A-B- the effect is cumulative and two units of pigments are produced.

Example:- Coat colour of Duroc-Jersey pigs.

4. Duplicate Dominant Epistasis (15:1)

Epistatic effects by dominant alleles cannot be confined to one gene pair. If two gene pairs effect a single character, both gene pairs may have epistatic effects on each other, so the phenotypic ratio of 9:3:3:1 becomes 15:1.

In the plant, shepherd's purse (*Capsella Bursa-pastoris*) two gene pairs affect the shape of the seed capsule. The alleles causing a triangular shape are dominant to the alleles causing an ovoid appearance. Thus a cross of heterozygotes for a triangular (Aa Bb) capsule produces 15 triangular and 1 ovoid capsules in F_2. Thus the dominant allele A and B hides the effect of ovoid (aa bb)

5. Duplicate Recessive Epistasis (9:7)

If both gene loci have homozygous recessive alleles and both of them produce identical phenotypes; the F_2 ratio 9:3:3:1 would become 9:7. In such a case, the genotypes aa BB, AA bb, Aa bb, and aa bb produce one phenotype. Both dominant alleles, when present together (AABB, AaBB, AABb, AaBb) complement each other and are called complementary genes which produce a different phenotype.

Example: Flower colour in sweet pea *(Lathyrus odoratus)*

6. Dominant and Recessive Epistasis (13:3)

Only two F_2 phenotypes result when a dominant genotype at one locus (eg A) and recessive genotype at the other (bb) produce the same phenotypic effect. Thus A-B-, A-bb and aa bb produce one phenotype and aa B- produce another in the ratio of 13:3.

Example: Feather colour in poultry.

Check Your Progress - 2.

Name different types of gene interactions.

Note: a) Write the answer in the space is given below.

b) Compare your answer with the one given at the end.

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19.5. MODIFYING GENES OR MODIFIERS.

Epistasis is not only one gene hiding the effect of another gene in particular but it is due to the increased or decreased enzyme activity that results in the appearance of colour or other complex events. Bearing this in mind a large group of genes can be considered as modifiers. Broadly, they can be defined as the genes that change the phenotypic effect of other genes in quantitative fashion. Certain breeds of cattle have spotted coats(skin) while others have coats of solid colour (black or white). These variations are due to the effect of a single pair of alleles acting as modifier genes. The gene for spotting is recessive (s), while the gene for solid colour is dominant (S). Simple mendelian inheritance determines the presence of S or s in F_2 progeny. The degree of spotting is variable continuously and follows the multiple gene principle.

19.6. PLEIOTROPISM

The multiple phenotypic effects of a single gene is called 'pleiotropism' and is useful to understand the relation between different developmental processes. Many genes seem to have pleiotropic effects. For example, a single mutation in a plant may be involved in the expression of a leaf shape, flower and fruit maturation etc. Single amino acid substitution of valine for glutamic acid in haemoglobin causes marked pleitropic effects, leading to impaired mental function, heart failure, rheumatism, abdominal pain etc.

Atavism: Through epistatic effects and other gene interactions traits may remain hidden for generations. Occasionally a 'throw back' occurs in a strain of domestic animals or plants. That is an ancestral trait is expressed unexpectedly. The reappearance of traits after several generations was discussed scientifically by Charles Darwin and was called 'atavism'. For example the progeny of 'wild type' in appearance occur in the rock pigeon *(Columbia livia)* when a chance combination of genes removes this effect of epistatic genes, that have suppressed the expression of certain ancestral traits in the show birds. Rock pigeon, is a European pigeon from which most domestic pigeons have been developed.

19.7. POLYGENIC (MULTIPLE GENES) INHERITANCE

The early genetic laws were based on clear differences in the appearances of phenotypes. e.g. tall, or short, green or yellow seeds etc. The quantitative differences were also noted in many characters. These quantitative variations were difficult to analyse genetically, since their phenotypic range appeared to be continuous. Early geneticists such as Bateson and De Vries therefore proposed that continuous characters were not genetically produced and were therefore not inherited; only large discontinuous variations were caused by genes.

Soon after the rediscovery of Mendel's experiments two groups of thought arose 1. Mendelians, who proposed that all evolutionary, important, heritable differences are qualitative and discontinuous.2. Biometricians, who proposed that the heritable variation is quantitative and continuous. The controversy between Mendelian and biometricians was resolved by Danish geneticist Johannsen, who identified numerous sizes in the princess bean ranging from light to heavy weighing seeds. He had grown 19 pure lines with varying sizes from light to heavy seed weights. Each line showed average seed weight but individually distinct, e.g. line 1 was the heaviest with an average weight of 64 centigrams but individuals varied from 50.2-64.9 centigrams. Line II was the lighter with a mean weight of 34 centigrams. From the experiment of Johannsen, it was confirmed that the continuous variation observed for various characters (quantitative) can be the result of both genotype and environment. So certain characters that exhibit quantitative variation are governed by multiple or polygenes.

The genetical studies of qualitative and quantitative traits are called qualitative genetics and quantitative genetics, respectively. The major differences between them are presented in the following table.

Qualitative genetics	Quantitat e genetics
1. It deals with the inheritance of traits of kind viz. form or structure.	1. It deals with the inheritance of traits of deggree viz. height or length.
2. It displays discontinuous variation	2. A spectrum of phenotypic classes occur which contain continuous variation.
3. Each trait is governed by two or many alleles of a single gene.	3. Here each trait is governed by polygenes.
4. The phenotypic expression is not in-influenced by environment	4. Environment effects the phenotypic expression
5. It concerns with individual matings and their progeny.	5. It concerns a population of organism consisting all possible kinds of matings.

19.7.1. Quantitative Inheritance - Seed Colour in Wheat

Nilsson-Ehle (1909) studied the inheritance of colour of wheat grain. The variety with a dark-red kernel when crossed with a white kernel showed some plants with intermediate shade of red in F_1 generation. The F_2 plants showed colours ranging from deep red to white. About 1/16 F_2 plants were white, 14/16 were intermediate between red and white parents. Thus the continuous gradations were recognized between the extremes of the parents as shown below.

Red		White
AA BB	x	aa bb
AB		ab

F_1 (Medium) Aa Bb F_1 Selfing)

F_2	AB	Ab	aB	ab
AB	AA BB Red	AA Bb Dark	Aa BB Dark	Aa Bb Medium
Ab	AA Bb Dark	AA bb Medium	Aa Bb Medium	Aa bb light
aB	Aa BB Dark	Aa Bb Medium	aa BB Medium	aa Bb light
ab	Aa Bb Medium	Aa bb light	aa Bb light	aa bb white

On the basis of colour intensity it was shown that four had more colour than F_1, six were intermediate like F_1 and four were lighter than F_1. Thus, this suggests that the segregation of two gene pairs controls a polygenic trait. Therefore, the F_2 phenotypic ratio becomes 1:4:6:4:1. The concept of multiple genes for quantitative inheritance is one of the most important principles of genetics. It is strengthened by the use of statistical methods derived by R.A. Fisher, Sewal Wright and others. Thus the inheritance of ploygenic traits depends on the cumulative or additive action of many genes. Thus a polygenic trait can be measured, and be subjected to statistical treatment.

A classical study of quantitative inheritance to establish multiple gene hypothesis was made by R.A. Emerson and E.M. East. A maize variety with ears averaging 6.6 cm. in length was crossed with a variety having ears of 10.8 cm. The F_1 progeny were intermediate in earlength, averaging. 12.1 cm. The F_2 represented a wide spread of variation between the two. Histogram in the Figure 19.2 represents graphically the characteristic pattern of multiple gene inheritance.

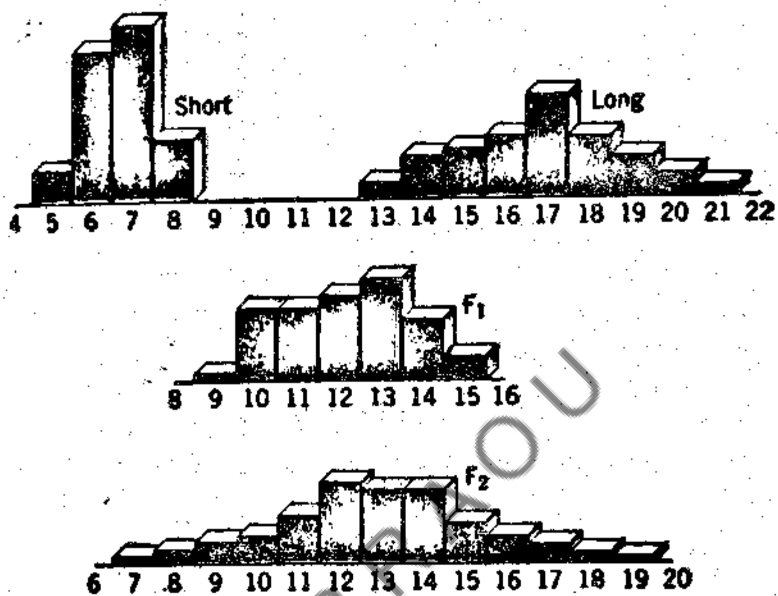

Fig. 19.2 . Graphic representation of the characteristic pattern of multiple gene inheritance.

The quantitative characters may be due to more than two pairs of independent genes. A rough estimate of genes determining the quantitative character can be made by using the formule $(1/4)^n$ this represents the fraction of the progeny resembling the parents,where 'n' represents the pairs of gene controlling the character.

Number of gene loci	1	2	3	n
fraction of F_2 as extreme as one parent	1/4	1/16	1/64	$(1/4)^n$

19.8. SUMMARY

Genes do not always behave as separate units exhibiting individual effects; but they do interact among themselves resulting in modified classical Mendilian ratio of 9:3:3:1 of F_2. When more than one pair of genes are involved in character expression one gene that hides the effect of the other is called epistatic. The masked gene is said to be hypostatic. The Six types of epistatic gene interactions are: i)dominant epistatis(12:3:1) ii) recessive epistasis (9:3:4), iii) duplicate genes with cumulative effect (9:6:1) iv) duplicate dominant epistasis (15:1), v) duplicate rekecessive epistasis (9:7) and vi) dominant and recessive epistasis (13:3).

The group of genes that change the phenotypic effect of other genes in a quantitative fashion are called modifiers, whereas genes that show manifold effects are terms as pleiotropic genes.

Polygenes or multiple genes show continuous quantitative variations in inheritance. The intensity of the trait depends upon the environment and the joint action of the several allelic pairs of genes with cumulative effects.

19.9. CHECK YOUR PROGRESS: MODEL ANSWERS

1. A gene which masks the effect of one or both members of a different pair of genes is said to be epistatic. The masked gene may be termed hypostatic.
2. The different types of gene interactions are - Dominant epistasis, Recessive epistasis, Duplicate genes with cumulative effect, Duplicate dominant epistasis, Duplicate recessive epistasis, Dominant and recessive epistasis.

19.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Write briefly about the modified Mendelian ratios.
2. What are multiple genes or polygenes? Write briefly about polygenic inheritance.

II. Answer the following questions in 15 lines each.

1. Write about the dominant and recessive epistasis.
2. Write briefly about modifying genes or modifiers.
3. Explain briefly about pleiotropism.

UNIT-20 : LINKAGE AND CROSSING OVER

Contents

20.1. OBJECTIVES

After going through this unit you will be able to:

1. understand the phenomenon of linkage and crossing over,
2. analyse the relation between crossing over and chiasma formation,
3. know about construction of linkage maps,
4. describe and differentiate the terms interference and coincidence and
5. describe the phenomenon of sex linkage.

20.2. INTRODUCTION

Mendel's law of independent assortment is applicable only when genes are located on separate chromosomes. He couldn't have succeeded in framing the laws, had the genes been linked together. The phenomenon of linkage was developed by the post- mendelian geneticists. The chromosomes are the carriers of genes that are inherited together, was established by Sutton between 1902 and 1912.

T.H. Morgan (1866-1945) and his associates supported the theory through experiments and established the theory of linkage. Linkage was first defined as the tendency for non-allelic genes in the same chromosome, to enter the gametes together in higher proportion than in an independent assortment.

Later it became established that the number of linkage groups in species of animals and plants correspond to number of pairs of chromosomes. For example 7 linkage groups in peas (2n=14). 4 in *Drosophila* (2n=8), 23 in man (2n=46) etc.

20.3. LINKAGE

In 1906, Betcson and Punnet analyzed the results of a cross between two varieties of sweet peas-purple flower with long pollen grains and red flowered with round pollen grains. The F_1 hybrid is purple and long. In F_2 progeny, Bateson observed purple, long; red, round occurred more frequently, whereas pur ple, round; and red long; appeared less frequently. In another cross, when sweet peas with purple flowers and round pollen, were crossed with those having red flowers and long pollen the paren tal combinations were in higher numbers. Thus, Bateson and Punnet developed a theory called 'coupling' and 'repulsion'. The condition in which the dominants enter the gametes in greater than dandom proportion is called 'coupling' and the tendency for one dominant and one recessive gene to enter the gametes is called 'repulsion'. The genes ab/++ are said to be in a coupling configuration and the genes a+/+b are in a repulsion phase. The purple, long X red, round cross represents 'coupling' whereas the purple, round X red, long cross illustrates 'repulsion'.

Thus linkage can be defines as "a mechanism by which a group of genes on the particular chromosome, show a tendency to remain linked together, without assorting independently or each other during gametic formation". Due to linkage, a group of parental genes are inherited together into the offspring.

Thus the genotypes of the parental and flist filial generation of the Bateson and Punnet cross may be written in the following fashion.

Parents : Purple, long X Red, round

$$\frac{R\ Ro}{R\ Ro} \times \frac{r\ ro}{r\ ro}$$

F_1 $\quad \frac{R\ Ro}{r\ ro}$ $\quad$ Purple, long

Test cross : Purple, long X Red, round

$$\frac{R\ Ro}{r\ ro} \times \frac{r\ ro}{r\ ro}$$

Test Cross Progeny :	Purple, long	192	Non-cross overs
:	Red, round	182	
:	Purple, round	23	Cross overs
	Red, long	30	

The test cross ratio indicate that there is a tendency for the dominant alleles (R RO) to pass together into the same gamete instead of resulting in four phenotypes in 1:1:1:1 ratio. This tendency of dominant alleles to inherit together was explained as gametic coupling by Beteson and Punnet.

The linkage may be of geno types in an individual heterozygous for two precise of gene which are linked. The arrangement of two dominant genes as one member of the chromosome pair; with the two recessive genes on the other is referred as Cis arrangement. The second type having dominant of the first gene pair and the receiver of the other on one chromosome of the pair, with the recessive of the first gene pair and the dominant of the second gene. On the other chromosome is called 'trans' arrangement. (Fig.20.1)

Fig.20.1. Cis and trans arrangements for two pairs of linked genes.

Thus the gametes purple, round (R ro), Red, Long (r Ro) are crossover gametes and termed recombinants.

Check Your Progress - 1.

Define linkage.

Note: a) Write your answer in the space given below.

b) Compare your answer with the one given at the end of this unit.

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Kinds of Linkage: The linkage is of two kinds - complete linkage and incomplete linkage.

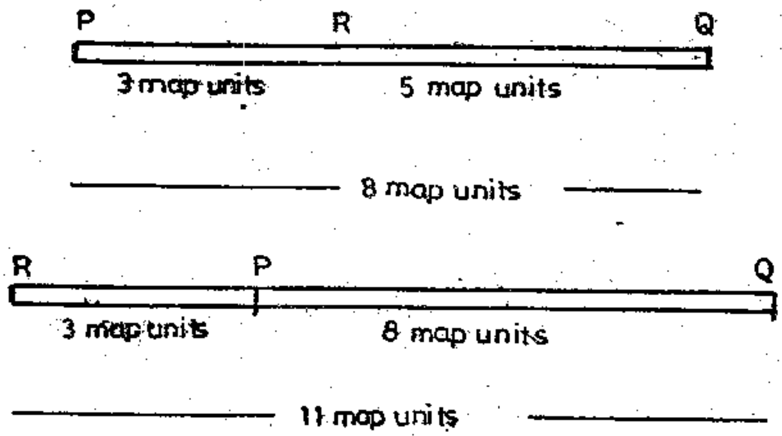
1. Complete Linkage: When the linked genes are so closely located on the chromosome, they inherit in the same linkage groups for two or more generations in a regular fusion and they are called completely linked genes and the phenomenon is called 'complete linkage'. For example, the genes for bent wings (bt) and Shaven bristles (Svr) on the fourth chromosome in *Drosophila* exhibit complete linkage.

2. Incomplete Linkage: The linked genes don't always stay together as non-sister chromatids may exchange segments of varying length with one another by the process of crossing over. The linked genes which are widely located in chromosomes and have chances of separation by crossing over are called incompletely linked genes and the phenomenon is called 'incomplete linkage'. For example, in pea, *Zea mays*,tomato, female *Drosophila* etc.

20.4. CROSSING OVER

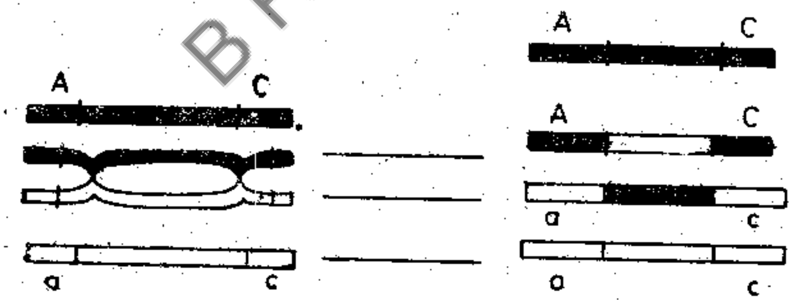
The linkage is broken down due to the phenomenon called crossing over, occuring during meiosis. Crossing over can be defined as a mechanism by which a group of genes on one chromosome exchange their loci with a similar group of genes on the homologous chromosomes during cell division. This involves crossing of two non-sister chromatids and is called chiasma formation. The chromatids in which crossing over has occured have new combinations of genes and are called cross overs. A crossover involves only two of the four chromatids of pair of homologous chromosomes. Crossing over and independent assortment are the important mechanisms for the generation of new combinations of genes.Crossing over will be relatively more frequent, if distance between two genes is more than in a case where the distance between two genes is lesser. The maximum frequency of recombination between linked genes is 50%.

If one assumes that the probability of a cross over between two loci, is directly proportional to the distance between the loci, probability of cross over = K (distance).



For example when loci P and Q are linked with a map units of 3 between P and R, 5 map units between R and Q and the total distance between P and Q is 8 map units.

But there will be systematic deviation from additivity as the distance become large, when double cross overs occur at significant frequencies. from the following diagram it is evident that though cross over occur, they will not be recorded as double cross overs.



Check Your Progress - 2

Frequency of crossing over will be relatively more if distance between two genes is_____________ than in a case where the distance between two genes is _____________.

Note: a) Compare your answer with the one given at the end.

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20.5. RELATION BETWEEN CROSSING OVER AND CHIASMA FORMATION

To explain the relationship between crossing over and chiasmata, two main theories have been proposed.

In the classical interpretation the chiasmata are produced due to accidentl physical crossing of homologous chromatids that will produce separation of chromosome segments. Thus the chiasmata precede the genetic crossing over that occurs between diplotene and first anaphase. Thus chiasmata are not the result of the crossing over.

The chiasma type theory was presented by Janssens and developed by Belling and Darlington. This theory proposed that crossing over occurs during the eary meiotic stages (pachtene).

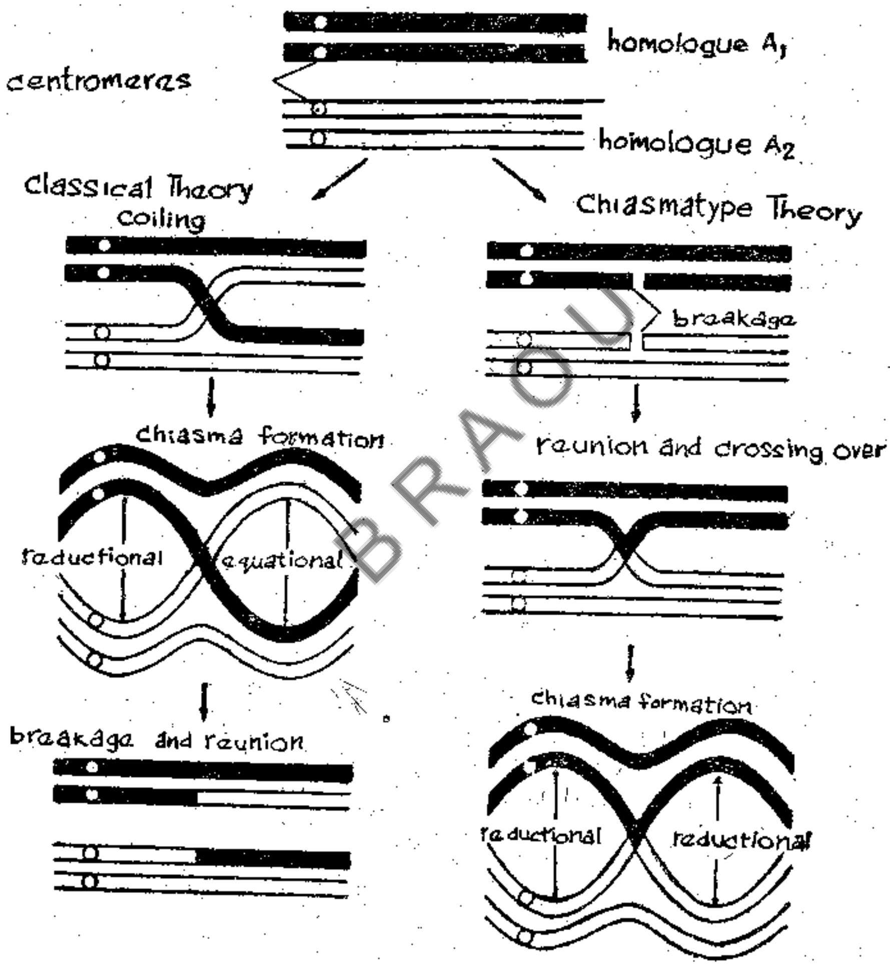


Fig.20.2 Differences between the classical theory of chiasma formation and the chiasma type theory in explaining the origin of observed chiasmata in pairing between two homologous chromosomes.

20.6. CROSSING OVER AND LINKAGE MAPS

Morgan stated that the tendency of linked genes remaining together in original combination is due to their location in same chromosome. The degree or strength of linkage depends upon the distance between the linked genes in the chromosome. Morgan's concept about the linkage developed the theory of linear arrange ment of genes, in the chromosomes which helped the cytogeneticists in the construction of genetic or linkage maps of chromosomes.

Geneticists use map units to describe distance between linked genes. A map unit is equal to one per cent of cross overs. They are also called Morgan units. One Morgan unit represents 100 per cent crossing over.

20.6.1 The Three-point Cross

The most commonly used method in genetic mapping is the trihybrid test cross. The gene symbols and phenotypic characters are given below.

Gene Symbol	Phenotype
+	Normal Wing (dominant)
Cu	Curled wing (recessive)
+	Normal thorax (dominant)
Sr	Striped thorax (recessive)
+	Normal bristles (dominant)
Ss	Spineless bristles (recessive)

The following example in drosophila is given to determine the correct gene sequence.

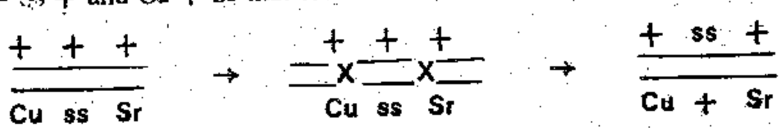
Test cross: Normal, Normal, Normal X curled, Spine less, Striped

♀ +++/Cu Ss Sr ♂ Cu Ss Sr/Cu Ss Sr

F_2 Phenotype	Maternal Chromosome			Type
Normal,Normal,Normal	+ + +	430	88.2%	Non-Cross overs
Curled, Spineless, striped	Cu Ss Sr	452		
Normal, Spineless, striped	+ Ss Sr	45	8.3%	Cu-ss
Curled, normal, normal	Cu + +	38		
Normal,Normal, Striped	+ + Sr	16	3.3%	Single cross overs Ss-Sr
Curled, Spineless, Normal	Cu Ss +	17		
Normal, Spineless, Normal	+ Ss +	1	0.2%	Double cross overs
Curled, Normal, Spineless	Cu + Ss	1		
		1000	100.00	

Some of the important considerations are given below.

1. The first two classes are phenotypically like the parents. They are therefore, non-cross over type.
2. Since non-cross overs are more frequent than cross over cells, +++/Cu Ss Sr and Cu Ss Sr/Cu Ss Sr files occur in majority numbers.
3. The double cross over individuals result from two cross overs that are resultant between first and third genes on the maternal chromosome.
4. The double cross overs are smallest progeny groups that are used to determine gene sequence. These are + Ss + and Cu + Ss that can be derived in this manner.



5. The true distance between Cu and ss is therefore, 8.3 + 0.2 =8.5 (single cross overs + double cross overs). The true distance between Ss and Sr is 3.3 + 0.2 = 3.5 (single cross overs + double cross overs). The distance between Cu and Sr is 8.3 + 0.2 +3.3 +0.2 = 12.0.

With the cross outlined above, the genetic mapping of the *Drosophila* chromosome can be made as follows.

Cu	Ss	Sr
o	8.5	12.0

20.7 INTERFERENCE AND COINCIDENCE

According to the law of probability the frequency of double cross overs between genes should be equal to the cross over frequency at region I and regional II. Actually this is not observed. The following frequencies were obtained.

Region	Genes	Frequency of chromosomes	Percentage of cross overs	Map distance
I	Cu Ss	0.083	8.3	8.3+0.2= 8.5
II	ss Sr	0.033	3.3	3.3+0.2= 3.5
Double cross overs	Cu Ss Sr	0.002	0.2	

The cross overs in region I and II being independent, 0.3 per cent double cross overs would be predicated, but only 0.2 per cent were observed. Thus difference of the kind, where the actual double cross overs observed are less than the calculated one is very common or crossing overs at one region interferes with the crossing over of neighbouring region, suggesting the phenomenon called 'interference'. Interference appears to be unequal in different parts of a chromosome, and among the several chromosomes of a given genotype. In general interference appears greatest near the centromere or at the ends of the chromosome. The degree of interference and coefficient of coincidence is expressed as

$$\text{Coefficient of coincidence} = \frac{\text{observed frequency of double cross overs}}{\text{expected frequency of double cross overs}}$$

$$\text{c.c. in the above example} = \frac{0.002}{0.003} = 0.67$$

As interference increases, coincidence decreases. Coincidence values very between 0 and 1. Absence of interference gives a coincidence of one and complete interference results in a coincidence of zero. Interference value between 0 and 1 indicate the positive interference. In this example interference is 1 – 0.67=0.33.

20.8 CYTOLOGICAL BASIS OF CROSSING OVER

Genetic evidence for linkage became well established before the cytological basis was demonstrated. Genetic crossing over is due to exchange of parts between homologous chromosomes. The cyto logical demonstration was finally shown in 1931 from the experi ments of Stern in Drosophila and H.B. Creighton and Mc Clintock in maize.

Stern could find some marker chromosomes in his cultures. A part of Y chromosome was broken off, and was attached to X chromosome. He found another distinct feature i.e. a part of the X chromosome had been broken off by X-ray treatment that became attached to the small chromosome IV. Females with red bar-shaped eyes were mated with the males having carnation (flesh) colored normal shaped eyes. The genetic and cytological results were recorded. The material from flies that suggested crossing over through the appearance of phenotypes also showed microscopic evidence of exchanges between homologous chromosomes. The physical or cytological basios of crossing over was thus established, as shown in Fig.20.3. Here the two cross over products produced by the female are one long X chromosome and a short X fragment with attached Y section. When combined with the normal long X chromosome of the male the cross over females are thus cytologically distinct from the non cross overs.

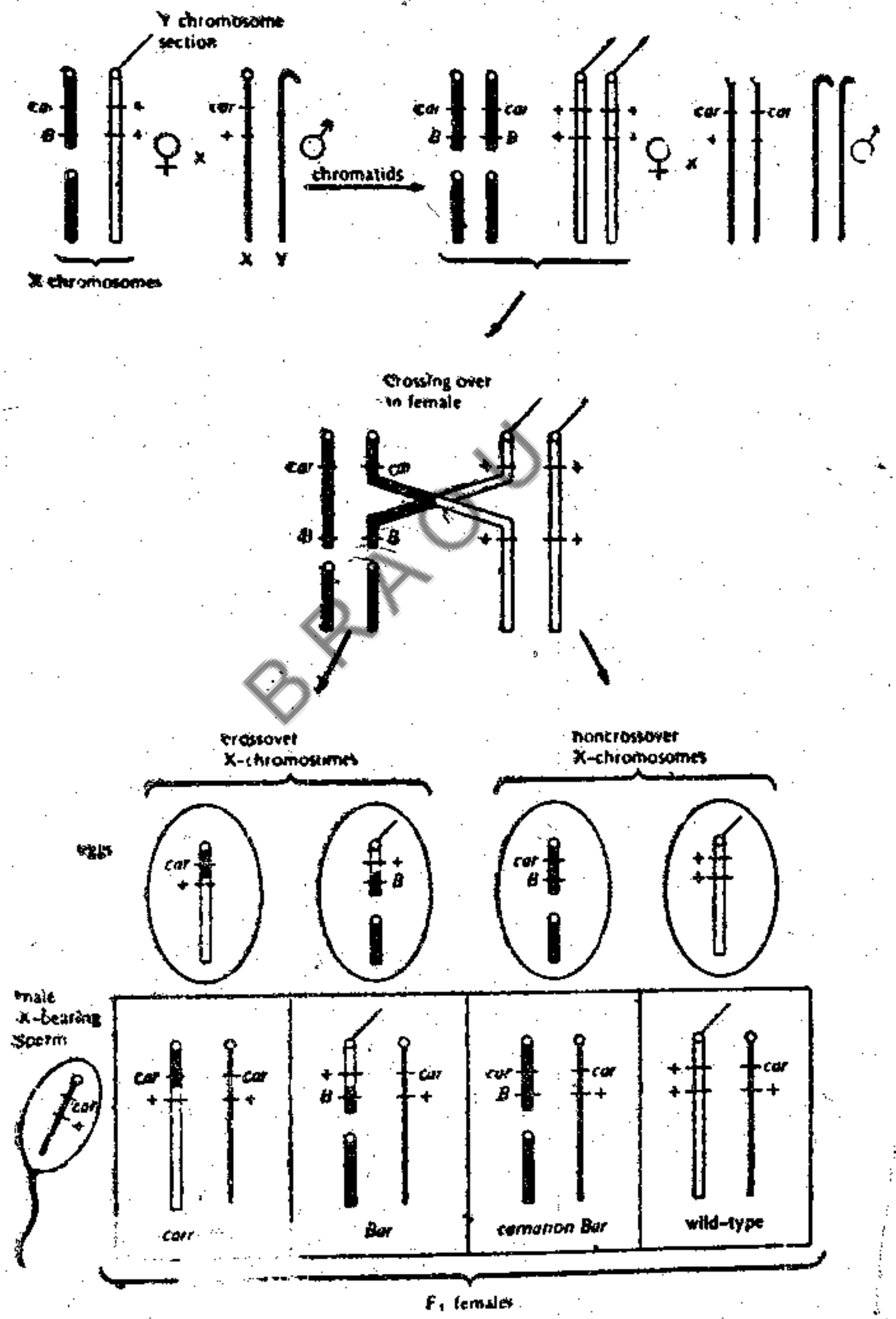


Fig.20.3 Expe... ...wing the correlation between genetic and cytological crossing over ... ...n two sex linked loci in *Drosophila melanogaster*.

20.9. FACTORS AFFECTING CROSSING OVER

Many extrinsic and intrinsic factors have been found to affect the rate of crossing over. They are sex, age, temperature, water content, ionizing radiation, certain chemicals, heterochromatic regions etc.

Gower (1933) have shown that in *Drosophila* mutation suppressed crossing over completely. Bridges (1927) demonstrated that with the increased age of *Drosophila* crossing over was depressed. Plough (1917) has shown that at temperature between 17°C and 29°C, the crossing over was constant, but below or above that temperature range, the rate of crossing over increased. According to Sybenga (1922) low water content reduces the chiasma frequency. Ionizing radiation increases the rate of mittic and meiotic crossing over in *Drosophila*.

20.10. SIGNIFICANCE OF CROSSING OVER

1. The frequency of crossing over is of great use in constructing genetic maps of the chromosomes.
2. It produces direct evidence for linear arrangement of linked genes on chromosomes.
3. It increases the frequency of genetical variation which are the raw materials for organic evolution.

20.11. SEX LINKAGE

When parallelism was discovered between X chromosome cycle and sex determination, it was assumed that genes other than sex determiners were also located on the X chromosome. The genes which are located exclusively on X chromosome are called X linked or sex linked genes. The first experimental evidence for sex linkage came with the discovery of white eye mutant in *Drosophila* by T.H.Morgan (1910). The white eyed males when mated with red eyed females, the F_1 flies were found to be red eyed but F_2 included red and white in the proportion of 3 red : 1 white, but all white flies were males. One half of the F_2 males have white eyes and another half had red, but all females had red eyes.

20.11.1. Sex Linkage in Humans

Early Greek philosophers noticed that some human traits tend to skip a generation. A characteristic that was observed in a father but not in any of his children, would reappear in males of the next generation. This distinctive criss-cross pattern from father through daughter to grandson is now interpreted as evidence of sex linkage.

Sex linkage has been indicated for more than 200 traits in humans. Examples are haemophilia, color blindness, optic atrophy(degeneration of the optic nerve), juvenile glaucoma (hardening of the eye ball), myopia, defective iris, juvenile muscular dystrophy etc. The explanation given for sex linked inheritance in *Drosophila* applies to traits in humans that are associated with genes on the X-chromosome.

The criteria for identifying sex linked recessive traits from pedigree studies is as follows. 1. The trait occurs more frequently in males than in females. 2. The traits are transmitted from an affected man through his daughters to half his grandson. 3. An X-linked allele is never transmitted directly from father to son. 4. All effected females have an affected father and a carrier mother.

Check Your Progress - 3

Define sex linked genes. Name any five sex linked traits in humans.

Note: a) Write your answer in the space given below.

b) Compare your answer with the one given at the end .

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20.11.2. Y chromosome Linked Genes or Holandric Genes

They occur normally on the Y chromosome. The gene that determines excessive hair development on the ears(hypertrichosis) is holandric.

Histocompatibility antigen genes are located on Y chromosome in human, the mouse, rat, guinea pig etc. The genes controlling spermatogenesis are on the long arm of Y chromosome.

20.12. SUMMARY

Genes that are located on the same chromosome don't assort independently during meiosis. Instead, they tend to segregate together. Such genes are said to be linked. The degree or strength of linkage depends upon the distance between the linked genes in the chromosome. The arrangement of a wild form of a gene in a heterozygote is called a coupling or Cis-configuration and the wild type gene is called the repulsion or trans-configuration. The linkage is broken down due to the phenomenon of crossing over occurring during meiosis. The process of crossing over produces new combinations(recombination) of genes.It involves two of the four chromatids of the pairs of homologous chromosomes. The chromatids in which crossing over has occurred have new combinations of genes and are called cross overs.

The location of genes on a chromosome can be mapped by three factor crosses. A map unit can be defined as the linkage distance that yields one percent recombination. The phenomenon of interference is inversely proportional to coefficient of coincidence and varies between 0-1. Genes other than sex determiners which are located on sex chromosomes are said to be sex linked or X-linked. The genes which are located on Y-chromosome are called 'holandric' genes.

20.13. CHECK YOUR PROGRESS : MODEL ANSWERS

1. Linkage is a machanism by which a group of genes on the particular chromosome show a tendency to remain linked together, without assorting independently of each other during gametic formation.

2. More, less.

3. The genes which are other than sex determines and are located exclusively on 'X' chromosome are called 'X' linked or sex linked genes. Examples of sex linked human traits are: haemophilia, color blindness, optic atrophhy, myopia, defective iris.

20.14. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. Explain the cencept of linkage of genes with suitable examples.
2. Write about crossing over and chiasma formation.
3. Write about linkage maps with suitable examples.

II. Answer the following questions in about 10 lines each.

1. What are sex linked genes? Explain with examples.
2. Explain the terms interference and coincidence.
3. Write briefly about crossing over and its significance.

UNIT-21: CYTOPLASMIC INHERITANCE

Contents

21.1. OBJECTIVES

After going through this unit you will be able to:

1. understand that some factors in the cytoplasm of the egg cell are responsible for inheritance of few characters both in animals and plants,
2. define and distinguish between true cytoplasmic inheritance, maternal effects, cytoplasmic inheritance influenced by nuclear genes and daver modifications.

21.2. INTRODUCTION

The transmission of characters from parents to offspring is due to genes, present in the nucleus, situated on the chromosomes.As we have alredy seen this transmission is according to the Mendelian laws of inheritance. These genes are called nuclear genes and the inheritance is known as nuclear inheritance or chromosomal inheritance.

Eventhough this kind of inheritance is normal in all living organisms, there is another kind of inheritance which is controlled by the genes present in cytoplasmic organelles like chloroplasts and mitchondria. As these are situated outside the nucleus in the cytoplasm, this is called cytoplasmic inheritance. This is also called extra-nuclear inheritance. This kind of inheritance does not follow Mendel's laws, so this is also called non-Mendelian inheritance.

21.3. CYTOPLASMIC INHERITANCE

In the case of Mendalian inheritance the result of a cross between two organisms A and B will be the same whether A is male or female parent and B female or male parent. That is to say the results of normal cross and reciprocal cross do not show any variation. But this is not the case with cytoplasmic inheritance. In this the result of reciprocal cross will be different from that of normal cross. It means the results of a cross between A (female) and B (male) will be different from a cross between A (male) and B (female). In one cross A is the female parent and in the other B is the female parent. The fact that the results of reciprocal cross are different from normal cross, is an evidence for cytoplasmic inheritance.

Of the two gametes, the egg and the sperm, in most of the cases, the cytoplasm of the zygote comes only from the egg cell but not from the sperm. There are reports that sperms also possess some amount of cytoplasm e.g. *Epilobium, Oenothera, Nepata* But the role of this in cytoplasmic inheritance is negligible. So the cytoplasm of the egg cell is the source of cytoplasmic inheritance. Besides, sometimes the early growth and development of the embryo is so much influenced by the surrounding maternal tissue that the progeny resembles very much the female parent sometimes. These are called meternal effects and are under the control of maternal genes but not under the control of growing embryo. Hence these are called maternal effects.

Factors controlling cytoplasmic inheritance are located inside cell organelles like chloroplasts and mitochondria. These organelles have their own DNA and RNA which are differnt from nuclear DNA. These undergo mutations. These cytoplasmic factors controlling cytoplasmic inheritance are collectively called plasmon. In contrast to nuclear genes these are called plasmagenes.

Cytolasmic inheritance is usually studied under 4 heads. They are (1) true cytoplasmic inheritance (2) maternal effects (3) cytoplasmic inheritance influenced by genes and (4) Daver modifications.

Check Your Progress - 1

What is meant by cytoplasmic inheritance?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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21.3.1. True Cytoplasmic Inheritance

Plastid colour in *Mirabilis jalapa:* Plastids are cytoplasmic organelles of plant cells. Their number per cell may vary from one to many. The plastids never arise *de novo* in any cell. They arise from preexisting particles in the cytoplasm, which are very microscopic and are known as proplastids. The plastids are self replicating organelles. When the cells divide, these plastids also get distributed into the two cells more or less equally. Plastids with chlorophyll are called chloroplastids or chloroplasts and these are green in colour. Some plastids are colorless and are called leucoplastids or leucoplasts.

Correns and his coworkers in 1908 studied the inheritance of plastid colour in *Mirabilis jalapa.* The plant has three different kinds of leaves-green, albino and variegated. Green leaves possess chloroplasts, albino leaves have leucoplasts. Variegated leaves have both chloroplasts and leucoplasts and hence show green and white patches. In the same plants there may be three different kinds of branches-green, albino and varigated. Sometimes entire plants may be green or albino or variegated. However albino seeedling cannot survive, as there is no photosynthetic activity in such plants and so such plants do not reach maturity. In plants with all the three kinds of branches at maturity albino branches get the food supply from the rest of the plant body.

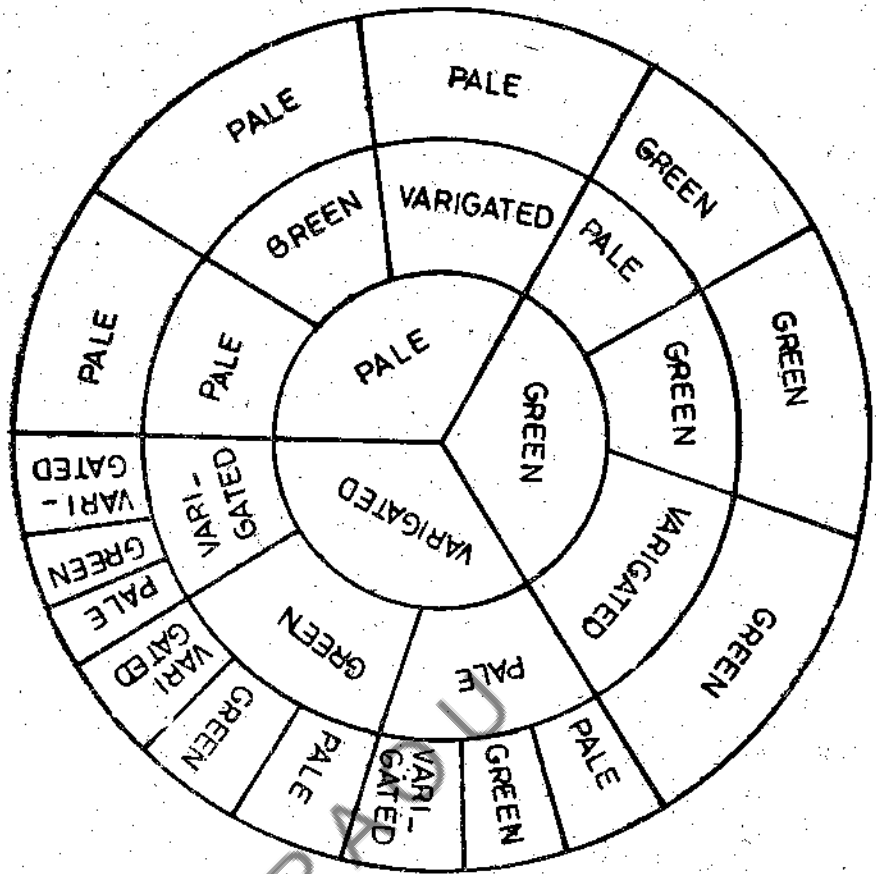


Fig.21.1 Maternal inheritance of plastid colour in *Mirabilis jalapa*. The central circle represents the type of branch that produces flowers which are pollinated. The intermediate circle shows branch from which pollen is used and the outer circle represents the type of *progeny*.

Correns and his coworkers have found that the inheritance of plastids colour does not follow Mendel's laws but it always follows the character of the female parent. Thus when the flowers on a green branch were pollinated with pollen from the three kinds of branches, all the seeds give rise to green seedlings. Likewise, when the flowers on a albino branch were pollinated with pollen from the three kinds of branches, the result was all were albino seedlings only. The flowers on the variegated branches when pollinated with pollen from all the three kinds of branches, gave rise to the three different seedlings-green, albino and varigated (Fig.21.1). Thus the progeny always resembled the branch on which the seeds are borne i.e. the female parent. Thus the inheritance of plastid colour in *Mirabilis jalapa* is strictly maternal, the sperm has very little cytoplasm and no proplastids at all. The egg cell, on the other hand, contains cytoplasm and the proplastids. The egg cells on the green branches contain proplastids for chloroplasts, on the albino branches proplastids for leucoplasts and on the variegated branches, some egg cells contain proplastids for chloroplasts and some proplastids for leuoplasts and still others both types of proplastids. This is due to random distribution of proplastids during the formation of the egg cells. At the same time, it should be understood clearly that formation of chlorophyll or its absence, is however controlled by nuclear gene only and cytoplasm has no role in it at all.

Proplastids are known to contain DNA, the hereditary matetrial. This was established by scientists like Ris, Chum, Edelman. When such DNA undergoes mutations, changes are seen in the plastid colour. These mutations are not under the control of nuclear genes.

Thus the inheritance of plastid colour in *Mirabilis jalapa* is controlled by DNA present in the plastids which are situated in the cytoplasm outside the nucleus.

21.3.2. Maternal Effects

Maternal effects can be understood as changes brought about in organisms by maternal genes or by the tissues surrounding the developing embryo. These effects may slowly fade away as the organism grows or as in some cases, may survive the life time of an adult. We will try to understand two cases where such effects were observed and studied.

Eye color in the flower moth: E.Caspari in 1936 studied the maternal effects in the flower moth *Ephestia kulniella*. In this moth the colour of the eyes is dark brown and the larva have pigmented parts. These two characters are due to a pigmented precursor called kynurenine which is produced by a dominant gene A. A homozygous recessive (aa) moth has no kyhnenine, as a result, the larvae have no pigment on their bodies and the eye colour of the moth is red.

In a cross between a homozygous rcessive (aa) as a female parent and heterozygous (Aa) as a male parent, the results were as follows:

larvae	1 Pigmented	:	1 non pigmented.
Adult	1 dark brown eyes	:	1 red eyes.
Genotypically	1 Aa	:	1 aa

A reciprocal cross was made with heterozygous (Aa) moth as female parent and homozygous recessive (aa) as male parent. Surprisingly, all the larvae were pigmented as though Kynurenine was present in all larvae. But when these larvae developed into adults only half of them had dark brown eyes and the other half had red eyes. Genotypically, they were as in the normal cross Aa 1: aa 1.

Body pigmentation in larvae can only be due to the presence of pigment precursor kynurenine. We have already seen that homozygous recessive (aa) do not produce kynurenine. How is it then in the reciprocal cross larvae with genotype aa they also had pigmented bodies? This can be explained like this. All the eggs produced by dark brown eyed heterozygous (Aa) female parent receive the pigment precursor, kynurenine, whether they received A or a. This kynurenine is located in the cytoplasm of the egg cell. All the eggs receive gene a. As a result of the fertilisation, two kinds of zygotes, Aa, aa are formed. All these, whether Aa or aa, have kynurenine in the cytoplasm. So all the larvae, irrespective of the genotype Aa, or aa have pigmented parts, to begin with. Those larvae with Aa, continue to have kynurenine and so develop into adults with dark brown eyes. On the other hand, in the larvae with aa, as kynurenine is no more produced, they slowly lose pigmentation, and adults will have only red eyes.

So even though the larvae do not have the dominant gene A, still they have pigmented bodies because of the fact that the mother is heterozygous (Aa), and its effect is seen in the cytoplasm of the eggs having kynurenine. So, for sometime, its effect is seen in the larvae and it slowly fades away.

1st cross

♀ a a × ♂ Aa

red eyes | dark brown eyes

nonpigmented larvae | pigmented larvae

½ Aa
larvae coloured.
Adults dark brown eyes

½ aa
larvae not coloured
Adults red eyes

Reciprocal cross

♀ A a × ♂ aa

All larvae coloured.
(Both Aa and aa).

½ adults dark
brown eyes.

½ adults
Red eyes

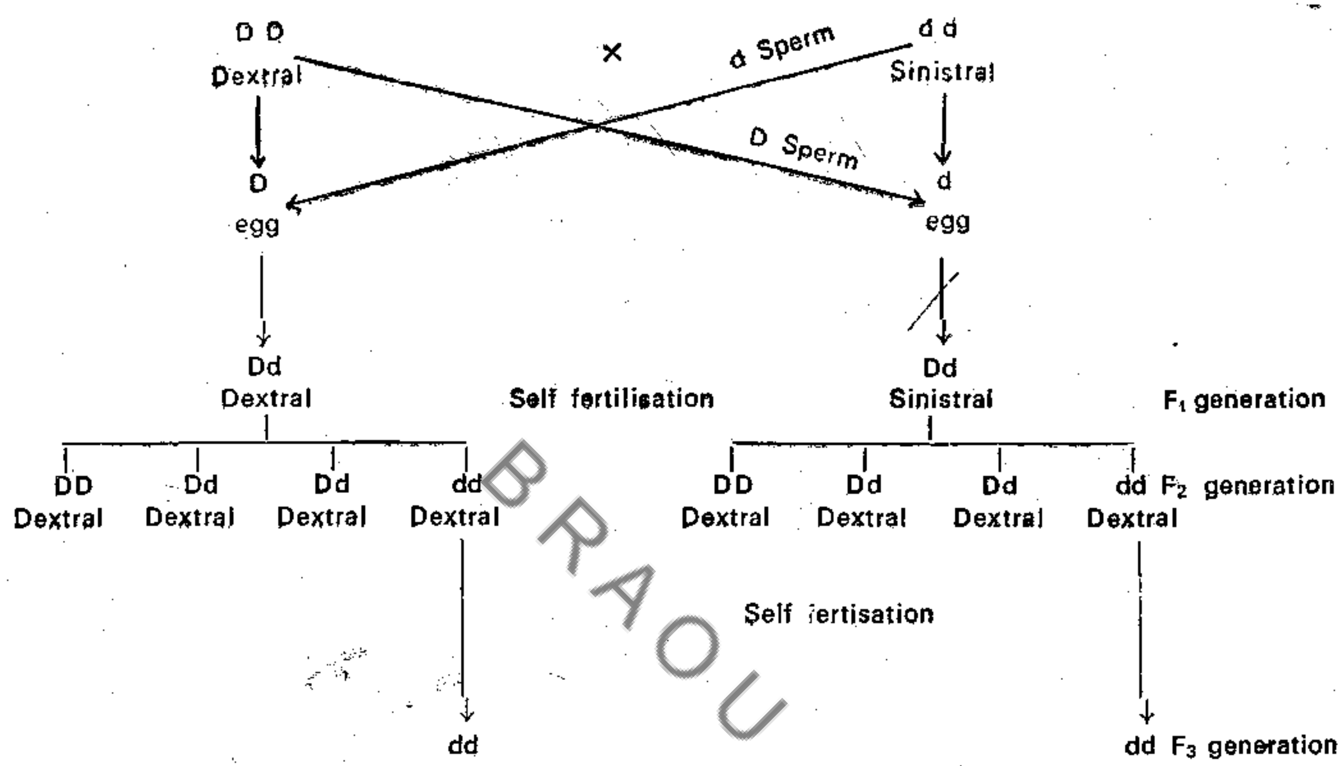
Coiling in snails: We will now study another case of maternal effects, where the effects persist throughout the life of the individual.

A.H.Strutevant (1923) studied the inheritance of coiling of a snail species called *Limnaea peregra*. The shells of these snails show two different types of coiling-one type coil to the right called dextral, another coil to the left called, sinistral. This character of coiling is controlled by a pair of alleles, Dextral (D) being dominant, over sinistral (d) which is recessive.

However the coiling of any snail is determined by the genotype of the maternal parent but not by the genotype of the concerned individual.

These snails are hermaphrodite. Fertilisation can be brought about by conjugation between two individual snails or through self-fertilisation. In a cross between dextral (DD) male and sinistral (dd) female, all the progeny showed sinistral coiling eventhough their genotype is Dd. When these heterozygous sinistral (Dd) snails of F_2 generation show dextral coiling even though 1/4 among them had genotype dd, because the genotype of female parent is Dd. However when these snails with genotype dd get self fertilised in the F_3 generation, sinistral coiling occurs.

Thus it will be seen that in F_1 generation eventhough the genotype is Dd, it showed sinistral coiling as its female parent is sinistral with dd. Thus again in F_2 generation, snails with genotype dd showed dextral coiling as the female is sinistral with Dd. So the genotype of the female parent determines the type of coiling of the snail, but not its own genotype. Hopwever, such a maternal effect is seen in one generation only. For instance, snails with genotype dd in F_2 generation eventhough show dextral coiling, their progeny in F_3 all show sinistral coiling and all of them have the genotype dd.



Inheritance of dextral (DD) and sinitral (dd) Coiling in *Limnaea peregra* showing maternal effect.

3. Cytoplasmic Inheritance Influenced by Nuclear Genes

Recent investigations have revealed that certain characters which are cytoplasmically inherited are influenced by nuclear genes. The nuclear genes are plasmagenes together control the inheritance of such characters. We will consider three examples to understand this type of inheritance. They are 1. Iojap trait in maize 2. Kappa particles in *Paramecium* 3. Cytoplasmic male sterility.

Iojap trait in maize: in maize there is a character in which the leaves show alternate green and white stripes. This trait is called japonica. This is controlled by a gene present on chromosome No.8. This is a recessive trait and so is seen in homozygous recessive (jj) condition only. Normal green leaves may be JJ or Jj. This shows normal Mendelian inheritance.

In the state of Iowa in U.S.A a trait similar to japonica was observed in which the leaves showed long green and white stripes alternatingly, and was by a gene located in chromosome no.7. This trait is called Iojap (Io stands for Iowa state where this was first observed and jap stands for japonica which it resembles). This also is seen in homozygous recessive (ij ij) state. The homozygous dominant Ij Ij or heterozygous Ij ij are green in colour. In contrast to japonica trait, iojap is transmitted to the progeny through the female parent. For instance, in a cross between green maize (Ij Ij) as the female parent and Iojap striped (Ij Ij) as the male parent, the progeny contained 3 different kinds of plants-green, colourless and green and white striped and all of them are genotypically Ij ij. So this character is seen in the progeny only when the female parent is ij ij or ij Ij. In such plants, the cytoplasm of the egg, is now known to contain two different kinds of plastids 1. normal chloroplasts and 2. abnormal plastids. If the egg has only normal chloroplasts, then green plants are produced. If only abnormal plastids are present then colourless plastids result. If the egg contains both normal chloroplasts and the abnormal plastids, then plants with Iojap stripes are produced. There is some relationship between the gene or chromosome no.7 and plastids in the cytoplasm of the egg cell. The plastids undergo mutation and so abnormal plastids are produced.

1st Cross

Ij Ij   x   ij ij
Green   Striped
↓
Ij all green

Reciprocal cross

ij ij   X   Ij Ij
Striped   Green

↓	↓	↓
Ij	Ij ij	Ij ij
Green	colourless	stripped

Kappaparticles in *paramecium*: Sonneborn in 1943 studied the inheritance of two alternative strains in *Paramecium aurelia*. They are Killer and sensitive. The Killer strain is so called because when these two are present in the same petridish with culture medium, the Killer strain produces a toxic substance,which is released into the medium, as a result of which those of the sensitive strain get killed. This toxic substance is called paramecin.

Paramecium reproduces through binary fission, as a result of which, clones with a similar genotype are produced. Besides, *Paramecium* also reproduces sexually through conjugation. During conjugation, a cytoplasmic bridge is formed between the two paramecia. The diploid micronucleus of the *Paramecium* undergoes meiosis and two haploid micronuclei are produced. These haploid micronuclei are exchanged through the cytoplsmic bridge. Thus, two genetically different micronuclei fuse and individual paramecia separate as exconjugants. As a result of nuclear exchange and fusion, both the exconjugants are genetically identical. Under normal conditions, little cytoplasm is exchanged during conjugation. Thus the exconjugants maintain their original cytoplasm but contain similar nuclei. Thus when the Killer and the Sensitive strains are involved in conjugation, after exchange of nuclei the Killer and the sensitive strains separate into Killer and sensitive strains once again. Through fission the exconjugant Killer produces a clone of Killer Paramecia and the sensitive exconjugant produces sensitive paramecia. It should be noted here that eventhough nuclear exchange has taken place between the Killer and the sensitive strains, and the Mendelian genes get segregated during successive generations after self fertilisation-the two strains retained their identity. From this it is clear that the Killer and sensitive characters are not under the control of nuclear genes. However when the conjugation is prolonged, there is a massive transfer of cytoplasm in addition to micronuclei and in such cases, the Killer character is transferred to the sensitive strain. Therefore all the progeny of the Killer-sensitive prolonged conjugation are Killers(Fig.21.2). Surprisingly during conjugation, the sensitive strain becomes resistant to the toxic substance paramecin. Here is one more proof for cytoplasmic inheritance. The extrachromosomal particle responsible for the Killer character has been called Kappa by Sonneborn.

These Kappa particles present in the cytoplasm are dependent upon at least one nuclear gene for their multiplication and existence. The size of these particles varies from 0.2-0.8 microns. In each *Paramecium* there may be 400 - 1600 Kappa particles. Sonneborn carried out experiments on conjugation between different strains in *Paramecium* and established the following facts regarding the inheritance of the Killer character and the Kappa particles.

1. Kappa particles are transmitted to the progeny through the cytoplasm.
2. In the Killer strain, besides Kappa particles in the cytoplasm, there is a dominant nuclear gene k.
3. When the *Paramecium* is homozygous, dominant KK or heterozygous Kk and Kappa are present in cytoplasm, then only paramecin is produced.
4. The sensitive strain is homozygous recessive kk with no Kappa particles in the cytoplasm.
5. The dominant nuclear gene k cannot form Kappa particle in the cytoplasm but even if one particle enters a *Paramecium* with K gene, then K helps in the multiplication of the Kappa particle.
6. When the Kappa particles are present in a sensitive strain KK, as the dominant gene is not present such particles degenerate.

The relation between genotype, plasmagenotype and phenotype in *Paramecium* is given below.

Genotype	Plasmagenotype	Phenotype
K K or K k	Kappa present	Killer
K K or K k	No Kappa	sensitive
K K	No Kappa	sensitive
K k	Kappa Present	unstable (Kappa particles disappear)

It is now established that these particles have DNA and RNA also.

From this we now see that the Killer strain is able to produce **Paramecin** due to the presence of Kappa particles in the cytoplasm. Eventhough these particles are independent of nuclear genes, their multinlicaton is controlled by the dominant nuclear gene K. Thus the Killer character, eventhough transmitted through the cytoplasm, is also influenced by the nuclear gene.

Normal conjugation (no cytoplasmic exchange) — Prolonged conjugation (with cytoplasmic exchange)

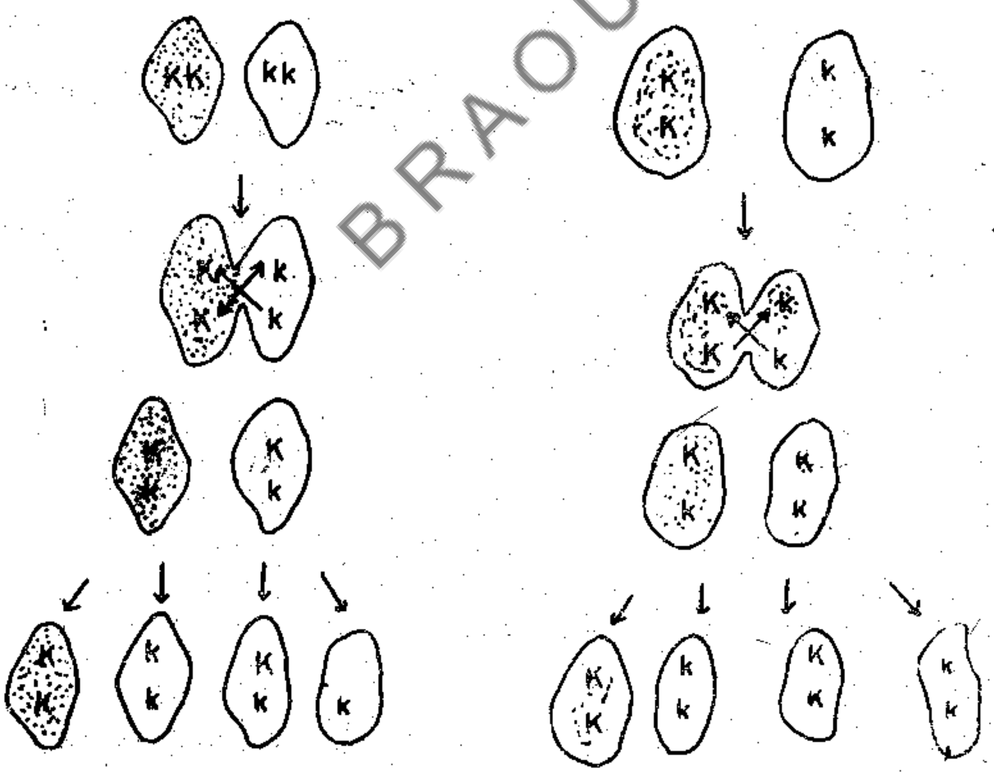


Fig.21,2. Inheritance of Kappa particle in *Paramecium*

Cytoplasmic male sterility: Male sterility, seen in some plants, is a condition in which the anther get shrivelled up and do not produce pollen grains, or if they do produce it, such pollen do not germinate. Generally, male sterility is controlled by nuclear genes. In maize there are over 20 varities which show male sterility and in each case a different gene is said to control their character.

In 1933 Rhoades discovered and studied a kind of male sterility which is inherited through the cytoplasm. This male sterility is transmitted to the progeny through the female parent. A maize plant with male sterility is pollinated by pollen from a normal plant. All the progeny happened to be male sterile plants. He selected one among the progeny as the female parent and repeatedly back crossed it with a normal plant of parental generation. Even after repeated back-crossing several times, male sterility continued to appear in the plants. This conclusively proves that the nucleus has no control over male sterility in this case and that the cytoplasm of the egg alone controls male sterility. Rarely male sterile plants produce fertile pollen grains in very small quantities. Reciprocal crosses using these rare pollen with normal maize plants where the male sterility is not present, produced normal plants with no male sterility (Maize is monoecious with separate male and female inflorescences). This is an additional proof that male dsterility is transmitted through the cytoplasm of the egg cell. But which organelles in the cytoplasm control male sterility is not clearly lunderstood. Besides in maize, nuclear genes are known which in a dominant condition suppress the cytoplasmically inherited male sterility. Thus the expression of male sterily depends upon the interaction between the chromosomal gene and the cytoplasmic factor.This kind of male sterility is also known in beet root, onion and wheat.

21.3.4. Daver Modifications

There is still another type of modifications which is transmitted through the cytoplasm of the egg i.e., through the female parent. These changes are induced by chemical agents or temperature in the plants and animals. But such changes are seen only for some generations and then disappear. For example in *Phaseolus vulgaris*,Hoffman found that morphological abnormalities in the leaf shape induced by chloral hydrate persisted for six generations in the female line and and then disappeared. Such modificatons are called Daver modifications.

Check Your Progress - 2.

What are Daver modifications ?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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21.4. SUMMARY

Cytoplasmic factors called Plasmon control cytoplasmic inheritance. These factors are located inside cell organelles like chloroplasts and mitochondria. In cytoplasmic inheritance, it is the cytoplasm of the egg that plays a crucial role. Therefore there will be a difference in the results between the normal cross and the reciprocal cross. Maternal effects are changes brought about by maternal genes in the growing embrygo and these may disappear after some time in the life of the individual or may be seen for one generation and disappear in the next. Some times cytoplasmic inheritance is influenced by nuclear genes. e.g: Miaze Iojap trait etc, Some times chemicals and temperature also bring about changes in the plant body which are transmitted through the female line. They disappear after a few generations. They are called Daver Modifications.

21.5. CHECK YOUR PROGRESS: MODEL ANSWERS

1. Inheritance controlled by the genes present in cytoplasmic organelles like chloroplasts and mitochondria which are situated outside the nucleus, in the cytoplasm, is called cytoplasmic inheritance.
2. Daver modifications are changes which are transmitted through the cytoplasm of the egg i.e through the female parent. These are induced in plants and animals by chemical agents or temperature. But such changes are seen only for some generations and then disappear.

21.6. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. What is meant by true cytoplasmic inheritance ? Exaplain this with suitable illustration.
2. Exaplain the inheritance of Killer character in *Paramecium*

II. Anser the following in 10 lines each.

1. What is meant by maternal effects ?
2. How is the character of shell coiling inherited in *Limnaea peregra*

UNIT-22: GENE STRUCTURE AND FUNCTION

Contents

22.1. OBJECTIVES

At the end of this unit you will be able to:

1. describe the structure and function of gene,
2. list out various mechanisms of exchange of genetic material.

22.2. INTRODUCTION

It was established beyond doubt by 1940, that the genes were carried on the chromosome. The primary genetic substance of the gene is the DNA, the carrier of genetic information. Genes which control heredity, are specific genetic factors arranged in a linear manner on the chromosome. Each gene can affect the character of a cell in a highly specific way. The hereditary characters are inherited by the offspring from the parents during meiosis (reduction division).

22.3. NUCLEUS

The nucleus in the cell was first described by Robert Brown in 1881 which is the largest of the cell organelles. The nucleus is present in all cells; except in few cases. In mature sieve tubes of higher plants and mammalion erythrocytes nucleus is present in early stages only and absent later on. In the bacteria and blue-green alggae, membrane bound well defined nucleus is absent, hence are designated as prokaryotic cells. However, localised region of DNA known as nucleiods are present.

The nucleus has a nuclear membrane enclosed in the nuclear sap containing chromatin which stain with basic dyes (aceto-carmine) and by Feulgen's technique indicating that it is made up of DNA. The chromatin comprise of a heavily stained region designated as heterochromatin and lighter region euchromatin. One or more spherical bodies known as nucleoli are present in the nucleus. During cell division, the nuclear membrane and nucleoli disappear with chromatin being organised into chromosome.

The nucleus is composed of nucleic acids(more of DNA and less of RNA) and proteins, small amount of lipids and other compounds. The composition of the nucleus vary with different nuclei. Protein forms the largest part of nucleus.

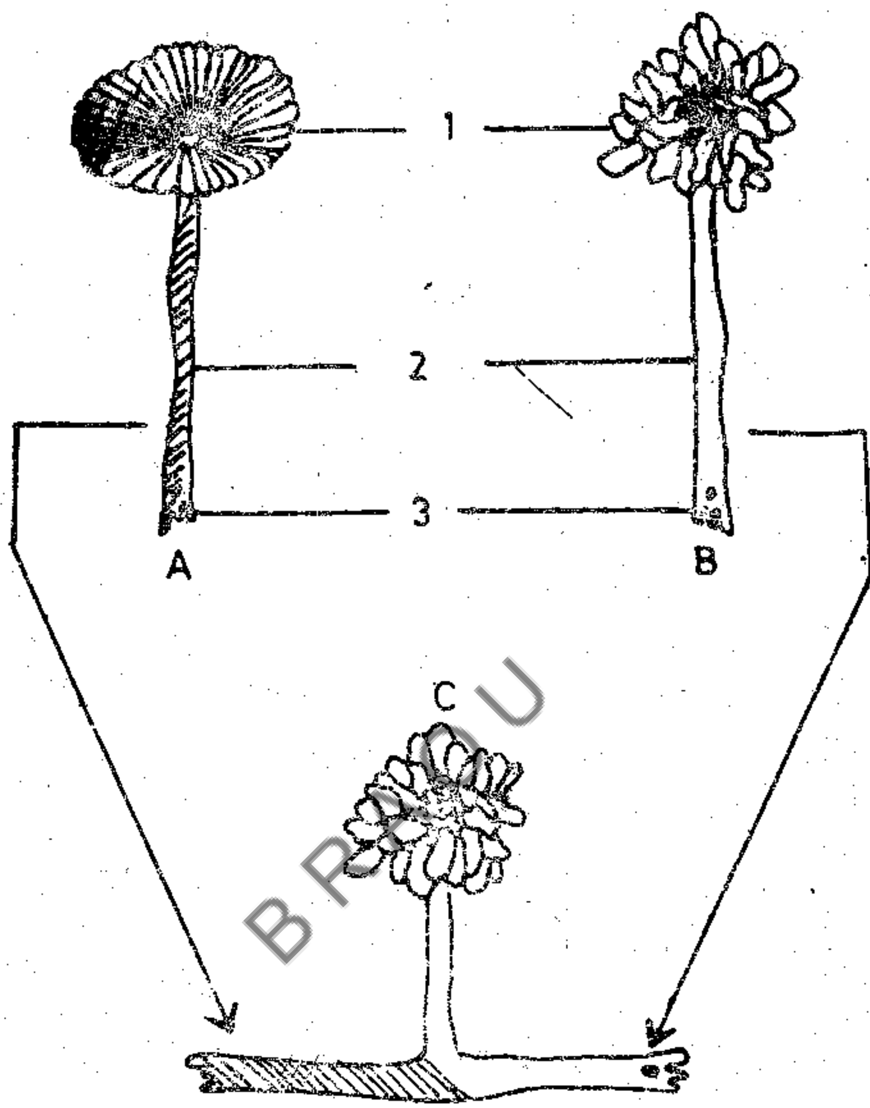


Fig.22.1 *Acetabularia*. The upper part shows the essential differences between the caps of the two species most commonly used in experiments. The lower part shows the result of fusing two decapited plants. The cap formed is intermediate between the two species. A. *A. mediterranea*. B. *A. crenulata*. 1. Cap. 2. Stalk 3. Rhizoid

Usually the proportion of DNA is more than RNA in nucleus. The protiens are composed of two major groups. The basic proteins (histones) or acidic proteins. The basic proteins, protamines and histones are found in nuclei of higher plants and animals. By ionic-binding with DNA, they form the nucleo-protiens and nucleio-histones, responsible in controlling gene activity. The non-basic or acid protiens which form 70% of the nuclear protiens, do not bind with DNA but form protein of nuclear sap, ribosomal proteins and partly chromosomal protein.

The nucleus is a complex structure and controls living cells. The chromosomes present in the nucleus have the bulk of genetic material DNA. The regulation of cellular activities is controlled by DNA, present in the chromosome. The DNA and RNA molecules which are responsible for synthesis of enzymes and proteins, expression of genetic information and the control of cellular function are all synthesized within the nucleus.

The importance of nucleus as the major centre which controls the reproduction and other activities of living cells was demonstrated by Hammerling. He conducted a series of experiments by using a single-celled mushroom like marine algae, *Acetabularia*, measuring 4-6 cm in length. The single huge cell is differentiated into a branched prostrate rhizoidal portion containing a

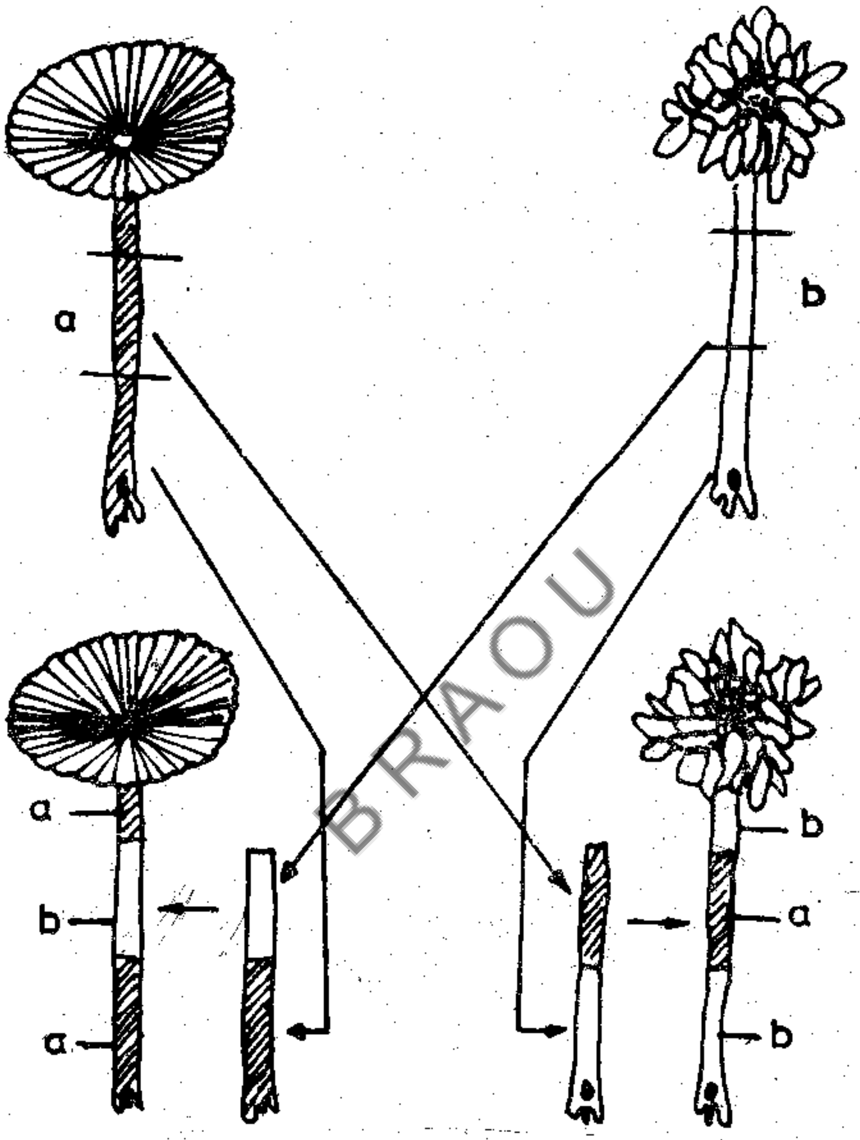


Fig.22.2. Grafting experiments with *Acetabularia*. The diagram shows two sets of experiments in which a piece of stalk of one species is grafted onto the rhizoid (containing the nucleus) of another species. The cap formed resembles most strongly that normally produced by the nucleus containing host. a. *A. Mediterranea* b. *A. Crenulata*

large nucleus, a long narrow vertical stalk with a terminal cap of 1 cm. in diameter. When the plant is decapitated it regenerates the cap characteristic of the species because the nucleus is still retained in the rhizoid. It is easy to produce nucleated and anucleated portion of the plants. When two decapitated plants both providing the rhizoidal end are grafted with each other, the 'hybrid' individual gives rise to a new stalk and cap. The cap of hybrid, possesses characters intermediate to both species. For example, *A. Mediterranea* which has a cap with

80 compartments with rounded edges and quite distinctive from *A. Crenulata* which has fewer segments in the cap. When the rhizoidal portions of these two species fuse, the cap formed by the graft hybrid is intermediate in its cap character, because of the presence of two different nuclei (Fig.22.1). When pieces of stalk of one species is grafted to another, the new hybrid developing from it will have the characteristic cap of the host plant (Fig 22.2). In this figure the rhizomatous portion with the nucleus belongs to *A. mediterranea* and it is grafted with the stalk not containing the nucleus of *A. crenulata*, the cap that is formed of this hybrid is characteristic of *A. mediterranea*, because the nucleus containing rhizomatous portion belongs to a *A. mediterranea*. Similarly in this figure the nucleus containing rhizomatous portion of *A. crenulata* decides the type of cap formed. These experiments clearly show that the genetic material contained in the nucleus determines the structure (morphological form of the plant). In other words, characteristics of the individual are controlled by the genetic material in the nucleus.

In bacteria and blue-green algae, the nucleus is devoid of a nuclear membrane, and a single circular chromosome is present. The other cell organelles in these prokaryotes do not contain membranous envelopes. But, in the eukaryotes a definite number (usually more than two) chromosomes are found in the nucleus and bounded by a nuclear membrane.

In higher plants during mitosis, the chromosomes consisting of largely euchromatin, divide longitudinally and the separated halves move to the opposite poles of the cell and form two identical daughter nuclei. Thus during nuclear division, each chromosome is duplicated and is associated with DNA synthesis.

The growth, development, structure of living organisms are guided by the hereditory information embodied as a code in the nucleic acids. These macromolecules accurately decipher and reproduce the information contained in them.

22.4. GENE

Gene may be defined as a segment of a DNA molecule. In other words, it is one of the basic units of heredity, each of which forms a discrete part of the chromosome. Genes exist in different forms and are known as alleles. Gene has a specificity of structure, location chemical nature and function. Genes can be located on the chromosome together or on different ones.

Genes with related function are adjacent and not randomly located. Studies on viruses and bacteria have indicated that a large fraction of the genes are situated in groups and carry out related function. In the T4 bacteriophage the genes responsible for the synthesis of head protein are in one region and those of tail fiber are situated in another region.

22.4.1. Chemical Nature of Gene

Deoxyribonucleic acid (DNA) is the primary genetic substance of the gene. It is a long macromolecule. The long chain polymeric molecule directs the amino acid sequences in proteins. The nucleic acid, DNA carries the genetic information.

The earliest clue on the significance of DNA as the conveyor of genetic information was made clear from the experiments of the English Biologist Fred Griffith(1928). He carried out experiments on *Diplococcus pneumoniae*,the bacterium causing pneumonia. It produces smooth glistering colonies. During his studies, he found a nonvirulent mutant strain of this bacteria which was devoid of the smooth capsule. The mutant produced small rough dull colonies.

Later in 1944 the substance which could cause the transformation of rough type into smooth type was isolated by Avery, Macleod and McCarthy. When DNA of the smooth type was introduced into rough type, it produced the capsule and transformed the mutant to virulent type. The introduction of the genetic material from one cell to another is called as transformation. Avery's discovery led to the important confirmation that DNA was exclusively found in the nucleus. The amount of DNA in a set of chromosome is constant for a given organism. DNA in diploid or sporophyte is twice the amount of that present in a haploid cell or a gamete. DNA, the genetic material is metabolically stable.

Further evidence for DNA being hereditary material came from studies of Hershey and Chase in 1952 on the T_2 phage, which proved again that DNA is the basic hereditary material. When Phage particles infect a bacterial cell, its protein coat is left outside and the viral nucleic acid enters the cell. The viruses cannot reproduce on their own. The viral nucleic acid takes over the cell's genetic machinery and produces the viral protein and nucleic acid to form new viruses.

Besides these experiments, the role of nucleic acid in heredity was tested in some virus hybridization experiments. Due to the experiments by Heinz Fraenkel Conrat in 1955 it was found that the virus particles can be dissociated into the protein and nucleic acid components. These components reassociate to become viable viruses when recombined. On the basis of this ability of viruses, an experiment was conducted, to verify whether the nucleic acid, or the protein is the genetic material.

The nucleic acid and protein components of TMV (Tobacco Mosaic Virus) and HR (Holmes-Ribgrass virus) was dissociated. The nucleic acid of TMV was mixed with the protein of HR virus, which resulted spontaneously in the regeneration of a 'hybrid' virus. In the same way, another hybrid virus was obtained when the nucleic acid from HR virus was mixed with protein of TMV. These two 'hybrid' viruses were allowed to reinfect their respective host cells. When these hybrid viruses replicated in the host cells, the TMV nucleic acid produced the progeny virus consisting only of TMV RNA and TMV protein, while the HR nucleic acid produced the HR progeny. These experiments revealed the significant observation that only the nucleic acid determines the kind of progeny produced.

Once the importance of nucleic acid was established by microbial genetics, many efforts were made to unravel the molecular nature of DNA, the basis of all life. Cellular DNA is present exclusively in the chromosomes, however, a little of it is found in mitochondria and chloroplast.

22.4.2. Base Pairing

The proteins which are linked with DNA in chromosomes are known as basic proteins or histones which are rich in the amino acids, lysine and arginine and have a net positive charge. On hydrolysis, these molecules yield nucleotides. Each nucleotide is composed of four nitrogenous bases, pentose sugar deoxyribose and phosphate. The nitrogenous bases are two of pyrimidines, viz. cytosine (C) thymine (T) and two purines viz, adenine (A) and guanine (G) (Fig. 22.3). In DNA molecule, one pyrimidine base pairs with purine base i.e. A.T. or G.C.

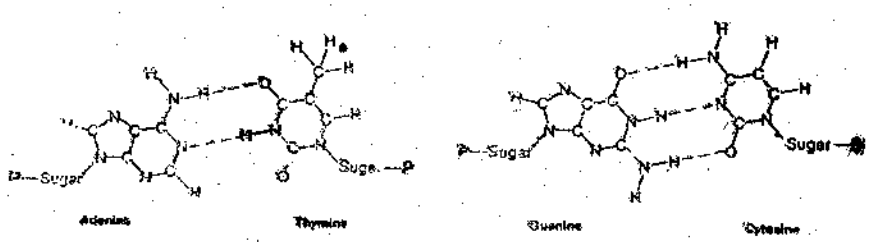


Fig. 22.3. The base pairs of DNA. The AT pairs has two hydrogen bonds; the GC pair has three.

In DNA, the number of molecules of guanine (G) is equal to the number of molecules of cytosine(C). The number of molecules of adenine (A) is always equal to the number of molecules of thymine (T) (Fig.22.4).

$$A + C = G + T$$
$$\text{(where } A = T \text{ and } G = C\text{)}$$

The bases (purine and pyrimidine) are attached to the deoxyribose at the 1-carbon atom, whereas phosphate is attached at 5-carbon atom. The phosphate group is bounded to the 5 carbon atom, one sugar is covalently bonded to the 3-carbon of the next (Fig. 22.5). Two such DNA strands are twisted around each other just like a spiral staircase.

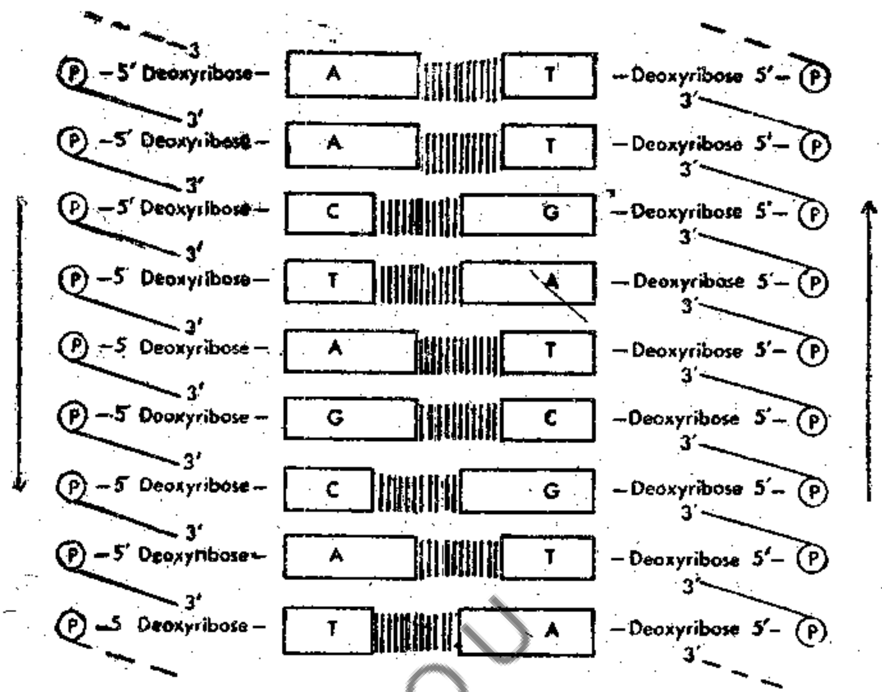


Fig.22.4. Diagram of the DNA double helix showing the specific pairing of the bases.

The individual polynucleotide chain exhibits a distinct polarity with 5' and 3'·end. The base pairs are properly fixed in the double helix, so that a maximum hydrogen bond can be formed. To achieve this the two chains run in opposite directions. Starting from the 'top' molecule one chain will run in the direction5'-3' while the other chain 3'-5'. This is important for gene activity.

22.4.3. The Double Helix

The two polynucleotide chains of the primary genetic material is a regular helix. The diameter of the helix is $20A^O$ and each chain makes a complete turn over $34A^O$. The internucleotide distance is $3.4A^O$. The sugar of the nucleotide is deoxyribose and are called deoxyribose nucleotides. The sugar of each nucleotide is joined by a phosphate group to the sugar of adjacent nucleotide. Four main nucleotides exist, each containing a deoxyribose residue, a phosphate group and a purine or pyrimidine base (Fig 22.6).

The molecular structure of DNA was proposed by Watson and Crick in 1953 for which they shared a Nobel Prize in 1962. Franklin and Wilkins confirmed the structure by X-ray diffraction method.

There is a wide variation in the base ratios of (A-T), (G-C) contents of different DNA molecules. DNA of higher plants and animals have excess of (A-T) over (G-C). Viruses, bacteria and lower plants are rich in both (A-T) and (G-C). The base ratios of taxonomically related organisms are similar and not random. The gene difference between plants and animals is due to the variation in the base ratios. The DNA molecules with high (G-C) are more resistant to heat than (A-T) rich molecules. Heat denatures DNA and the complementary strands separate from each other due to breaking of their hydrogen bond.

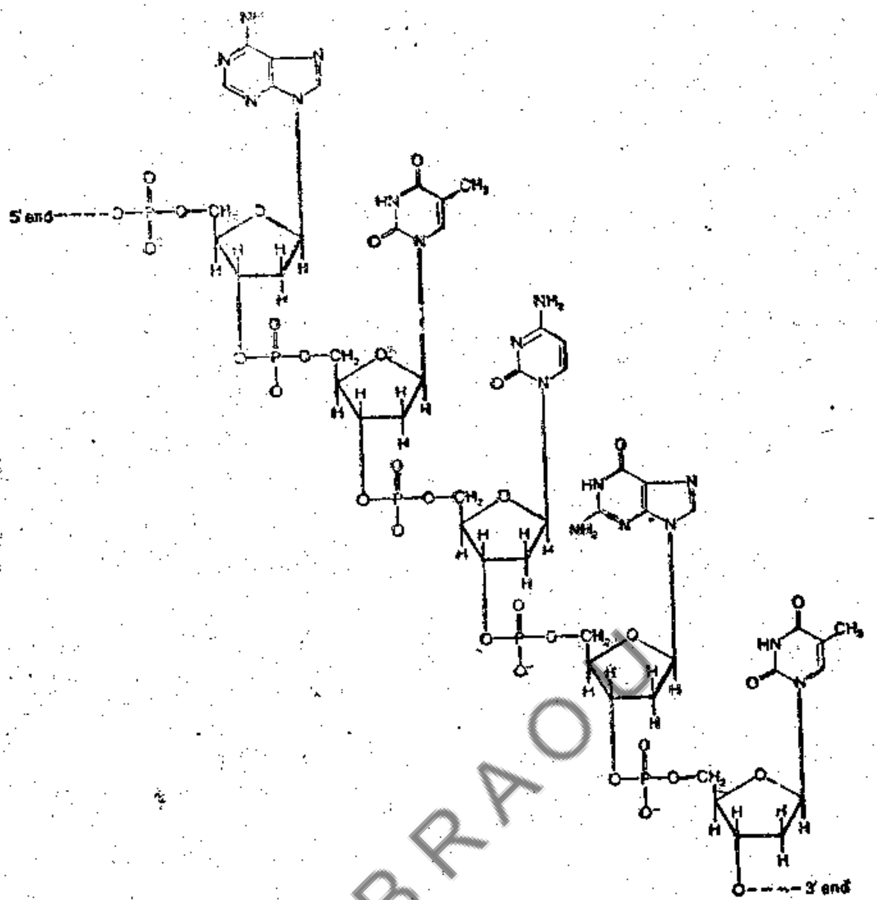


Fig. 22.5. A polynucleotide chain. The diagram indicates that a polynucleotide is constructed by the formation of a phosphate diester linkage between the 5' carbon of one sugar and the 3' carbon of its neighbouring nucleotide. The chain has one free phosphate group on the 5' carbon, the 5' end and one free 3' OH on the other terminal sugar, the 3' end. Interactions between different chains c within the chains are effected by bonding between the free purine and pyrimidine bases.

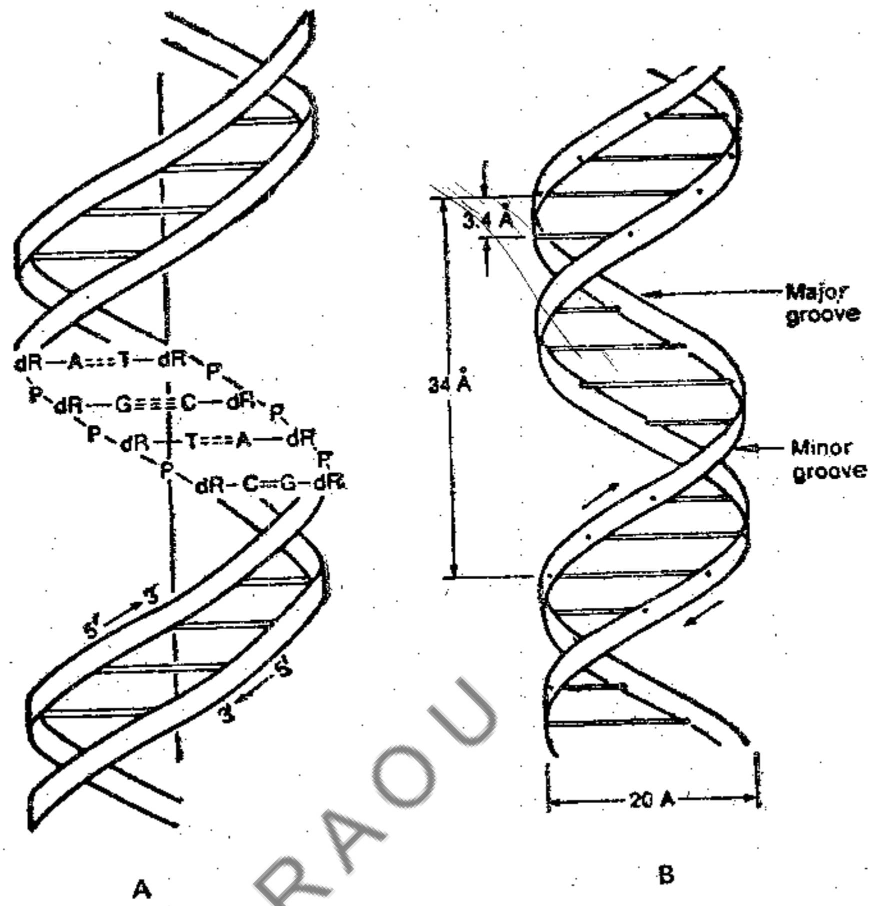


Figure 22.6. The double helix. A. A schematic diagram of the DNA molecule showing the base pairing and the helical structure. The polarity of the ester linkages of the two chains is shown by the arrows. B. The two chains are held together by hydrogen bonding between the complementary base pairs. Approximately 10 base pairs lie between two exactly overlying regions of the molecule. Note that there are two grooves in the helix.

22.4.4. Replication of DNA

The function of DNA is the self directed reproduction of itself. The complementary shape suggests self replication. During replication of DNA the double helix unwind themselves. The base units break apart at hydrogen bonds. Each separated strand serves as template for the formation of the complementary strand. The duplication and separation is a simultaneous process. In the presence of DNA template the precursors deoxynucleotide triphosphates for DNA synthesis are enzymatically formed.

DNA synthesizing enzymes are found in microorganisms, animals and plant cells. The enzyme DNA polymerase can synthesise DNA but it requires all the nucleotides. Adenine, Thymine, Guanine and Cytosine (or 5 methyl cytosine of higher plants) in the triphosphorylated form (ATP, TTP, GTP, CTP) and magnesium. A portion of DNA molecule is needed to act as a template. When these are mixed with enzymes DNA polymerases *in vitro* appreciable amount of DNA are synthesised. Pyrophosphate is split out of each added nucleotide as given in the following equation.

$$\text{deoxynucleotide triphosphates} \xrightarrow[\text{DNA template}]{\text{Enzymes, } Mg^{++}} (DNA)_n + \text{pyrophosphate.}$$

This reaction is reversible, but it is assumed that DNA synthesis is made irreversible by a phosphate that destroys pyrophosphate as rapidly as it is produced. The DNA formed will be identical to the DNA template molecule employed to initiate this reaction. Hydrogen bonds break between complementary bases of opposite DNA stands. Simultaneously the DNA polymerase adds a new nucleotide from available deoxynucleatide triphosphate (d-ATP, and TTP) to the new strands pyrophosphate (Pyro-p) is split out from the triphosphate. Each nucleotide added must contain a base(adenine, thymine, cytosine, guanine) complementary to the base in the parent strand to form a new stable double helix resulting in two helices identical to the original.

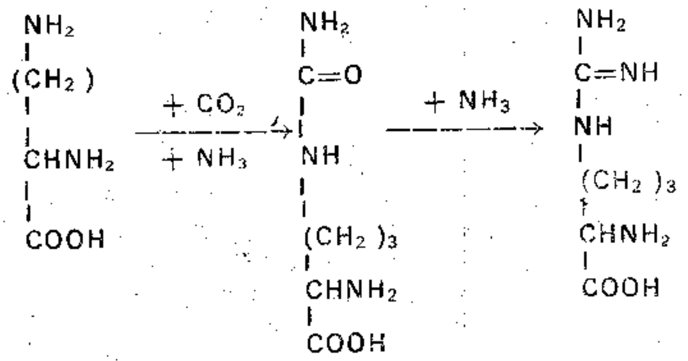
22.5. GENE ACTION

In 1909, Sir Archibald Garrod studied some hereditary diseases which he called as"inborn errors of metabolism" revealed that a relationship existed between gene and enzyme. Garrod published a book in which he discussed in detail a hereditary disease Alkaptonuria. This is a rare hereditary disease in few individuals known as Alkaptonurics. The individuals suffer no particular bad effect until late in life. The major symptom is blackening of urine on exposure to air due to a large amount of excretion of homogenetistic acid(alkapton) in the urine, which is oxidised into a black compound. In the normal individual, homogenetistic acid breaks down into acetoacetic acid. A chain of metabolic reactions in several stages from the precursor compounds take place to form the final product acetoacetic acid. The initial precursors are the proteins (from meat and egg diet) which are broken down to phenyl alanine, this in turn forms tyrosine. The last two are amino acids. Tyrosine break down to homogenetistic acid which finally produce acetoacetic acid. In Alkaptonurics there is a block in the metabolic system and so acetoacetic acid is not formed.

The direct experimental evidence on gene action comes from the work of Beadle and Ephrussi in the mid 1930's experimented with the eye colour of *Drosophila* and concluded that the genes were involved in the expression of the colour. *Drosophila* is a highly evolved and complicated organism having many complex reactions and substances. Therefore Beadle selected in 1940 the simple red bread mould *Neurospora crassa* to proceed with the work on genes.

Beadle and Tatum(1941) found that *Neurospora* could grow on a minimal medium. This fungus require some inorganic nitrates and other inorganic salts, a simple sugar and biotin a vitamin, a chemically complex molecule. The wild strain of *Neurospora* has the ability to synthesize the complex organic molecules from simple ones. Beadle and Tatum reasoned that the synthesis of the complex organic molecules was genetically controlled.They came to the conclusion that the nutritionally deficient mutants of *Neurospora* were unable to synthesise those metabolites and could not grow in the minimal medium lacking the particular metabolites. These mutants therefore needed an exogenous supply of substances to be included in the culture medium on which they were grown. They designed a complete medium by incorporating the particular vitamins, amino acids and other constituents necessary for growth of the nutritionally deficient mutants.

A number of mutants were obtained in *Neurospora* which could not grow unless the amino acid arginine was supplied i.e., arginine-less mutant. Biochemists have worked out the biochemical pathway of orginine synthesis in a sequential manner. Arginine is formed from citrulline, which in turn is synthesised from ornithine as follows.



Ornithine Citrullin Arginine

Among the seen originine-less mutants of *Neurospora* formed independently of each other; four of the strains could grow on the minimal medium if any one of the amino acid, i.e. ornithine or citrulline or arginine was supplied, whereas two could grow with the addition of either citrulline or orginine and one strain grew only arginine was provided (Fig.22.7). This is due to the genetic blocks caused by the absence of genes and nonsynthesis of specific enzyme concerned at different steps. A given enzyme being under the control of a specific gene brought out the hypothesis 'one gene-one enzyme' theory. Beadle and Tatum along with Joshua Lederberg were awarded the Nobel prize in medicine and physiology in 1958. The studies on *Neurospora* strengthened the findings that genes control sequentially related reactions.

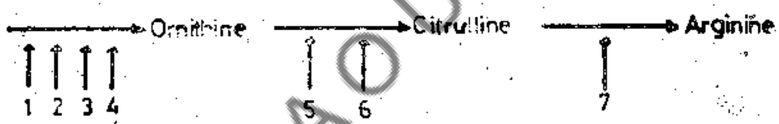


Fig.22.7. Seven-arginine-less mutants in *Neurospora* and their supposed site of action. Mutant 7 which blocks the conversion of citrullin to arginine will grow only in a minimal medium to which arginine is added, whereas mutants 1,2,3 and 4 grow with the addition of any one of the 3 substances.

Check Your Progress - 1

"Inborn errors of metabolism" revealed that a relationship existed between ______________ and ________________.

Note: (a) Write your answer in the space given above.

(b) Compare your answer with the one given at the end.

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22.6. MECHANISM OF EXCHANGE OF GENETIC MATERIAL

Many mechanisms exist for the exchange of genetic material between individuals.

a) Crossing over: The mechanism of crossing over involve the breakage of homo logous chromosomes and diagonal reunion. Thus, due to crossing over genes or gene groups initially found on paternally derived chromosomes get exchanged with material parent, resulting in new combinations. Seggregations of genes during crossing over helps in finding the location of the gene along the chromosome. It is possible to determine whether the gene is located on different or the same chromosome. When genes are located on the same chromosome, they seggregate together unless separated by crossing over. The crossing over seems to be a random event. Gene location on chromosome can be determined by the frequency of recombination.

It was thought earlier that crossing over occurred between genes and not within genes themselves; because crossing between two regions far apart is easier to detect than those at close proximity, unless a large progeny is examined.

Genes are stable and mostly copied during chromosome duplication. Mutation brings about heritable changes in the gene itself. Each gene has a number of sites at which mutation occurs. Like the order of genes on a chromosome the arrangement of mutable site in a gene is linear.

b) Transformation : Earlier work of genetics was done with large diploid organisms. Now the most suitable for the gene study are the haploid micro-organisms like the moulds, bacteria, viruses and the bactriophages (bacterial viruses). Because of their short life cycle a rapid testing of the progeny is possible.

(i) Lytic bacteriophages: The bacteriophages show genetic recombinations and are extensively studied. Plaques (circular holes) are produced by viruses on *Escherichia coli* the common colon bacterial colony. The plaques are due to the death of the bacterial cells caused by viruses. A single virus particle attached to a bacterium multiply to several thousand virus particles and are released by the dissolution (lysis) of the bacterial wall after 15 to 20 minutes after phage infection. These are known as lytic bacterial viruses as their multiplication bring about the lysis of a bacteria.

(ii) Lysogenic bacteriophages: The phage known as lysogenic phage does not multiply on entering a host cell, instead the virus chromosome becomes integrated with the bacterial chromosome designated as prophage. Such virues are known as lysogenic viruses. The integration of the viral chromosome is brought about by crossing over between the host chromosome and a circular form of the viral chromosome by attaching at a specific region. Both chromosomes break and rejoin in such a way that the broken ends of the viral chromosome join the broken end of the bacterial chromosome instead of each other, thereby inserting the prophage into host chromosome. The prophage detach from the host chromosome by the reverse process. The two ends of the prophage pair prior to a crossing over even which affect the viral chromosome, the new viral genome then begins to multiply as if it is the chromosome of a lytic virus.

22.6.1. Conjugation

The nature of the gene has been revealed by many organisms lacking in conventional meiosis. The sexual cycle starts in *E.Coli* by the conjugation of male and female cells known as bacterial conjugation. The nature of mating process in *E. coli* has revealed rarely diploid cells, instead partially diploid cells. Crossing over between the female parent chromosome and a male, or a fragment chromosome followed by seggregation, yields haploid cells. In the bacterial conjugation there is a direct one-way transfer of genetic material from the donor (male) to recipient (female) cell by intimate cellular contact.

The sex difference between male and female cells are determined by specific genetic factor called F(fertility) factor. When this factor is present F^+ are male cells. If the factor is absent, it is a female cell. The F factor exists in two alternative states, either as part of the *E. coli* chromosome or as a very small free circular chromosome that multiplies once for each cell division. In the latter case the F^+ cells are potentially male since they cannot transfer their gene to F cells. Only when the F factor is part of the chromosome can the male cells mate. F^+ cells containing integrated sex factors are called HFr (high frequency of recombination). The F agent is circular like the lysogenic chromosome. Both F chromosome and lysogenic phage chromosome are called episomes. It is defined as genetic particle that can exist either free or as part of a normal cellular chromosome. Evidence of episome is only found in bacteria. However, there are hints that they exist in higher plants and animals as well. A fixed end of the male chromosome specific for a given HFr strain always enters the female cell first.

The bacterial chromosomes are circular. Circular chromosomes also exist in many viruses. Plasmids are extranuclear DNA freely replicating circular chromosomal elements. Their size varies greatly, some small ones carry 1-3 genes while others have size upto 10-20% of the main chromosomes. Single strand circular molecules are present in some phages. There are some rod shaped molecules having the potential for converting into circular form.

22.6.2. Transduction

The transfer of genetic material from one bacterial cell to another (bacteria) has already been described. It is clear that the nuclear material (DNA) is responsible for the protein synthesis. In addition to the above, there is another method of exchange of genetic material.

The transfer of genetic material from one bacterial cell to another by means of a virus vector is known as transduction. Temperate phage is used in transduction as the virus is duplicated synchronously with the bacterial chromosome and does not kill the bacterial host. A bacterial DNA fragment from the donor is incorporated in the virus particle. When the phage- sensitive recipient cell differing genetically from the donor is infected with the transducting phage, the DNA from the donor bacterium may be incorporated into the recipient bacterium. Recombinations possessing the newly acquired character appear with a frequency of about 1 per 10^5 to 10^6 recipients. Sometimes a double cross over occurs between the recipient bacterial chromosome and donor viral chromosome in order to integrate the donor DNA to become integrated into the chromosome of the recipient bacterium. Transduction is yet to be observed in higher organisms.

Check Your Progress - 2

Define transduction.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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22.7. SUMMARY

The nucleus is the chief organell in the cell. During nuclear division, the chromosomes are duplicated. The regulation of cellular activity is controlled by DNA present in the chromosomes. A gene is the segment of DNA, therefore DNA is the primary genetic substance of the gene. The macromolecule DNA has a double helix. It has nucleotide composed of a 4 nitrogenous bases, a pentose sugar i.e., deoxyribose and phosphate. The nitrogenous bases are of two kinds (a) pyrimidines consisting of cytosine (C) and thymine (T); (b) purines consisting of adenine (A), and guanine (G). The molecular structure of DNA was proposed by Watson and Crick. The two chains of DNA run in opposite direction to each other. The individual polynucleotide chain exhibits a distinct polarity with 5' and 3' end. During DNA replication the double helix unwinds. Each separated strand serves as a template for the formation of the complimentary strand. Gene action was studied in *Drosophila* eye color and later in the red bread mould *Neurospora* crassa. From N. Crassa Beadle and Tatum found out that the stepwise synthesis of the complex organic molecules was genetically controlled. After the establishment of the relationship between specific gene and specific protein, the hypothesis one gene-one enzyme is modified to one gene-one polypeptide chain.

22.8 CHECK YOUR PROGRESS: MODEL ANSWERS

1. Gene, enzyme
2. The transfer of genetic material from one bacterial cell to another by means of a virus vector is known as transduction.

22.9. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines Each.

1. Describe briefly the gene and its function.
2. Describe the chief control centre in living cells.
3. What are the characteristic features of the double helix of DNA?
4. How the biochemical mutants helped us to understand the gene action?
5. Comment on the hypothesis "One-gene-one polypeptide chain".
6. Write in detail the differences between lytic and lysogenic viruses.
7. Describe briefly how genetic recombination occurs in bacteria.

II. Answer the following questions in about 10 lines each.

1. Describe briefly the different parts and functions of the nucleus.
2. Describe briefly the process of conjugation
3. Describe briefly the different processes of genetic recombinations.
4. Write an account on the DNA synthesis.
5. Give an account of the chemical nature of DNA.
6. Write briefly on the work of Beadle and Tatum.

UNIT-23: GENETIC CODE AND PROTEIN SYNTHESIS

Contents

23.1. OBJECTIVES

By the end of this unit you will be able to:

1. describe the components of protein synthesis apparatus,
2. explain the language of genetic code and characteristics of triplet codon and
3. describe the mechanism of protein synthesis.

23.2. INTRODUCTION

The genetic code consists of message included in DNA and different kinds of RNA. Therefore, it is necessary to learn more of nucleic acids at this stage. The structure of DNA is already given in detail in the previous unit, hence a brief account of it is presented here. The details about the structure and different kinds of RNA are dealt here.

23.3. PROTEIN SYNTHESIS APPARATUS

Chromosome in the nucleus which carry the hereditary characters contain mostly deoxyribose nucleic acid (DNA). Protein synthesis takes place under the direction of DNA which carries the genetic information of primary structure of protein. The DNA is made up of four nucleotides and the DNA macromolecule of several nucleotides forms double helix. The four types of bases viz. adenine (A), thymine, (T), guanine (G), cytosine (C), are arranged in definite linear sequence on the DNA molecule. The genetic information exists in the sequential arrangement of these bases. The bases are paired, one purine of one spiral of the helix always pairs with pyrimidine of the other spiral of the helix. In the case of DNA, adenine (a purine) is paired with thymine (a pyrimidine), while guanine (another purine) with cytosine (another pyrimidine) i.e., (A-T) and (G-C).

Ribonucleic acid (RNA) is present in the cytoplasm excluding the vacuole. It is a non-genetic nucleic acid. The pentose sugar in RNA is a ribose. The RNA contains four nitrogen bases. The thymine (T) presented in DNA is replaced by uracil (U) in RNA. The other bases are the same as that of DNA, i.e., adenine (A), guanine (G) and cytosine (C). Structure of RNA is less complex, but more varied than DNA. All cellular RNa are single stranded polynucleotides. The nucleotides are linked 3'-5' phosphate ester bonds (Fig.23.1).

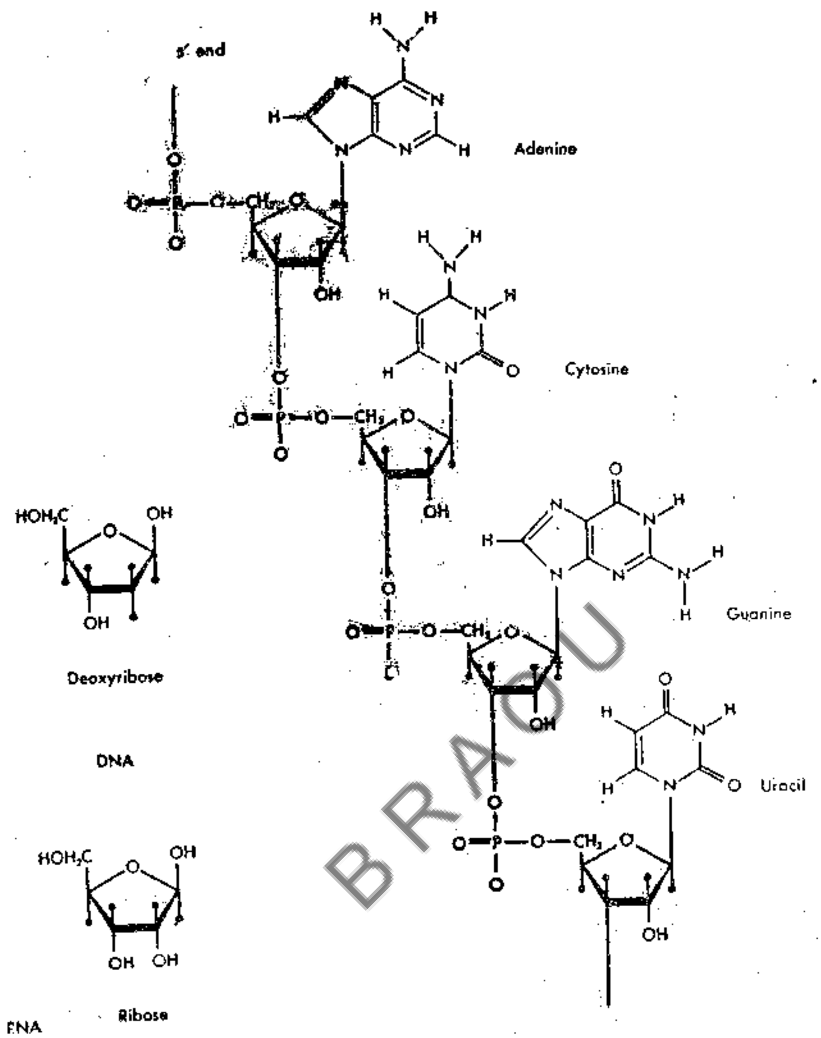

Fig. 23.1. The chemical formula of RNA.

In some cases RNA may form secondary structure as in some viruses (reovirus). A complimentary base pair forms hairpin loops so as to make the RNA molecules double stranded in certain regions like DNA type of base pairing (A-U) and (G-C) (Fig.23.2).

23.3.1. Synthesis of RNA

The synthesis of RNA complementary to a strand of DNA is known as transcription. The DNA helix is temporarily uncoiled for the synthesis of RNA (Fig. 23.3) and it is catalysed by the enzyme RNA polymerase. The genetic information is transcribed into specific mRNA molecules which translate them to specific proteins through tRNA. This process is known as translation

All the three forms of RNA. viz., ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA) are made on DNA templates. Enzyme RNA polymerase catalyses the synthesis of RNA. It uses nucleotide triphosphates (ATP, UTP, CTP and GTP). In the synthesis of RNA a primary template of DNA is necessary. As mentioned earlier the composition of RNA is identical to DNA, except for the presence of uracil (U) in RNA, which is equal to adenine (A) and guanine (G) to cytosine (C) of DNA.

$$\text{n nucleotidephosphates} \xrightarrow[\text{DNA template}]{\text{Enzyme, } Mg^{++} \text{ or } Mn^{++}} (\text{RNA})_n + \text{n pyrophosphate.}$$

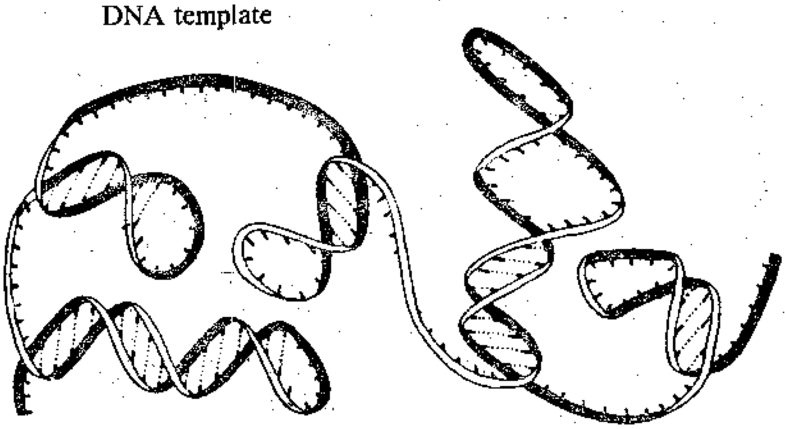

Fig. 23.2. Schematic folding of a RNA chain showing several double-helical regions held together by hydrogen bonds.

In prokaryotic cells RNA is liberated directly into the cytoplasm of the cell, whereas in eukaryotic cell the process is complex. The synthesis of mRNA occurs in the nuclear ground substance. The precursors of the other form of RNA is in the nucleolus and after transformation they emerge into the cytoplasm.

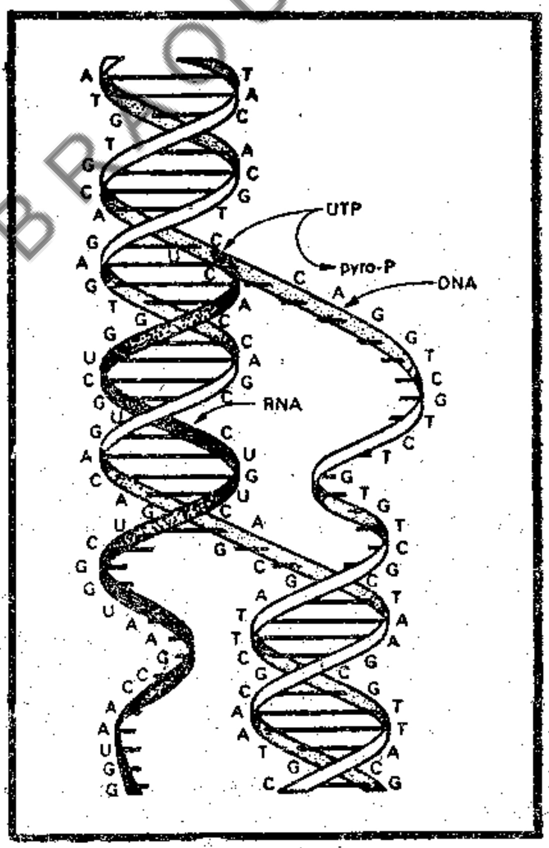

Fig.23.3. Synthesis of RNA using one of the strands of DNA as a template (transcription). According to this model, the DNA helix is temporarily uncoiled as RNA synthesis progresses along it. RNA polymerase molecules presumably become bound to the DNA and attach nucleotides from triphosphates such as UTP to the growing RNA chain, except that thymine (T) in DNA is replaced by uracil (U) in RNA.

The three forms of RNA (rRNA,tRNA, mRNA) are distinguished on the basis of their molecular size and function. They are defined in terms of the sedimentation constants (obtained through an ultracentrifuge), which are proportional to the logarithm of the molecular weights.

23.3.2. The Ribosomes and the Ribosomal RNA (rRNA)

The ribosomes are the site of protein synthesis, where amino acids are assembled in a specific sequence to produce polypeptide chains. Ribosomes were first observed by Robinson and Brown in 1953 in plant cells. Afterwards it was reported by Palade in animal cells. Ribosomes occur free in the cytoplasm and also attached to the endoplasmic reticulum known as rough endoplasmic reticulum. Both the free and membrane bound ribosomes synthesize proteins. The ribosomes attached to endoplasmic reticulum are active in protein synthesis, while free ribosomes are not Ribosomes consist of approximately equal parts of RNA and proteins in eukaryotic cells and catalyse the incorporation of amino acids into proteins in the presence of ATP, Magnesium (Mg^{++}) and other co-factors. The ribosomes are grouped into two major classes on the basis of their sedimentation coefficients. The ribosomes of eukarryotes have a sedimentation coefficient of 80S (Svedberg) and consists of two sub-units 60S and 40S (Fig.23.4).

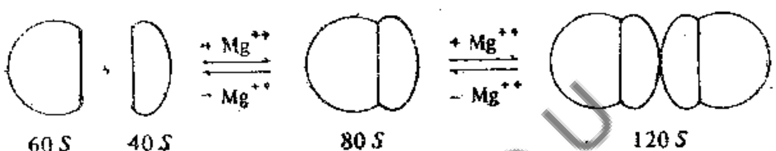

Fig.23.4. Diagram showing structure of ribosomes of eukaryotes.

The ribosomes of prokaryotic cells as well as from chloroplasts and mitochondria of higher organisms have 70S sedimentation co- efficient and dissociate into 50 S and 30 S sub-units (Fig 23.5).

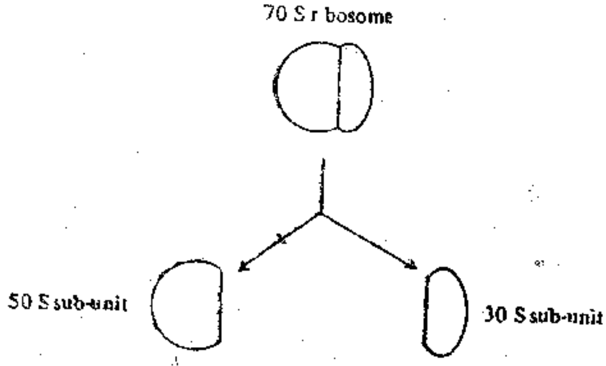

Fig.23.5. The structure of prokaryote ribosome from the bacterium *E. Coli*

The cytoplasmic ribosomes of plant cells are 20 nm in diameter, contain 40-50% RNA and 50-60% protein. Their molecular weight is about 4 x 10^6. And as mentioned earlier the ribosomes are composed of two subunits of unequal size. The small sub-unit lies to a side of the larger sub-unit. A characteristic cleft divides the sub-units into unequal regions. The Mg^{++} ions are necessary for their structural cohesion. When magnesium is limited; the ribosomal unit break down to particles and become inactive in protein synthesis. The ribosomes constantly dissociate into sub-units and reform. The dissociation is essential for protein synthesis to begin with when a number of ribosomes associate together on a RNA strand, it is known as polysome or polyribosome. In higher plants polyribosome may be the m ijor site of protein synthesis (Fig. 23.6).

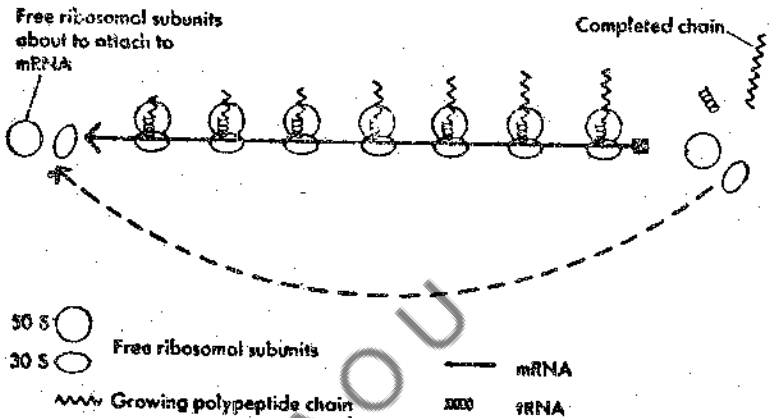

Fig. 23.6 Schematic picture of a polyribosome during protein synthesis. The mRNA molecule is moving from right to left.

The rRNA is present in ribosomes in which proteins are synthesised. The rRNA is present in (a) ribosomes free in the cytoplasm and (b) ribosomes attached to the endoplasmic reticulum. The rRNA constitutes about three fourths of the RNA in the cell. The rRNA molecules are single stranded polynucleotides, which are unbranched and flexible. They are coiled and folded within the ribosomes. The base composition of rRNA shows a general similarity from organism to organism. The rRNA serve as a template for protein synthesis.

23.3.3. The Transfer RNA (tRNA)

The tRNA is also known as solube RNA(sRNA) as it is soluble in 1 M Nacl. Its designation as tRNA is more appropriate because it is involved in the transfer of amino acids from cytoplasm to their proper position on the ribosomes. The tRNA forms 15% of the total RNA of the cell. The molecules of tRNA are the smallest and contain about 75-90 nucleotide bases. Their molecular weight is about 25,000 to 30,000, and sediments 4S. There are less than 60 distinct species of tRNA. There is at least one species of tRNA for each of the twenty amino acids forming proteins. They are synthesised on DNA and released to the cytoplasm.

The function of tRNA is to haul up the specific amino acid from the cytoplasm and transfer it to the mRNA template as distinguished by the codon and line up the amino acids for protein synthesis. The tRNA combines with amino acids in the presence of an enzyme and an energy source(ATP) to form tRNA- amino acid complex (initiation complex)-which is essential for protein synthesis. Directly the free amino acids cannot be incorporated into proteins unless tRNA-amino acid complex is formed. The first nucleotide sequence of a specific tRNA molecule reported was from yeast. It is, called as alanine tRNA as it is specifically attached to alanine and contains 77 nucleotides arranged in a unique sequence of high unusual bases with one or more methyl ($-CH_3$) groups Fig.23.7. The tRNA molecule is partially double stranded and the folded upon itself. In addition to A,G,C,U some methylated bases, pseudouridine (PSU) and others also occur on tRNA.

The molecule of tRNA resembles a clover leaf. with four arms. Each arm contains a helical hydrogen-bounded region and terminates in a loop. The region with in the loop is the reaction site. Each clover leaf has non-hydrogenated-bounded sections. The nucleosides at 3' end of tRNA (the acceptor stem) consist of ACC along with a fourth variable nucleotide. The 5' end tRNA has a guanine base. During protein synthesis the amino acid attaches itself to the 3' terminal A of the acceptor stem. Starting from the 3 end, the first lobe (loop) of the clover leaf is encountered. This loop is formed by seven unpaired bases and is involved in the binding to the ribosomal surface. Next to this , is a "Lump" which is variable in size and is designated as the variable loop. Next to this lump, i.e., variable loop is the loop called the anticodon loop, with seven unpaired bases. The three adjacent paired bases GCC comprises of the codon. The anticodon loop containing IGC anticodon triplet gets attached by hydrogen bonds to mRNA during protein synthesis. This anticodon of the particular tRNA carrying the concerned amino acid recognises the site (by complementary structural fit) on mRNA carrying the particular codon.

23.3.4. The Messenger RNA (mRNA)

The single stranded mRNA molecule forms a small amount (5% or less) of the total RNA of the cell. It is heterogenous in its molecular size. It contains 900-1500 nucleotides. It carries genetic message to the ribosomes, hence the name. This RNA is synthesized rapidly and broken down rapidly. The base composition of mRNA is similar to the organism in which it exists except that thymine (T) is replaced by uracil (U).

A single mRNA strand has the ability to function on several ribosomes simultaneously, so it is present in small amount in the cell. Many ribosomes are attached to a strand of mRNA, called polyribosomes.

23.4. THE GENETIC CODE

There are about twenty different kinds of amino acids which normally constitute different proteins. Single and double base coding will not cover all the twenty amino acids. A single genetic code involving each nucleotide, coding for a single amino acid, can code only for a protein with four amino acids, while two bases codes for sixteen amino acids in the formation of polypeptide. It was suggested by Crick, that each amino acid is specified by a triplet of nucleotides. It is proved experimentally by Nirenberg and Matthaei by using tailor made artificial triplets of RNA in cell free systems that a sequence of three base codes are necessary for a single amino acid.

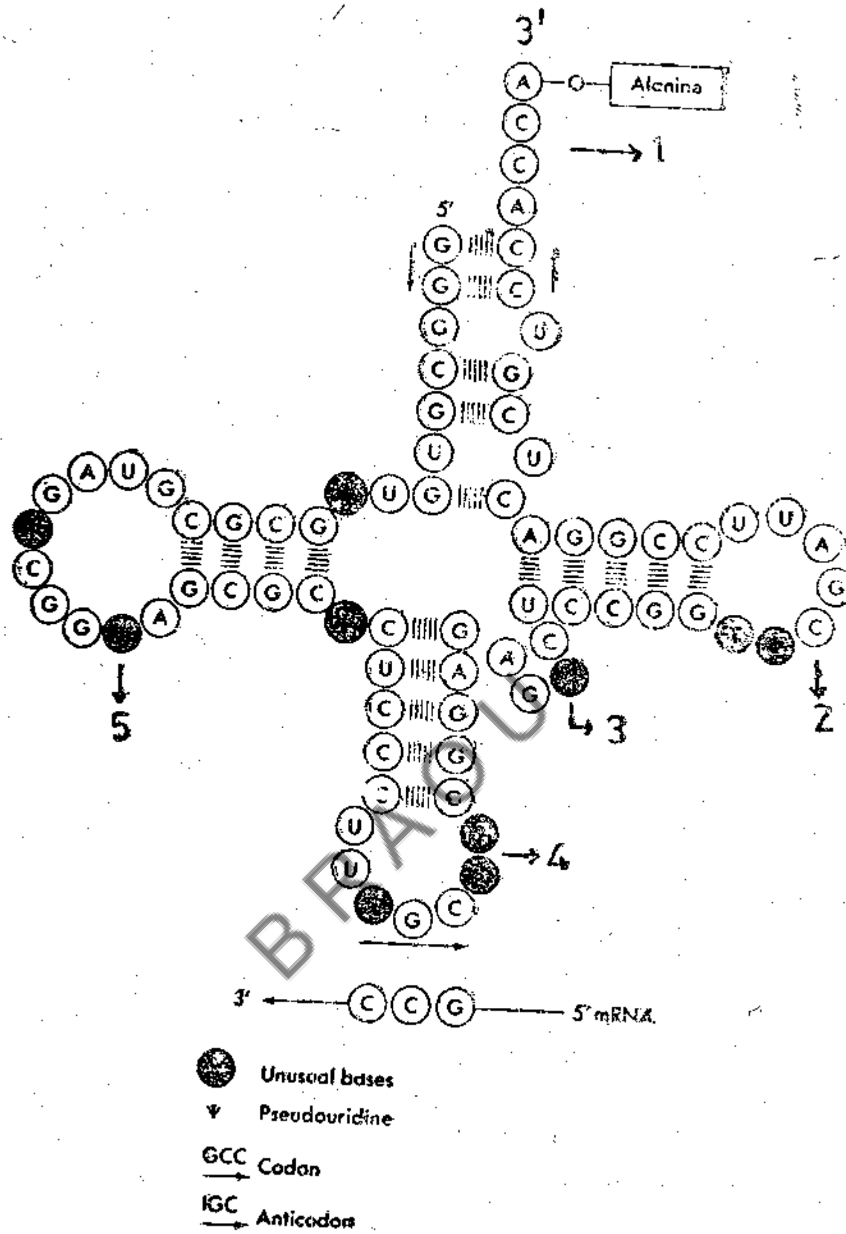

Fig.23.7. The complete nucleotide sequence of alamine tRNA showing the unusual bases and codon, anticodon position. 1. Acceptor stem. 2. TUC loop. 3. Variable loop. 4 Anticodon loop. 5. Dihydrouracil loop.

The involvement of a specific sequence of three bases of mRNA coding for a specific amino acid is called codon or triplet codon. So the genetic code exists in triplets. In fact, the triplet of nucleotides in mRNA do not code for an amino acid directly, but code through tRNA specific to amino acids. The codon sequence for several amino acids are listed in Table 23.1.

Table 23.1. The Genetic Code

5' end.	U	C	A	G	3' End
U	UUU Phe	UCU	UAU	UGU	U
	UUC	UCC	UAC Tyr.	UGC Cys	C
	UUA	UCA Ser	UAA Ochre	UG Non-sense	A
	UUG Leu	UCG	UAG Amber	UGG Trip.	G
C	CUU	CCU	CAU	CGU	U
	CUC	CCC	CACHis	CGC	C
	CUA Leu	CCA Pro.	CAA	CGA Arg	A
	CUG	CCG	CAG Glu	CGG	G
A	AUU	ACU	AAU	AGU	U
	AUC Leu.	ACC Thr	AAC AspN	AGC Ser.	C
	AUA	ACA	AAA	AGA	A
	AUG Met	ACG	AAG Lys	AGG Arg.	G
G	GUU	GCU	GAU	GGU	U
	GUC	GCC	GAC Asp	GGC	C
	GUA Val.	GCA Ala	GAA	GGA Gly	A
	GUG	GCG	GAG Glu	GGG	G

Two or more triplet code is represented for a single amino acid. In such cases the genetic code is degenerate. For example, code for phenylalanine is both UUU and UUC, while serine is coded by UCU, UCC, UCA, UCG, AGU and AGC. When the first two nucleotides are identical, the third nucleotide can be either cytosine or uracil and the codon will still code for the same amino acid. Often adenine and guanine are interchangeable. All the degeneracy need not be based on equivalence of the first two nucleotides (first two letters being the same). Leucine can be coded by UUA and UUG as well as by CUU, CUC, CUA and CUG (Table 23.1). When a specific codon for a given amino acid is altered (by mutation) to specify, another amino acid it is known as missense mutation. In addition to these certain altered codons also caused by mutation do not code for any amino acid at all. These are called nonsense triplets (mutations) and they help in terminating the polypeptide chain at a particular length.

The genetic code appears to be universal in all organisms. Another unique feature of the genetic code is its non- overlapping. This is achieved by some type of mechanism present in the mRNA that prevents the tRNA molecules from forming base pairs with purines and pyrimidines in the two adjacent codons. Similarly, during the transcription of mRNA on a DNA template, it starts and ends at specified points. All these events are controlled by operator and regulator genes.

Check Your Progress- 1

What is triplet codon? Explain.

Note: a) write your answer in the space given below.

b) Compare your answer with the one given at the end of this unit.

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23.5. THE OPERATOR THEORY OF GENETIC REGULATION

There is a genetic regulation of synthesis of enzymes. The amount of production of a given enzyme is proportional to the total amount of protein synthesis. The regulation of these inducible and repressible enzyme synthesis depends on the amount of mRNA formed during transcription. Jacob and Monad (1961) put forward a hypothesis, the operator theory to explain the genetic regulation of enzyme synthesis by induction and repression. This is known as Jacob and Monad model or Operon Model (Fig.23.8) on the synthesis of an inducible enzyme galactosides in *E.coli.* According to this theory the synthesis of mRNA can start at a particular site on the stands of DNA i.e. in one direction. These sites are called as operators, which regulate the synthesis of specific mRNAs. They determine whether transcription occurs or not. The structural gene coding for the enzyme group is controlled by a single operator and form a unit.

The structural gene and operator gene are collectively known as Operon. The activity of operator gene is controlled by specific regulator gene. The structural gene for enzyme groups are usually located adjacent to one another on the chromosome. These enzymes are coordinately induced or repressed. For example, the lactose operon consists of an operator gene (O) and three structural genes which coordinately code for the inducible enzyme galactosidase (Z gene).

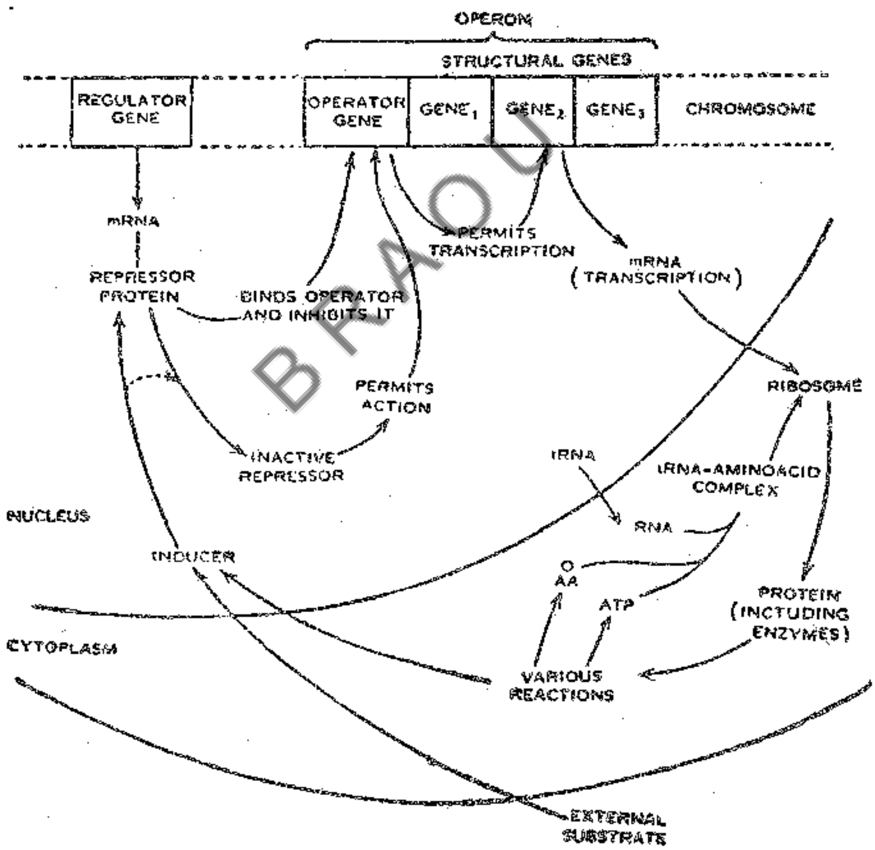

Fig.23.8. Operon model of regulation of protein synthesis (After Jacob and Monod. 1961).

galactoside permease (Y gene) and galactoside transacetylase (X gene). The regulator gene(i) may be located far away from the operator gene (or genes) on the chromosome with which it is associated (Fig.23.9). The regulator gene elaborates repressor substances which control operator gene to initiate the transcription of the structural genes and prevent mRNA synthesis and inhibit specific protein synthesis. The attachment of an inducer (corepressor) inactivates the repressor. When inducer is introduced, it combines with the repressor elaborated by the structural gene resulting in a product unable to interact with the operator. Because the repression of the operon is lifted, mRNA synthesis take place. There is evidence to suggest that the transcription of mRNA begins instead of the operator gene at a specific site on the DNA at or near the beginning of the first polypeptide, the Z gene in the case of Lac operon. This site is known as promoter gene.

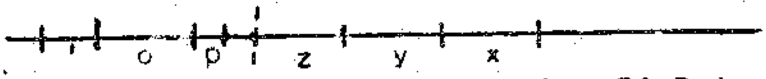

Fig 23.9 Schematic diagram of the lac operon containing the structural genes Z for B-galactosidase, Y for galactoside permease, X for transacetylase; and the operator site O, the regulator gene i and the promoter p. It is not clear whether the promoter is a separate gene site or part of the structural gene

The central dogma of molecular biology: The DNA regulates the cellular activities. Two important functions occur in the nucleus. The biosynthesis of DNA (or replication) is the first function in which the duplication ensures the genetic continuity. The other (transcription) is the biosynthesis of different RNA molecules which are responsible for the synthesis of enzymes and proteins. This function at the molecular level is known as the central dogma of molecular biology. In other words, the DNA (the gene) makes RNA; which moves to the cytoplasm and guides the synthesis of protein.

$$\text{Duplication of DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein.}$$

It is clear from the figure that this process involves duplication of DNA which transfers the genetic information contained in RNA (transcription) and the expression of the information in protein synthesis (translation). However this dogma is not absolute, since in some systems (RNA tumour viruses) RNA transcribes DNA. This enzyme is generally known as reverse transcriptase.

23.6. PROTEIN SYNTHESIS

The synthesis of protein involving DNA, and RNA consists of three essential steps. a) replication. b) transcription and c) translation. The first two (a and b) take place in the nucleus and the third (c) take place in the ribosomes. The same is summarised in Fig.23.10. The replication (a) has already dealt with earlier while describing RNA synthesis.

The process involved in protein synthesis can be broadly divided into four stages as follows.

1. Activation of amino acids with the formation of specific amino acyl tRNA molecules.
2. The molecules of amino acyl tRNA bind to mRNA-ribosome complex and initiate the peptide chain.
3. Elongation of the polypeptide chain.
4. Termination and release of the chain from ribosome complex.

The amino acids are activated by ATP. The arrangement of the activated amino acids on the mRNA template is brought about by the tRNA as detailed previously. The formation of an amino-acyl tRNA complex takes place in two steps. The amino-acyl-tRNA is highly specific for one amino acid, catalyses both the reactions. Each cell has at least twenty different activating enzymes.

$$\text{Amino acid} + \text{ATP} + \text{Aminoacyl tRNA} \longrightarrow \text{(Aminoacid-AMP-enzyme)} + \text{Pyrophosphate.}$$

$$\text{(Amino acid-AMP-enzyme)} + \text{tRNA} \longrightarrow \text{Aminoacyl-tRNA} + \text{AMP} + \text{Enzyme.}$$

In the presence of the enzyme, amino acid tRNA synthesis the amino acid and ATP form bound amino acid and adenylate by releasing pyrophosphate. The bound adenylate can react only with specific tRNA and release AMP and the enzyme.

The three forms of RNA have a special role in protein synthesis. The unpaired bases of rRNA are probably involved in binding tRNA and mRNA to both the sub-units of ribosomes. The end of a mRNA attaches to a ribosome. Then tRNA binds itself to the same end of mRNA by hydrogen bonds between particular three bases in each RNA molecule for example adenine-uracil, cytosine-guanine and uraciladenine. The three respective bases of mRNA with which rRNA unites are collectively referred to as codon and the complementary triplet in the tRNA as anticodon. This requires three initiation factors F1, F2, F3 and GTP. The three base sequences determine the acid composition of a synthesizing protein. The protein synthesis begins at a 5' terminal and of mRNA and terminates at the 3' end.

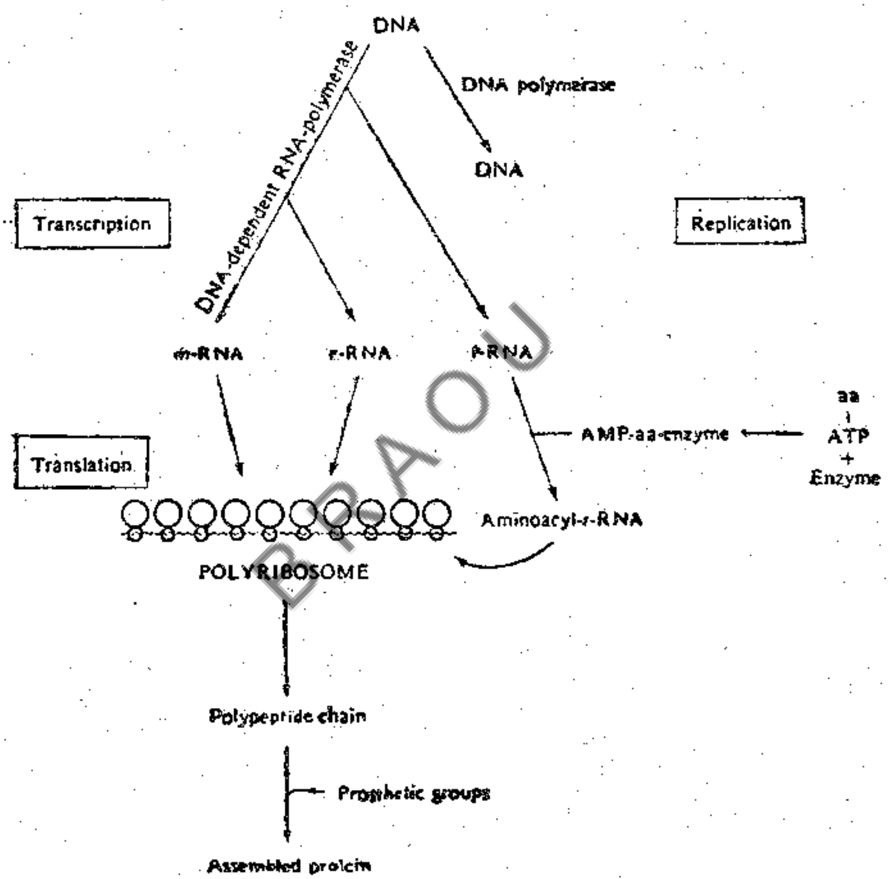

Fig. 23.10. The over all scheme of DNA, RNA, and protein synthesis, showing the relation of the primary processes of replication, transcription, and translation.

The initial step involves AUG codon of mRNA with an activated tRNA which attach with an amino acid formylmethionine on its CCA ending. The anticodon on such tRNA is UAC and it comes to lie in alignment by their hydrogen bonds and in the presence of several initiating factors F1, F2, F3 and GTP. The mRNA molecule moves along the ribosome along three more bases. As this occurs a new tRNA with its amino acid diffuses into position (Fig.23.11). This process continues till the genetically determined lifetime of mRNA after which, they break down by degradative enzymes.

A peptide bond is formed between the two amino acids formed side by side which is catalysed by a potassium dependent, enzyme peptide synthetase. Always initiation of a polypeptide chain starts from the terminal amino (NH_2) group, to the carboxyl (-COOH) end.

Check Your Progress - 2

Name the three essential steps of protein synthesis.

Note: a) Write your answer in the space given below.

b) Compare your answer with the one given at the end.

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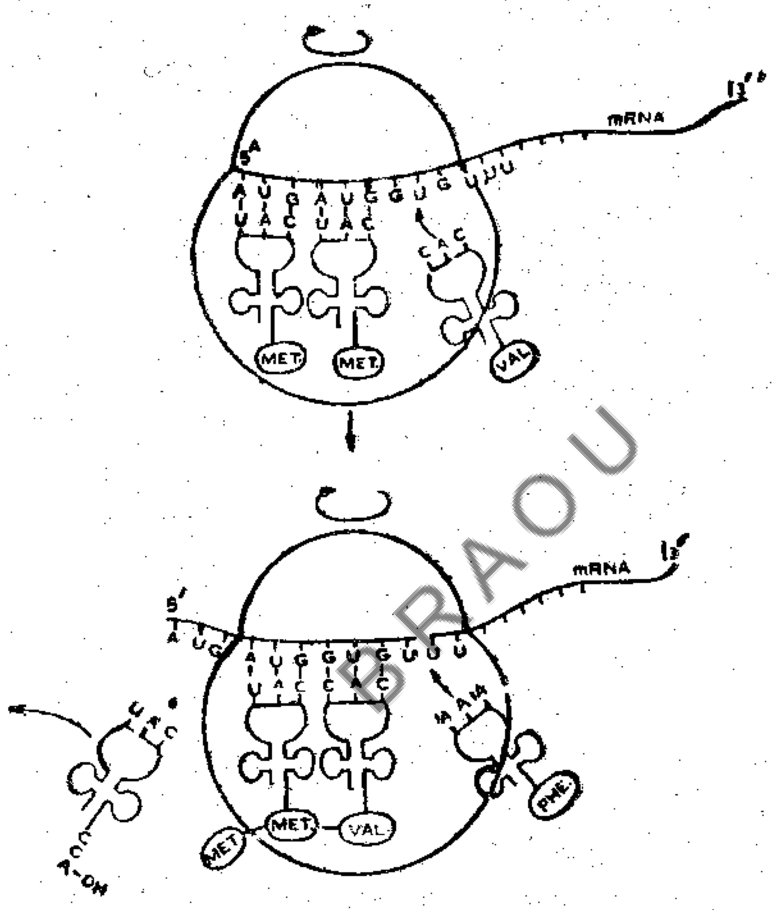

Fig. 23.11. The initial step of biding of tRNA to mRNA ribosome.

23.7. PROTEINS

Proteins are organic nitrogenous components of all the plants, animals and bacteria. They are large macromolecules consisting of a number of polypeptides (biopolymers) with molecular weight ranging from several thousands to several millions. Protein contain carbon, hydrogen, oxygen, nitrogen and frequently sulphur and rarely phosphorus and iron. The building block of proteins are the amino acids and they are grouped into macromolecules called polypeptides. The primary sequence of polypeptide chains are composed of different amount of the twenty amino acids. The peptide (-CO-NH-) bond which links the amino acids together is formed by the condensation of two amino group ($-NH_2$) of amino acid with the carboxyl group (-COOH) of another. Proteins have localized hydrophilic and hydrophobic area and have definite site for nucleophilic and electrophilic displacement, which imparts to them the unique catalytic activity.

and their molecular affinity. Proteins have four structural levels of organisation, which are referred to as primary, secondary, tertiary and quaternary structures.

Primary Structure: It is the linear polypeptide chain and is formed by linking of a sequence of amino acids with each other by peptide bonds in a line.

Secondary Structure: Each polypeptide chain may form sheet-like structure, or adopt a coiled or helical structure. The sheet- like structure is formed by hydrogen bonds between the oxygen and nitrogen atoms of the different group of polypeptide chains. These configurations are collectively known as B-structures, as described by Pauling by employing X-ray diffraction method. The regular arrangement of the polypeptide chain is brought by hydrogen bonding between groups of the same chain which results in the twisting forming usually in an x-helix.

Tertiary Structure: The tertiary structure of protein is three dimensional form and in many cases irregular, containing both helical and non-helical regions. The tertiary structures are folded into different configurations by the formation of hydrogen bonds, hydrophobic bonds and disulphide bridges between the two SH groups. The tertiary structure of protein is essential for the biological activity.

Quaternary Structure: The quaternary structure is composed of two or more polypeptide sub units, which are non-covalently linked . Of the four polypeptide chains in it, each of the two are composed of two species.

Proteins are classified on their solubility into simple proteins and conjugate proteins. Hydrolysis of simple proteins yield only amino acids. Albumin, Globulin, Protamines, Histones and Sclero- proteins are simple proteins. Conjugate proteins are grouped on the basis of the prosthetic groups to which they are attached. Proteins conjugate with nucleic acids, carbohydrates or lipids and form nucleo-proteins, muco-proteins or lipoproteins respectively. Proteins catalyze many enzymatic reactions. Large amounts of proteins are stored in plant tissues and supply energy and nitrogen during growth. Proteins are also classified into a)enzymes b) Storage proteins, c) contractile proteins, d) Structural proteins, e) hormones, f)toxins, etc.

23.8. SUMMARY

Proteins the macromolecules are organic nitrogenous components of all living beings. The synthesis of proteins is genetically controlled. The amino acids are building blocks of proteins. The DNA molecule consists of the genetic information for protein synthesis. A specific part of DNA called structural gene synthesises RNA. The synthesis of RNA complementary to a strand of DNA is known as transcription,and is catalysed by the enzyme RNA polymerase. There are three forms of RNA, distinguished on the basis of their molecular size and function. They are ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA). The ribosomes are the site of protein synthesis. The rRNA is present in ribosome and serve as a template for protein synthesis. The tRNA transfers the amino acid from cytoplasm to their proper position on the ribosomes hence the name. mRNA is the template that directs protein synthesis. The involvement of a specific sequence of three bases of mRNA coding for a specific amino acid is called as codon or triplet codon. Genetic code is non-overlapping.

The process involved in protein synthesis is broadly divided into four stages.

a. Activation of amino acyl with the formation of specific amino acid t RNA molecules.

b. The molecules of amino acyl tRNA bind to mRNA ribosome complex and initiate the peptide chain.

c. Elongation of the peptide chain.

d. Termination and release of the chain from the ribosome complex.

23.9. CHECK YOUR PROGRESS: MODEL ANSWERS

1. The ribosomes are the site of protein synthesis where amino acids are assembled in a specific sequence to produce polypeptide chains. The involvement of a specific sequence of three bases of mRNA coding for a specific amino acid is called codon or triplet codon.

2. a) Replication

b) Transcription

c) Translation.

23.10. MODEL EXAMINATION QUESTIONS

I. Answer the following question in 30 lines each.

1. Describe the process of synthesis of proteins from amino acids.
2. Describe the structure of RNA.
3. Given an account of the genetic code.
4. Describe the role of operator and regulator gene.
5. Write an account on the nature of proteins.
6. Describe the structural level of organisation of proteins.
7. What is the function of ribosomes.

II. Answer the following questions in 10 lines each.

1. Given an account of the synthesis of RNA.
2. Give an account of the role of tRNA in protein synthesis
3. Give an account of the function of mRNA.
4. Write briefly on the structure of proteins.
5. Describe the central dogma of molecular biology.

UNIT - 24: MUTATIONS

Contents

24.1 OBJECTIVES

After going through this unit you will be able to:

1. explain what mutations are, and how are they formed,
2. describe and different chromosomal mutations which involve changes in the chromosomal number from the one involving the change in the structure of chromosomes, and
3. understand the significance of mutations.

24.2. INTRODUCTION

There are two fundamental points on which evolution of plants and animals rest. They are (1) the presence of variations in the characters of the organisms (2) such variations are transmitted to the progeny faithfully. Now let us try to understand what is meant by variations. According to Darwin's theory of evolution, variation is the bed rock of evolution. First variations develop in the population and then nature selects those that are useful, they survive and multiply. On the other hand, those variations that are not useful, slowly get eliminated. During his wide travels Darwin observed numerous examples of sudden and distinct variations arising in populations. He named them 'Sports' and did not give them much importance in evolution. On the other hand, he considered small and continuous variations in organisms as being pivotal to evolution. Darwin thought that evolution is a slow and continuous process and small variations go on accumulating for generations to give rise to distinct varieties or species.

In 1880 Hugo de Vries of Holland studied the populations of *Oenothera lamarkiana* near Amsterdam for about 8 years. He saw among them numerous variations. All these variations were breeding true. Some were very tall and were called 'gigas' while other varieties were drawf called 'nanella'. He collected these varieties and cultivated them in his garden and observed the progeny for several generations. In those populations de Vries identified 7 sub species. de Vries gave the name mutation to those sudden and discontinuous variations in organisms which are heritable and he also believed that all those observed variations in *Oenothera lamarkiana* were, indeed mutations only.

In the year 1901, de Vries published all his observations in the form of a book entitled "The Mutation Theory". Of course, after nearly 50 years, scientists realised that what were termed as mutations by de Vries in *Oenothera* were not gene mutations but were chromosomal aberrations and polyploids. What is mutation? We have learnt that genes of the organism are located in chromosomes. The genes are made up of DNA. A gene is thought to be an entire DNA molecule that controls a particular reaction of the cell. Together all the genes of an organism control the life activities of the organism. At each cell division, the DNA molecule of the cell also gets duplicated accurately. Thus exact copies of the genes are produced. This continues millions of times without a mistake. Thus, we say generally, the genes are very stable.

But occasionally, sometimes a mistake may occur in the duplication of a gene. For some unknown reasons, which are yet to be understood fully, a nucleotide of a DNA molecule may be lost or may be substituted or an extra nucleotide may be added. Any one such change is enough to modify the structure of the DNA molecule. What is now formed is no more an exact copy of the original DNA. This in its turn changes the genetic message. This may cause synthesis of a new protein. This kind of change in the genetic message is called mutation. Thus according to the modern concept, mutations are hereditary changes due to alterations of genes or Mendelian factors. These are called gene mutations or point mutations. Sometimes it will be difficult to draw a line between gene mutations and chromosomal changes, which occur quite frequently. These chromosomal changes are also to be considered as mutations because due to these changes, not only genotypic and phenotypic variations are seen in the progeny but they aid in evolution also. So in this unit, while giving greater attention to gene mutations, we study chromosomal mutations and also cytoplasmic mutations.

24.3. GENE MUTATIONS

During the years 1909-1912 two scientists did considerable work which enhanced our knowledge of mutations. They are T.H. Morgan and R.A. Emerson. Morgan worked on the fruit fly, *Drosophila melanogaster,* at Columbia University, U.S.A., while Emerson worked on *Zea mays* at Cornell University, U.S.A. Morgan raised thousands of fruit flies in bottles, supplying them mashed banana. All those fruit flies had red eyes. Suddenly, Morgan found that one, among these thousands, had white eyes instead of red eyes. He also discovered that the particular white-eyed fruit fly was a male one. It became clear that the gene responsible for the red colour of the eyes, present on X chromosome had undergone mutation to give rise to white colour, which is recessive. This is a permanent change, and a clear example for gene mutation. Subsequently, hundreds of such gene mutations were discovered in the fruit fly. Gene mutations were observed in maize, snapdragon, bacteria and viruses and also in man.

A mutation may have only a minor effect in the cell. It may be so insignificant that it cannot be easily detected. At the other end, if a mutation affects the production of an important enzyme, such a change may cause the death of the cell. Such mutations are spoken of as lethal mutations. Most of the mutations studied by biologists lie between these two extremes, so that the effects of the mutation can be observed and at the same time it is not harmful to the cell.

Genes normally mutate only once, thus their alleles are formed. In fact, the contrasting pairs of characters observed by Mendel in *Pisum sativum,* namely Tall vs. Dwarf; Round, vs. wrinkled etc., are formed due to the mutation of the factors or genes. But there is no rule that a gene can mutant only once. Biologists have observed that a particular gene may mutate repeatedly many times. In such an event, a series of multiple alleles are formed. For example, biologists have found atleast 14 alleles for the white colour of the eye in *Drosophila.*

A mutant gene sometimes not only affects one particular character but may also affect certain other basic chemical reactions and thus other characters may also be affected. In *Drosophila melanogaster,* the gene mutation that brought about white eye colour also affected its fertility and longevity. Likewise in *Gossypium* as a result of a gene mutation, lintless seeds are produced. Along with this change, the plant's size, height and fertility also were affected. Such genes which affect several unrelated characters simultaneously are called pleiotropic genes. This phenomenon is called pleiotropy.

Classification of mutations

- Mutations
  - Gene mutations
  - Chromosomal mutations
    - Numerical
      - Chaanges in the number of sets of chromosomes
        - Polyploids
        - Haploids
      - Changes in the number of chromosomes
        - 1. Trisomics
          2. Tetrasomics
        - 1. Monosomics
          2. Nullisomios
    - Structural
      - Changes in the number of genes
        1. Deletion or deficiency
        2. Duplication
      - Changes in the arrangement of genes
        1. Inversion
        2. Translocation
  - Cytoplasmic mutations

24.3.1. Types of Mutations

Many of the gene mutations are recessive, and generally they are lethal. A few of them are dominant. Some gene mutations are useful to the plant to adjust itself to the changed environment and thus help in the evolution of the species. Sometimes mutations are neutral and go unnoticed. Besides mutations can be germinal or somatic, sexlinked or autosomal.

Germinal mutations are those that occur in the germ plasm i.e. in the reproductive parts of an organism. In 1791 in U.S.A as a result of germinal mutation in sheep, a short legged sheep arose. At present this variety predominates the world over. Another example for germinal mutation is drawf mutation in wheat. As germ cells are affected, the mutations are transmitted to the next generation through the gametes, each and every cell of the progeny possesses the mutation.

Mutations that occur in the vegetative parts of the organism are called somatic mutations and these, unlike the germinal mutations do not get transmitted to the progeny, and so are lost for ever with the death of the individual. Multiplication of the mutant somatic cell results in the mosaic of mutant and non-mutant tissue. In plants which are vegetatively propagated such mutations can be transferred and perpetuated. The famous apple variety called Golden delicious, arose as a somatic mutation and a few twigs bore such fruits. Vegetative propagation of those twigs resulted in the introduction of this new variety, which is now worldwide in distribution.

Depending upon the results, there are different kinds of mutations. The mutations may differ from the parents in morphological, physiological and biochemical characters.

When genes undergo mutation, changes in the morphological characters are commonly observed. Some mutations result in the change of one organ into another. For example, in *Drosophila*, as a result of mutation, the antennae change into legs. Certain mutations do not cause any visible changes but bring about a change in the physiology of the organism. For example, in the fungus, *Neurospora* as a result of mutations the nutritional needs of the mutants got changed. In bacteria, mutations may result in either increasing or reducing their virulence, also, it may result in production of penicillin resistant or bacteriophage resistant varieties.

As we know gene is a linear stretch of DNA molecule, may be an entire molecule or a part of it. Changes in the molecular level of the DNA molecules result in mutation. This change at molecular level sometimes bring about observable changes. Such changes can occur at any place in a gene. A change in the sequence of the nucleotides in DNA, brings about a change in the genetic code and thus mutation occurs. Once change has taken place it can be transmitted to the progeny. Changes in the nitrogeneous bases of the nucleotides can be brought about in many ways.

1. Addition of a pair or more of nucleotides to the DNA. This is called insertion. An addition of even a single pair of nucleotides makes all the difference. To understand this, here is an analogy.

Intended message.	:	Please say where you are.
Actual message.	:	Please stay where you are.

Here an insertion of a single letter 't' in the word 'say' brings about an entirely different meaning.

2. Sometimes a pair of nucleotides may be missing. This is called deletion. Consider the following analogy.

Intended message	:	Will send friend to get the book.
Actual message	:	Will send fiend to get the book.

When the letter 'r' is deleted in the word friend, an entirely different meaning results.

3. At times two or more nucleotide pairs may exchange places. This is called inversion. Consider the following analogy.

Intended message	:	No arms will be supplied.
Actual message	:	No rams will be supplied

The two letters 'a' and 'r' of the word arms exchange their place, giving a different meaning.

4. Yet another way is called substitution. Here one nucleotide may get substituted for another. Read the two following messages.

Intended message	:	Ram now going to Madras.
Actual message	:	Ram not going to Madras.

The letter 'w' in the word 'now' is substituted by 't' in the actual message giving an altogether different meaning.

Gene Mutation in Man: A very subtle change at the molecular level of the DNA brings about mutation of the gene and it may result sometimes in far reaching changes, with serious consequences. To illustrate this point let us consider a case of gene mutation in man.

The red colour of red blood cells is due to the oxygen-carrying pigment, haemoglobin, which is a protein. The haemoglobin molecule is made up of two halves, each of which contains about 4000 atoms arranged in some 300 aminoacids of 19 different kinds. The red blood cells which carry haemoglobin are biconcave discs.

Mutation in Haemoglobin: There is a gene mutation in haemoglobin as a result of which an abnormal haemoglobin, naemoglobin S is produced. The haemoglobin S is not efficient enough in carrying oxygen as the normal haemoglobin. Individuals having this haemoglobin S suffer from a disease called sickle cell anaemia, a genetic disorder. In such persons, the red blood cells are sickle shaped instead of being biconcave discs and the victim suffers all symptoms of extreme oxygen shortage, weakness, emaciation, kidney and heart failure. All these disorders are due to the fact that haemoglobin S is inefficient in carrying oxygen.

Part of amino acid chain of normal haemoglobin		Corresponding amino acid Chain of haemoglobin S.
histidine		histidine
Valine		Valine
Leucine		Leucine
Leucine		Leucine
Threonine		Threonine
Proline		Proline
glutamic acid	replaced by	Valine
glutamic acid		glutamic acid
lysine		lysine

IMGRAM of Cambridge University, United Kingdom worked on this problem, to find out where exactly the chemical change has taken place in the haemoglobin molecule. His careful and pains-taking study showed that one half molecule of the haemoglobin S differs from the normal haemoglobin in only one amino acid. At one place in the chain of amino acids, glutamic acid is replaced by valine, an amino acid essential to many proteins including haemoglobin but disadvantageous at this particular spot.

The evidence is clear that the mutant or changed allele responsible for the sickling of red blood cells causes valine unit to be substituted for glutamic acid unit at one point in the molecule. A change of only one amino acid in such a large molecule of 300 amino acids, without altering the rest, can bring about enormous change in the nature of the protein and result in so many disorders in the victims.

Check Your Progress - 1

Sickle cell anaemia, a genetic disorder caused due to ______________________ in haemoglobin

Note:(a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

Haemophilia: Another example for gene mutation in human beings is haemophilia. This is a disorder in which the blood cells fail to clot after an injury or clots very slowly. In extreme cases the victims may bleed to death even from a small cut.

This has a royal history. It probably arose as a gene mutation in Queen Victoria. Her ancestors are not known to have this disease. From her, this trait become widely distributed in the many royal families of Europe. This is a sex-linked gene mutation and is carried on X chromosome. Invariably it is the male who suffers from this disorder while the women are generally only carriers. The Y chromosome in the male has no role to play in this.

24.3.2. Frequency of Gene Mutation

Scientists have long been interested in finding out how often genes mutate as that would give them some idea about how often new kinds of organisms appear on this planet.

From their studies, scientists know that there is a wide range of variations in the rate of gene mutation. Some genes mutate frequently, once in every 2000 gametes. Other genes do not mutate even within several billions of cell divisions. Between these extremes, most genes, including those of human beings mutate once in a hundred thousand to a million gametes in each generation. In bacteria, the mutations rate may be as low as 1 in ten billions.

From this data, it may appear that mutations do not occur frequently enough to provide much genetic variability. However, it should be borne in mind that this data applies only to one gene. We do not exactly know how many genes there are in any organism. Apparently it must be in large numbers. In human beings, it is estimated that there are about 10,000-genes. In that case, if each gene mutates at about the same rate, then more than one in every 100 gametes would have a mutation of at least one gene. If the number of individuals in each species is in millions, then mutation in each generation can be very large indeed. Thus mutations provide a large amount of genetic variations in organisms.

24.3.3. Spontaneous and Induced Mutations

Mutations may be autogenous or exogenous. Autogenous mutations are caused in organisms due to internal conditions. These are also called spontaneous mutations. The exact causes that are responsible for spontaneous mutations are not clearly known. In some plants like *colchicum*, due to metabolism, substances like colchicine are produced. Such substances can bring about mutations.

On the other hand, a large number of mutations are caused by external agents. These exogenous mutations are also called induced mutations. Some of the causes that are effective in increasing the mutation rate are given below.

Radiation: High energy radiations such as X-rays, gamma rays, neutrons, ultraviolet light have all been shown to increase mutation rate.

For the first time, it was the american biologist H.J. Muller who in 1927 subjected *Drosophila melanogaster* to X-ray radiation to induce mutations. From his experiments conducted at Indiana University, U.S.A., Muller estimated that formation of induced mutations when x-rays are used, is about 150 times to those naturally occurring in *Drosophila*. He also found out that by far the larger number of such mutations are harmful. The x-rays and gamma rays are shortest and hence the most energetic wave lengths of radiation energy. These energetic waves are capable of distorting the base sequence of DNA molecule by substitution, deleting or rearranging the nitrogenous bases. This in its turn impairs the cell's ability to synthesize proteins and produces errors in coding. The implications of this are far reaching and the effects could be even lethal. Radiation cannot be seen but its effects, specially those on living matter are easily detected. The severe injuries caused by the atomic bombs dropped on Hiroshima and Nagasaki in Japan are well known. Even though it is now 40 years since those bombings, mutations caused by radiations are still visible in the newly born babies.

2. Chemicals: After the discovery of mutagenic effects of radiation, scientists began the search for chemical substances that induce mutations. Thom and Steinberg in 1939 reported that nitrous acid can induce mutations in the fungus, *Aspergillus*. Auerbech and Robson reported in 1946 that nitrogen mustard gas and related substances can increase the rate of mutation in *Drosophila*. To day there is a long list of oils and chemicals which are mutagenic. Contaminated vegetable oils like groundnut, mustard, castor are now regarded as mutagenic. Hydroxylamine, Ethyl methyl sulphonate are powerful chemical mutagenics.

3. Temperature: It is reported that when the temperature of the reproductive cells is increased within a narrow range the organism can tolerate, there will be an increase in the rate of mutation.

24.4. CHROMOSOMAL MUTATIONS

These are also called chromosomal aberrations. Like gene mutations, these also arise suddenly in nature. These are of two kinds. They are (A) Changes in the number of chromosomes (B) Changes in the structure of chromosomes.

24.4.1. Changes in the Number of Chromosomes

(1) Euploidy: In the cells of all higher plants and animals there will be two sets of chromosomes, one derived from the male gamete and another from the female gamete. The set of chromosomes from one gamete is called a genome. Thus the cells of all higher plants are diploid, or, we may say have two genomes. The number of chromosomes for each plant and animal is fixed. Chromosomal mutation that involve sets of chromosomes is called euploidy. If the number of sets of chromosomes in any plant is more than two, then it is called polyploidy and such plants are called polyploids. On the other hand if the number of chromosome sets is one then it is a haploidy

Types of Euploidy

Type	No. of chromosome sets	Example
Haploidy	One(n)	A B C
Diploidy	Two(2n)	A A B B C C
Polyploidy	More than 2	
a) Triploidy	Three (3n)	AAA BBB CCC
b) Tetraploidy	Four (4n)	AAAA BBBB CCCC
c) Pentaploidy etc.	Five (5n)	AAAAA BBBBB CCCCC

Polyploidy: This is very widely seen in the plant world. It is estimated that nearly half of the plant genera are polyploids. Many of our economically important plants are polyploids eg., Plantain, Pine apple, tobacco etc. Among grasses, two thirds are polyploids. The variety 'gigas' in *Oenothera lamarkiana.*, identified by De Vries as a mutation, is in fact not an example of gene mutation, but a polyploid. The normal diploid *O. lamarkiana* has 2n = 14, while gigas has 28 chromosomes. This is an autotetraploid. Polyploids are rare in the animal world.

Polyploids are of two kinds. In some, polyploids arise as a result of duplication of the chromosome sets of the same species. These are called autopolyploids. When chromosome sets of two or more species combine to form a polyploid, it is termed as allopolyploid.

Polyploids may arise among diploids due to any of the following reasons. 1. An egg cell may get fertilised by more than one sperm 2. As a result of failure of meiosis in spore mother cells, the gametes so formed will be diploid. Fusion of such gametes result in polyploids. 3. During mitosis in a diploid somatic cell, if the chromosomes duplicate and divide but there is failure of cytokinesis, a tetraploid cell forms. Normal divisions of such a cell give rise to a tetraploid tissue. The flowers borne on such plants produce diploid gametes. Fusion of such gametes bring about tetraploids.

Triploids are formed when two gametes, one with haploid number of chromosomes and another with diploid number are united, or when a egg cell is fertilised by two sperms. In triploids there will be three genomes (3n). But in these plants as meiosis is highly irregular sterlity is prevalent. However such plants can be propagated vegetatively. Some of our common, economically important plants are triploids eg. Musa, Grape, annanas, rose, chrysanthemum, *psidium.*

Tetraploids have 4 sets of chromosomes. In Autotetraploids, the diploid chromosomes themselves get doubled. These are formed when abnormal gametes with diploid chromosomes unite in fertilisation. Eg. *Arachis hypogea*, coffee, potato. Allotetraploids are formed in a different manner. Fertilisation between gametes belonging to two different species results in a diploid plant. Such a hybrid plant generally will be sterile. However, when such diploid chromosomes get doubled naturally or artificially, the result is a allotetraploid. Of the four sets of chromosomes in that plant, two sets belong to one species, and two other sets to another species. This tetraploid will be fertile plant. To understand this, let us consider the development of a new genus *Raphanobrassica.*

In 1927 Karpachenko crossed *Raphanus sativus* (2n = 18) with cabbage, *Brassica oleracea* (2n=18). The result was a hybrid (2n = 18) which is a sterile plant normally. However occasionally this produces abnormal diploid gametes. Such fertilisation resulted in a new plant which is fertile. This is *Raphanobrassica* (2n=36). Of the 36 chromosomes 18 belong to *Raphanus sativus* and 18 to *Brassica oleracea.* Thus a allotetraploid is born.

Raphanus sativus X *Brassica oleracea*
2n= 18 2n= 18
(R) (B)

F1 hybrid 2n = 18
9 R + 9 B.
Raphanobrassica. 4n = 36
18 R + 18 B.

When compared to diploids, tetraploids have leaves, flowers, fruits and seeds of larger size*.

There are many economically important plants which have even more sets of chromosomes. In the sweet pea, *Triticum vulgare* there are hexaploids (6x). In the sugarcane, there are octaploids (8x) different genomes in them.

Induced Polyploids: There are some agents, which when applied to plants induce polyploidy. By applying some chemicals, through temperature shocks and causing mechanical injuries to tissues, polyploidy can be induced. Chemicals like colchicine, acenaphthene are believed to interfere with the spindle formation in cell division, thus leading to polyploidy. Colchicine is an alkaloid present in the bulbs of *Colchicum autumnale* of Liliaceae. This is applied to buds, roots of plants in very dilute aqueous form. As a result, the chromosomes during metaphase do not move to the poles but remain scattered in the cytoplasm.

Even cytokinesis is prevented, and with duplication of chromosomes, their number goes on increasing. In the tomato plant, when the stem tips are cut, disorganised cell division takes place and callus is formed. Most of the cells of such tissue are tetraploids, while the rest of the plant is diploid. Thus mechanical injury also induces polyploidy. In plants like maize, polyploidy can be induced by temperature shocks, which also disorganises cytokinesis.

Variations in larger numbers are seen in polyploids than in diploids.Due to polyploidy, new physiological characters arise, ultimately leading to the formation of a new species. E.B.Babcock examined 196 species belonging to the genus *crepis*. He found out eight percent of these arose as polyploids. Autopolyploids may not contribute much towards formation of new species. But allopolyploids certainly help in evolution of new species as there are different genomes in them.

Haploidy: In all higher plants and animals, the organisms are diploid and haploidy is confined only to the gametes. But occasionally haploid plants are seen in nature. Like gametes, these have one set of chromosomes or one genome only. In angiosperms haploid plants are seen in *Brassica, Datura, Gossypium, Oryza, Triticum, Oenothera, zea mays* etc. In animals, haploids are very rare. Haploids are seen in ants, honeybees occasionally. Haploid plants may arise due to any of the following reasons.

1. An egg cell, not fertilised by a male gamete, develops into an embryo and then into a plant. The pollen grains supply only stimulus, but not male gamete. This development is called female parthenogenesis.
2. Sometimes a male gamete after entering the egg cell without any role of the egg cell, may grow into a haploid embryo. This is called male parthenogenesis.
3. Sometimes a synergid cell-a haploid cell of the embryo sac - may develop into an embryo.
4. Sometimes both the male gametes may enterr embryo sac unite with the diploid secondary nucleus; thus forming a tetraploid endosperm mother cell. As for the egg cell, which received no sperm, may get stimulus and grow into a haploid embryo.

* An allopolyploid in which the total chromosome complement of the two species is combined to form a fertile hybrid is called amphiploid. eg. *Raphanobrassica*.

Haploid plants do not grow well and are usually sterile. They are smaller in size than diploid plants. As only one set of chromosomes are present, meiosis usually is irregular. Rarely haploid gametes are produced, which on fertilisation result in diploid plants.

Check Your Progress - 2

Mention the types of euploidy and number of chromosome sets associated with each type.

Note: (a) write your answer in the space given below.

(b) compare your answer with the one given at the end.

....

....

....

....

....

....

....

Heteroploidy or Aneuploidy: Sometimes some organisms may have chromosomes, fewer or more than the exact multiple of basic number of haploid number of chromosomes of the organism. Such an organism is called aneuploid or heteroploid and that condition is called aneuploidy or heteroploidy. The number of chromosomes in such a plant may be a little more or less than the normal diploid number. Heteroploids are seen in plants and animals as well in human beings. *Datura, maiz* wheat, tomato are examples of heteroploid plants. In *Drosophila* several kinds of heteroploids are seen. Addition or deletion of chromosomes brings about phenotypic as well as genotypic changes.

Sometimes the gametes of the same species may have different haploid chromosome number. Fusion of such gametes results in heteroploidy. Irregularity in meiosis, results in formation of gametes with different chromosome number. This may be due to

1. Non-disjunction of homologous pair during meiosis as a result of which both the chromosomes reach one pole only.
2. At the beginning of meiosis there may be failure in pairing of homologous chromosomes.

Thus the gamete formed may have chromosome number like n_1, (n+1), (n-1), (n+2), (n-2) etc. When gametes like these fuse, it results in different types of heteroploids. They may be 1.Trisomics, 2. Tetrasomics, 3. Monosomics, 4. Nullisomics. Diploid plants are called disomics.

Variations involving individual chromosomes within a diploid set.

Type	No. of chromosomes	Examples
Disomic	2n	AA BB CC
Trisomic	2n+1	AA BB CC
Tetrasomic	2n+2	AA BC CCCC
Monosomic	2n-1	AA BB C
Nullisomic	2n-2	AA BB

Trisomics: These have one extra chromosome 2n+1. One of the chromosomes may have three homologous chromosomes. If there are two extra chromosomes, belonging to two different chromosomes, it is then called double trisomic (2n+1+1). These are seen in *Datura*, *maize*, tomato. Bridges recognised two trisomics in *Drosophila* XXX and XXX.

Tetrasomics: There are organisms with (2n+2), i.e. 4 homologous chromosomes for one of its chromosomes. eg. *Datura*. These look like trisomics.

Monosomics: In these plants the number of chromosomes will be one short of the diploid number (2n-1). In *Nicotiana* (2n=48) 24 monosomics are identified. Likewise in *Triticum vulgare* (2n=42) 21 monosomics are identified. Bridges identified some monosomics in *Drosophila*.

Nullisomics: In these plants one entire homologous pair may be missing (2n-2). These are formed when self pollination takes place in monosomics. Some nullisomics like those of tobacco cannot survive, while those of wheat survive.

Aneuploidy in man: Human beings normally have 46 chromosomes. Sometimes aneuploidy is seen in some individuals. Such persons may have less than 46 chromosomes or more.

In some individuals there is only one 'X' chromosome. Such individuals have only 45 chromosomes. This condition 'XO' is called Turner's syndrome. These are underdeveloped females with broad shield like chest, underdeveloped breasts and female external genitalia, abnormalities of skin, skeleton and stature.

Another abnormality is where individuals have one extra chromosome. They have 47 chromosomes. They have three sex chromosomes 'XXY'. This condition is called Klinefelter syndrome. The males having syndrome have very small testes, are long- legged, with slightly developed breasts.

Some individuals have 47 chromosomes. The 21st chromosome has three homologous chromosomes. This condition is called Down's syndrome. Such individuals are called Mongoloid idiots'. This is also an example for trisomic.

Addition or deletion of even a single chromosome, as we have just now seen, will have serious consequences for the individuals.

24.4.2. Changes in the Structure of Chromosomes

This expression and inheritance of the sum total of characters of an organism is determined by the number and sequence of genes present on the chromosomes in its cells. We have seen how changes in the number of chromosomes affect the characters of organisms. Likewise changes in the architecture and morphology of the chromosomes also affect the characters. These alterations are the result of the changes in the sequence of genes on the chromosomes. These are also called chromosomal aberrations. These chromosomal aberrations are caused by breaks in the chromosomes or chromatids. When broken ends rejoin to restore the original chromose structure and gene sequence there will be no change at all. However, when the broken ends are either lost or get joined to a wrong chromosome or in a wrong manner to the same chromosome, then chromosomal aberrations occur. Much of our knowledge about chromosomal aberrations came from the study of two organisms. They are *Drosophila melanogaster* which has only four pairs of chromosomes, and *zea mays* where there are ten pairs of chromosomes each pair of which is long and distinct. Thus these two are ideal for the study of the morphology and structure of chromosomes.

A. Changes involving the number of genes: a) Deletion or deficiency: Sometimes a part of the chromosome may get broken. This may happen at the apex of the chromosome (terminal break)or anywhere in the chromosome (intercalary break). When such bits are lost in the cytoplasm, some genes are also lost with it. If a chromosome has a gene sequence A B C D E F G H I,a break occurs between CD and FH, and the bit DEF is lost; then a new chromosome is formed with the gene sequence as A B C G H I, which will be shorter than the original. This deletion affects some characters of the organism.

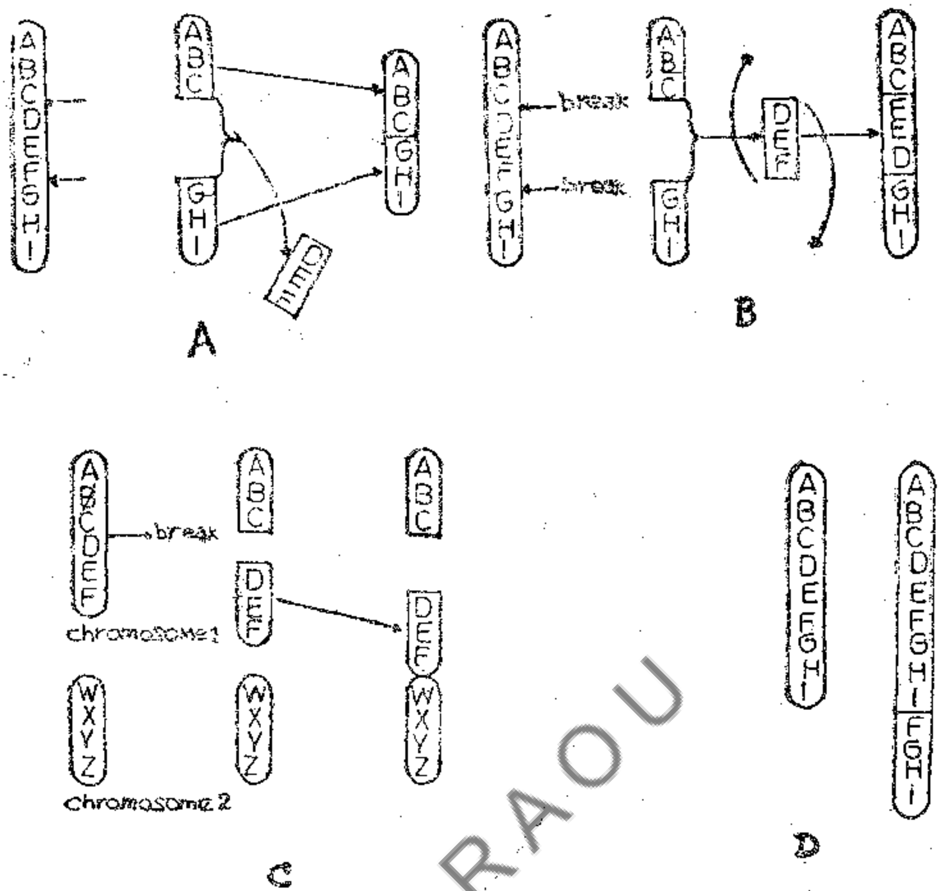

Fig.28.1. Diagram showing different types of chromosome mutation. Each type of mutation results in alteration in the number and/or sequence of genes on the chromosome. A. Deletion. B. Inversion. C. Translocation. D. Duplication.

b) Duplication: The presence of a part of a chromosome in excess of normal complement is called duplication. A broken section of a chromosome may get attached to a normal homologous chromosome. Consequently some genes are present in more than double doses. A B C D E F G H I is the normal chromosome. An addition of a broken bit FGHI to it results in a chromosome. A B C D E F G H I/F G H I Duplications are more frequent in nature than deletions. These are not harmful to the organism but result in morphological changes.

B. Changtes Involving Arrangement of Genes: a) Inversion: In this there is a break in the chromosome at two points. The broken section rotates by 180^{0} on its own axis and reunites with the same but in a reverse order. Thus there is neither gain nor loss of genes in the chromosome. A B C D E F G H I is a chromosome in which a break occurs between C D and F G. After inversion, it would be A B C D E F G H I. Inversions may include centromere calle pericentric inversions; those that do not include centromere are paracentric inversions. These are helpful for formation of new species.

b) Translocation: This involves the transfer of a part of a chromosome or a set of genes to a non-homologous chromosome. It results in a change in the sequence and position of genes. Let us say, there are two nonhomologous chromosomes A B C D E F and W X Y Z. A break in the former between C D occurs and the bit D E F so formed gets attached to the letter. So a new chromosomes D E F W X Y Z is formed.

Due to structural changes like inversion and translocation, structural hybridity of chromosomes is formed. This brings about changes which have lasting effect.

Cytoplasmic Mutations

We have already seen that there are plasmagenes in the cytoplasm. Also it is now established that organells like chloroplasts mitochondria have DNA. The plasmagenes also get mutated and transmit characters accordingly. Variegated part of plants are now recognised as due to mutations in chloroplasts. Different strains of yeast are formed due to mutations in the mitochondria present in the yeast.

24.5. SIGNIFICANCE OF MUTATIONS

Undoubtedly mutation is the principle factor in organic evolution. It is mainly due to mutations that there is so much of genetic variability without which evolution may not have possible. It is also true that a very high percentage of these mutations are harmful and a small percentage nearly 25% are useful. It is these useful mutations that bring about changes in the organisms so that they can adapt themselves the environment and can stand competition in natural selection, so that their progeny survives. All the now existing plants and animal are those formed due to mutations and have come out successful in the struggle for survival. Thus natural mutations played and continue to play a crucial role in the evolution of organisms.

Induced mutations are useful in developing new varieties of plants suitable to different environmental conditions. As so many variations are before him, it is possible for the plant breeder to select those that have desirable characters for his hybridisation experiments through which he can raise superior varieties.

List of cultivated species which arose through polyploidy from wild ones.

Crop	**Common name**	**Level of ploidy**
1. *Avena sativa*	Cultivated oats	Hexaploid
2. *Triticum aestivum*	bread wheat	allohexaploid
3. *Sorghum vulgare*	cultivated sorghum	tetraploid
4. *Nicotiana tabccaum*	tobacco cultivated	allotetraploid
5. *Gossypium barbadense*	sea island cotton	allotetraploid
6. *Brassica juncea*	rai	allotetraploid

List of mutant varieties of crop plants released in the India.

Type of crop		**mutant varieties**
1. Wheat	NP 836	Sharbati Sonara
2. Pea	Hons	
3. Rice	Jagannath	IIT 60, k 84, HM 95
4. Castor	Aruna	
5. Cotton	Indora-2, MCU-7	
6. Groundnut	TG 1	

24.6. SUMMARY

Mutations are heriditary changes. These are caused due to alterations of genes or Mendelian factors hence called gene mutations or point mutations. Chromosomal changes are also to be considered as mutations because due to these changes genotypic and phynotypic variations are seen. Chromosomal mutations are of two kinds. They are (A) changes in the number of chromosomes (B) changes in the structure of chromosomes. Changes in the number of chromosomes include euploidy, polyploidy, haploidy heteroploidy. Changes in the structure of chromosomes include deletion or deficiency, duplication, inversion, translocation. Mutation is the principle factor in organic evolution. It is mainly due to mutations that there is so much of genetic variability, with out which evolution might not have been possible. Induced mutations are useful in developing new varieties of plants suitable to different environmental conditions.

24.7. CHECK YOUR PROGRESS: MODEL ANSWERS

1. Gene Mutations.

2.

Type	**No.of chromosome sets**
Haploidy	One (n)
Diploidy	Two (2n)
Polyploidy	More than 2
a) Triploidy	Three (3n)
b) Tetraploidy	Four (4n)
c) Pentaploidy	Five (5n)

24.8. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Write briefly about chromosomal mutations which involve changes in the number of chromosomes.
2. What are gene mutations? How does they influence plant and animal life?

II. Answer the following questions in 10 lines each.

1. Write about the gene mutations in man.
2. Write about structural changes of chromosomes which involve arrangement of genes.

UNIT-25: BRIEF ACCOUNT OF GENETIC ENGINEERING

Contents

25.1. OBJECTIVES

By the end of this unit you will be able to:

1 understand the techniques of genetic engineering,

2 explain the potential applications of recombinant DNA technology.

25.2 INTRODUCTION

Biologists have made experimental progress during their lifetime in developing better varieties of plants, lower animals and in understanding the mechanisms of genetic change. In 1974 Fletcher have pointed out the doubling time for scientific knowledge in general is 10 years, 5 years for life science and 2 years for genetics. Thus a good deal has been known by now about genetics from molecular processes involved in recombination, transduction, mutation, transcription, translation, DNA replication etc. Recent advances in molecular and cell biology have raised the hope that a new technology may soon be developed to correct and improve the genotype by directly acting at DNA level. The bulk of human genetic material is under going a slow decline in quality due to several reasons. So the need for genetic improvement lies in these general approaches - 1. over riding the expression of defect producing genes, 2. directed recombination and genetic engineering. Therefore, the most recent rapidly advancing frontiers of molecular biology is 'genetic engineering', where the normal genetic make-up of a cell responsible for specific function is altered by manipulating the genome to fulfil the new functions. It involves a wide range of techniques, an important one being the selective alteration of DNA in bacterial cells by new genes donated from any organism like animal or plant, so that the altered cell will produce non bacterial protein with the reconstructed DNA of the recipient bacterium. The modified bacteria are referred to as 'recombinants', because in addition to their own genes, they contain complete or a part of gene from plant, animal or human cells. Thus the new tools of genetic engineering and biotechnology have revolutionized the chemical industry and they can also change the face of agriculture, forestry, animal breeding and medicine in the coming years. Its important achievement and possibilities for the future of mankind also lies in the synthesis of artificial genes and making them function, as if they were the natural genes of the cells into which they have been transplanted.

Recombinant DNA and gene cloning technologies provide a method for molecular biologists by which genes or segments of chromosomes can be isolated, replicated and studied by nucleic sequencing techniques, electron microscopy and other analytical techniques. There are various procedures for gene cloning.

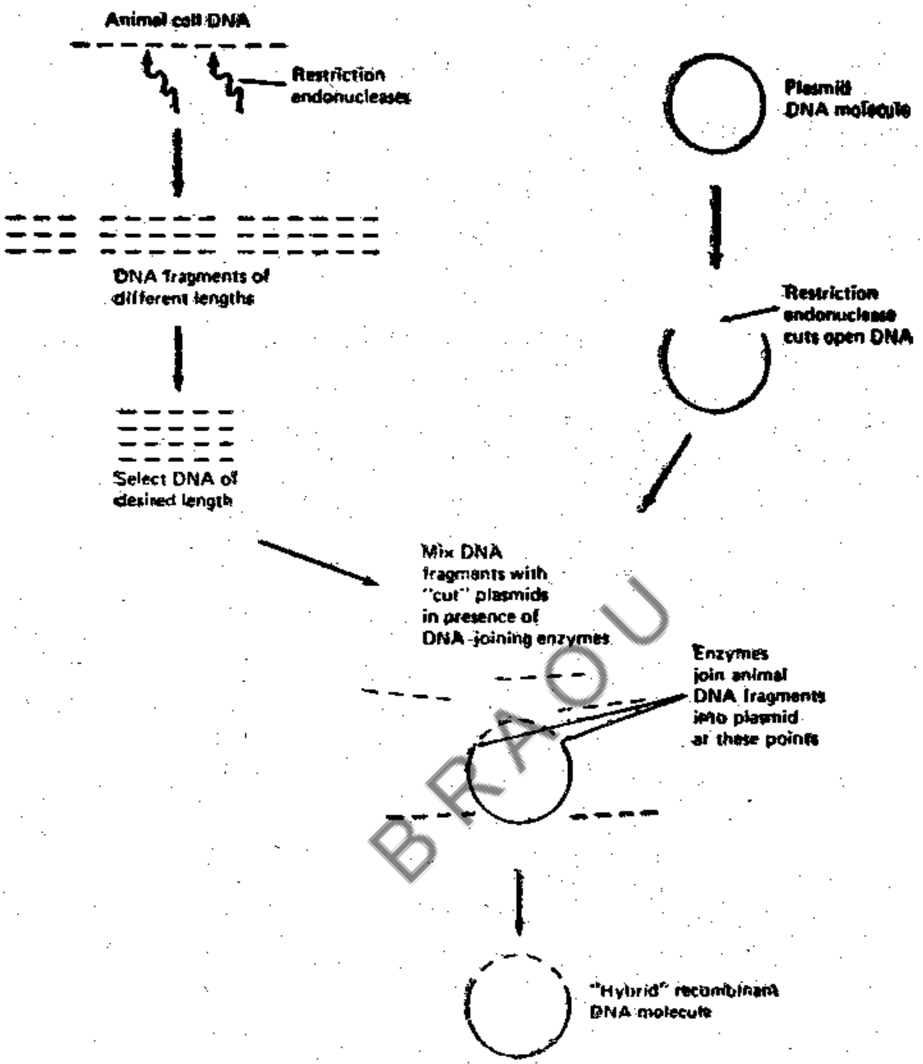

Fig.25.1 Simplistic view of generation of recombinant DNA

25.3. IDENTIFICATION AND ISOLATION OF DESIRED GENE

DNA possesses nucleotides that code for many proteins. In actual sense only a small amount of DNA in each chromosome codes for proteins. A special type of enzymes called restriction endonucleases (nuclear scissors) are used to cut DNA of the donor into small fragments at a particular point. Thus they can produce a few hundreds to a few thousand DNA fragments. A number of endonucleases that permit the break of DNA strands of known length, have been isolated. Once the DNA is fragmented, the desired DNA sequence or gene must be identified. Thus the desired DNA fragment is inserted into a virus or plasmids. Plasmids are autonomous extranuclear genetic elements (circular DNA) existing in bacterial cytoplasm.

Plasmids are also cut open by specific restriction endonucleases. The previously isolated eukaryotic DNA fragments from the donor are inserted into plasmid DNA by the enzymatic methods (Genetic Technology - a New Frontier, 1982). The final result is the generation of a hybrid recombinant DNA molecule (Fig:25.1) - a bacterial plasmid DNA molecule with a hybrid plasmid can be further subcultured into an unlimited number of additional colonies.

Check Your Progress - 1

A special type of enzymes called _____________ are used to cut DNA of the donor into small fragments at a particular point.

Note: (a) Writing your answer in the space given above.

(b) Compare your answer with the one given at the end.

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25.4. GENE CLONING

various procedures for gene cloning are in use in order to clone a piece of DNA.

(a) Cloning vehicles: Plasmids and phages are two vehicles or vectors for carrying genes into bacteria. As such, they have become tools of genetic engineering; for if specifically selected DNA could be introduced into these vectors, it would then be possible to transfer into bacteria the blue prints for proteins- the building blocks of genetic characteristics. Plasmids are extra-chromosomal genetic elements that replicate autonomously in the bacterial cell. Some plasmids have the ability to integrate into the host's chromosome, called episome'.

(b) Cloning of recombinant DNA: Once the recombinant DNA has been formed, the next step is to transform a bacterial strain with it. The isolation of transformed cells carrying recombinant plasmids are important. The transformed cells are cloned by plating, and allowed to form colonies that can be tested by replica-plating technique

Gene Activation

Gene splicing and the manufacture of recombinant DNA has been considered a great technological achievement of the century. Viruses containing recombinant DNA can be made to infect new organism. For example a gene from *Salmonella*, that is resistant to streptomycin was incorporated into a plasmid and was added to *E. coli*, that occurs in the intestine of man. Thus gene transfer has found wide applications. Treatment of diabetes using bacterially produced insulin was successful in curing the disease. Yeast cells have been used in the human system.

25.5. PLANT GENETIC ENGINEERING

From the modern methods of cloning and gene transfer, it may be possible to improve the many field crop plants and to create new strains of high yielding plants. Plant genes have been isolated and incorporated into bacteria to create recombinant DNA molecules. Further, these recombinant molecules have been transferred back into eukaryotic plant cells in protoplast cultures. Plant cells possess a cellulose cell wall. If the isolated plant cells are exposed to cellulase enzyme, the cellulose cell wall gets dissolved, such cells without cell was can be used just as any microorganism for introducing any plasmid containing recombinant DNA molecules. These experiments have been performed in cells from the flowering plant *Petunia*. *Figure* 25.2 summarizes some strategies that are being used in plant genetic engineering.

25.6. ACHIEVEMENTS

1. Drugs and Chemicals: Genetic engineering technology introduced a revolutionary way of manufacturing functional proteins such as interferons, antibodies, blood clotting factors, insulin and growth hormones. A newly emerged aggressive field of research and development known as biotechnology largely depends on the new tools of genetic engineering. In 1981, the genetic company in US was successful in inducing yeast cells to make interferon to be injected into cancer patients. Along with this, the production of monoclonal antibodies work as potential and precise weapons for the war against cancer. Bacterially produced human growth hormone is used to treat dwarfism in man. Genetically engineered microbes can produce an enzyme urokinase that is important in the treatment of brain and lung cancer. Human insulin, sold with the trade name 'Humulin' one of the first products to be commercially manufactured in 1982 using genetic engineering (recombinant DNA) technique, is pure and specific, and more effective, with lesser side effects. Vaccines have been developed against hepatitis virus, which causes liver cancer.

2. Crop improvement: Moderate success has been achieved for growing cells in tissue culture in the mature plants. In some crop plants attempts are being made to produce a hybrid of suitable variety. Some of the chloroplast genes RuBPcarboxylase were cloned in bacteria and their experiments were studied. The genes far plant storage proteins were successfully cloned in bacteria. Nif gene (nitrogen fixing gene) is being tried to be transferred from bacteria to plant cells. Scientists have already succeeded in producing wonder plants such as 'Sunbean'(sunflower bean) and 'Pomato'(potato+tomato) by combining two totally unrelated plants. Similarly a gene from french bean was transferred to Ti plasmid from *Agrobacterium tumefacians*,a plant tomour causing bacterium. This plasmid was returned to the bacterium which was used to infect tobacco plant causing crown gall or plant tumour. The cells cultured from this tumour formed a new plant which was grafted to the tobacco plant. Thus this bean gene was successfully expressed in a newly formed 'Tobean'. Protoplast fusion has made it possible in crossing tomato and potato to make a pomato and sunflower and french bean to form 'sunbean'.

3. Pollution control: Pollution can be controlled by enhancing the growth and activity of microbes present and by adding new microbes to pollution sites. Attempts are made to select the microbes that can degrade the chemicals released in the environment. The genetically engineered microbes and plants may be useful in the removal and degradation of various toxic chemicals from the environment.

Check Your Progress - 2

Name few biologically functional proteins which are manufactured by using genetic engineering technology.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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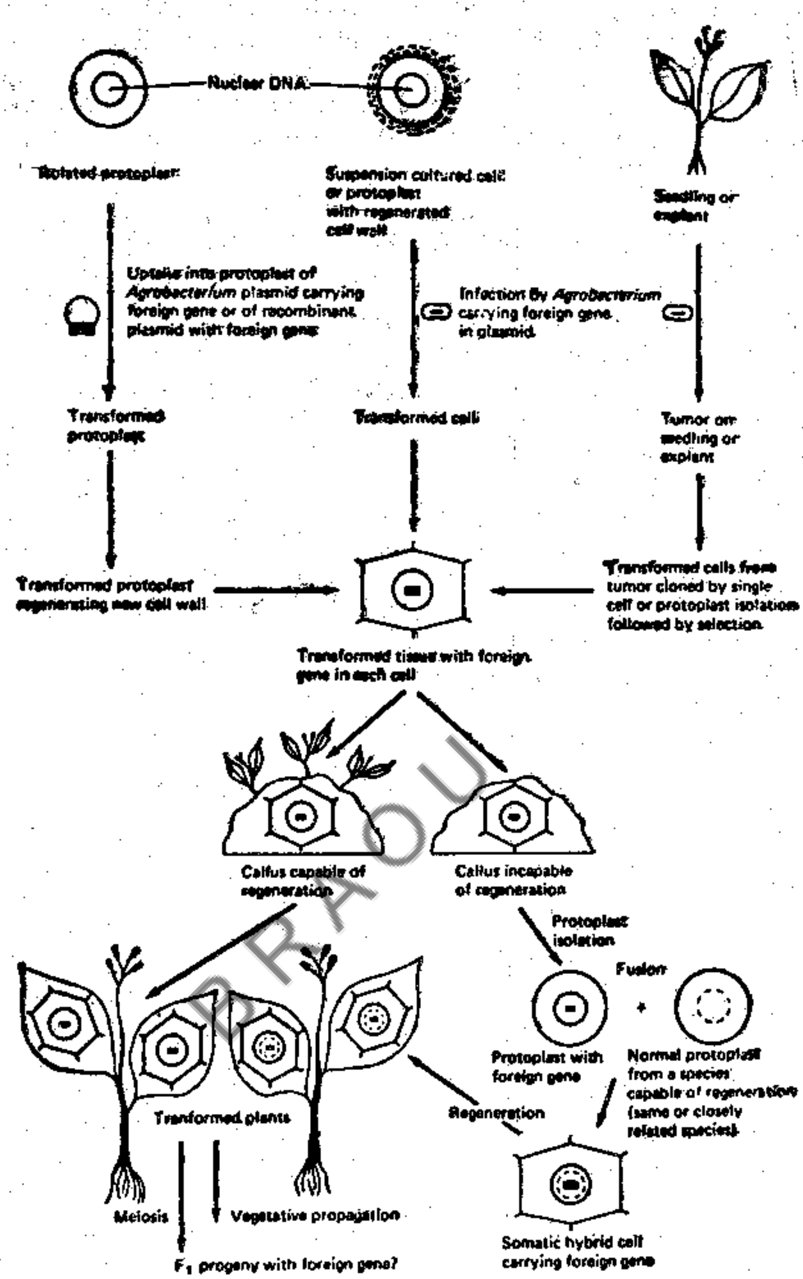

Fig.25.2. Strategies for transformation in higher plants using *Agrobacterium*, *Agrobacterium* plasmid, and recombinant plasmids. Three experimental systems are shown as alternative recipients for foreign genes depending on the vehicle used. If the capacity of plant regeneration is lost in the transformed callus, it could be restored following the fusion of the transformed protoplast with a normal protoplast of the same or different species.

25.7. PROSPECTS

1. Curing and correcting human diseases: One important goal of recombinant DNA technology is to cure human genetic disease. The gene therapy lies in the correction of defective genes. Vaccines for leukemia, hepatitis, influenza, cholera can be obtained through recombinant DNA technique.

2. Plant genetic improvement: Transforming non-legumes into nitrogen fixers can substantiate the necessity for chemical fertilizers. Recombinant DNA technology holds the promise to enable the creation of new crop with high yield potentials, insect and disease resistance. The suitable plasmid vectors for plant genes should be developed. Further, developing new varieties with disease, drought and salt resistance are also envisaged in the near future by the gene transfer through this technology.

25.8. RECOMBINANT DNA AND GENE CLONING HAZARDS

Many biologists recognized this technology to be useful in the study of structure and function of genetic material. They also wondered whether it may introduce potential hazards (for example, the gene responsible for transformation to malignary, carried by tumour virus when cloned in an *E. coli* plasmid, if this *E. coli* strain serves as vector for the transmission of cancer causing genes. So the opponents believe the organism produced may be introduced into the ecosystem. Thereby, after extensive discussion, the National Institute of Health (NIH), US, Department of Health, Education and Welfare established specific guidelines under which recombinant DNA research should be confined to the use or sterile technique and specifically designed laboratories to prevent vectors from escaping from the laboratories to the natural ecosystems. A permanent federal panel of scientists has been established to propose and monitor genetic engineering in human beings. In the United States any research concerning deliberate transfer of recombinant DNA into human subjects shall be reviewed and approved by the recombinant DNA advisory committee which acts as a watch-dog .

25.9. SUMMARY

Genetic engineering is a technique for isolating individual genes from plants and incorporating these genes in bacteria and yeasts. This technology depends upon the elegant manipulation of bacterial genome. It involves the synthesis of recombinant DNA, cloning and transmission of the of desired characters. There are so many potential applications of recombinant DNA technology, namely, the production of genetically superior crops and manufacturing biologically functional proteins such as interferon, antibodies, insulin and growth harmones etc.

25.10. CHECK YOUR PROGRESS : MODEL ANSWERS

1. Restriction endonucleases.
2. Interferon, antibodies, insulin, blood clotting factors are few biologically functional proteins manufactured by using genetic engineering technology.

25.11. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Write briefly about genetic engineering
2. What are the techniques involved in genetic engineering?What are their applications to the plants, animals and human beings?

II. Answer the following questions in 10 lines each.

1. How do you identify and isolate a desired gene?
2. Write briefly gene cloning.
3. What are the hazards of recombinant DNA and gene cloning technology?

BLOCK - III
ECOLOGY

UNIT-26: CONCEPT OF ECOSYSTEM AND PLANT ADAPTATIONS

Contents

26.1. OBJECTIVES

After going through this unit you will be able to :

1. describe the concept of ecosystem,
2. differentiate and describe various ecological adaptations of plants.

26.2. INTRODUCTION

No organism exists in a vacuum, but depends on other life and its physical environment. Ecology has been traditionally defined as the study of interrelationships between organisms and their environment. A modern view point, enunciated by a contemporary ecologist, Eugen P. Odum, defines ecology as 'the study of the structure and function of nature'. This definition attempts to avoid the distinction between organisms and environment by treating them as an integral unit or an organised whole. Further in this definition, nature includes all living forms of an area functioning with their non-living environment. Nature is thus perceived as an ecological system, in other words, ecosystem. So to speak, an ecosystem is composed of all the living organisms in an area plus the surrounding physical environment with which they interact. The 'eco' part of the word refers to environment, the 'system' part a complex of coordinated units.

26.3. THE ECOSYSTEM CONCEPT

The ecosystem concept is an extremely useful one since it permits to study the structure and function of an ecosystem in a quantitative manner. Now the ecosystem has become the basic functional unit in ecology. Ecologist refer to all living organisms in a given ecosystem as community. Within the community, organisms are grouped into populations. A population is a group of one species of organisms occupying a particular space at a particular time. The term ecosystem was first proposed by the English plant ecologist A.G. Tansley in 1935. But, of course, the concept of such an ecological system is by no means recent. As early as 1877 Karl Mobius mentioned the term biocoenosis while describing the community of organisms in an Oyster reef. S.A. Forbes in 1887 used the word microcosm as a synonym to biocoenosis. Great emphasis was placed on the concept of biocoenosis by the Russian ecologists during 1846-1903, who later expanded the term to geobiocoenosis.

What then is a system? A system is a collection of interdependent part of events that make up a whole. For example a radio consists of various transistors, transductors, wires, a speaker, and control knobs, among other things. Each part has specific function, yet the expression of their role depends on the proper functioning of all the other parts. The whole system fails to function unless there is some kind of input from the outside which the system can act to produce some output. For the radio the outside input is electrical energy, on which the system acts to pick up certain radio waves which are transmitted as output sound. Thus the radio with all its parts functions as a total system. The ecosystem functions in a similar manner.

According to Odum (1971) typical ecosystem has six components as 1) Inorganic substances (C,N,CO_2, H_2O etc.). 2) Organic compounds (proteins, carbohydrates, lipids, humic substances etc). 3) Climate regime (temperature and other physical factors). 4) Producers (autotropic organisms as green plants, phytoplankton, photosynthetic bacteria). 5) Macroconsumers or phagotrophs (heterotrophic organism, chiefly animals which ingest other organisms or particulate matter, 6) Microconsumers, saprotrophs or osmotrophs (heterotrophic organisms chiefly bacteria, and fungi). The first three constitute the biotic component, and the last three form the abiotic component. The inorganic constituents are synthesised into organic structures by the green plants(primary producers) through photosynthesis utilising solar energy. The green plants become the source of energy for the animals (herbivors), these herbivors become the source of energy for the flesh eating animals (carnivores). Animals of all types grow and add organic matter to their body; here the source of energy is the complex organic compounds taken as food. These animals are known as secondary producers. All these living organisms, whether plants or animals, have definite life-span and die. The dead organic matter provides food for the microorganisms which ultimately decompose the same and break the complex materials and liberate the inorganic components into the environment. These organisms are known as the decomposers. All this is known as the trophic or food and feeding structure of the ecosystem and in volves food chains and food webs. During the decomposition process of organic molecules, the energy which kept the inorganic components bound together in the form of organic molecules gets liberated and dissipated in the environment as heat energy. Thus in an ecosystem the inorganic materials, keep on circulating from the abiotic environment to the biotic environment and back to the abiotic state. This circulatory cycle or cycling of materials is known as 'biogeochemical cycle' The energy, however, enters into the ecosystem from outside i.e. from the sun, and is trapped by the primary producers which through food chain is passed onto the other organisms and ultimately the energy during the process of decomposition is dissipated into the environment and goes out of the ecosystem. The process is called flow of energy, like the cycling of materials, energy does not cycle but falls down in a series from a high level i.e., cascades in the ecosystem. It enters into the ecosystem in the form of light and passes out in the form of heat. Thus one basic feature of an ecosystem is that it is not a closed but an open system in that it receives energy from an outside source, the sun. From this open system, energy and matter continuously escape and are replaced in order that the system can continue to function.

Ecosystem is self-maintained and self-regulated. Living organisms and their environment are both dynamic in nature, their interactions produce changes in both. The organisms adjust to the environment through adaptational changes in their body or function, while the environment through physical or biogeochemical processes maintains its life supporting qualities and a proper balance in the ecosystem. There is thus a self-regulating mechanism in ecosystems of checks and balances. This is called homeostasis, and refers to the tendency for biological systems to resist chanse disorder or even a change and to remain in a state of equilibrium. The principles of cybernetics or of control such a feed back system are applied to understand the homeostasis.

Feedbacks may be positive or negative. For example in a balanced population, a predator like the snake eats up and reduces the prey population of frogs. It means that snake is interacting with frog in a negative feed back way. The frog population by virtue of its numbers is regulating the snake population. This is a positive feedback as the frog is responsible in a positive way. The positive and negative feedbacks strike a balance for maintaining an equilibrium.

The ecosystems have indefinite range and size. They are either terrestrial, freshwater, marine or man engineered (agricultural). Terrestrial ecosystems are generally named after the kind of habitat conditions, as for example, forest ecosystem, desert ecosystem, grassland ecosystem etc. Fresh water ecosystems are usually named according to size and nature of aquatic body, for instance, river ecosystem pond ecosystem and lake ecosystem. They are often interrelated. Biological transfers of nutrients and energy from one system to another system bring about interdependence of the systems. Organic compounds and nutrients also get transferred from one system to another by means of stream, rivers etc. The total of the ecosystems (minus any overlaps between them) constitutes the biosphere.

Man (*Homo sapiens*) in his quest for more amenities and to feed the ever increasing human population, has interfered with the natural ecosystems, and sometimes even tampered them to the extent that today the very existence of many organisms and environment is threatened.

Since man himself is an animal, and as such is an ingredient in ecosystem, even his own existence may be in jeopardy. Unless he develops an understanding of and respect for natural ecosystem. If man is to exist, he has to work within the framework of the ecosystems of which he is a part. In other words, he needs to develop an ecosystem approach to the management of the earth.

In order to get a sustained yield from an ecosystem a proper knowledge of the factors influencing the components and processes within the system is very essential. Ecology could become a predictive science if we are able to quantify the effects of factors influencing the components and processes of the ecosystem. One of the aims of international Biological Programme and Man and Biospher Programme, is to obtain quantitative data on contents, processes and factors within the ecosystem to understand their interactions and prepare predictive models for any type of ecosystem for a given set of environmental parameters.

26.4. PLANT ADAPTATIONS

The plants exhibit differences in their morphology, physiology, anatomy and reproductive structures to compromise with their environments. Such changes are known as adaptations, which enable them to withstand extreme conditions of environment. An adaptation is the net result of all environmental factors operating simultaneously on the organism/community. However, the intensity of any particular factor which is limiting the plant growth, brings the adaptational modifications in the plant. Competition of the life cycle of an individual or stabilization of the community results through a series of adaptations which have survival value. Adaptations of survival value are genetically fixed and heritable. Plants usually occur in groups and this groupism is actually in response to the same set of environmental conditions in which they grow. Groups of plants growing under the same set of environmental factors are called the ecological groups and these are indeed much broader groups without any airtight boundaries between them.

On the basis of their water requirements and more specifically on the amount of water available in their habitats, the plants are classified into three categories as 1. Hydrophytes 2. Mesophytes, and 3. Xerophytes.

26.5. HYDROPHYTES

These are the plants that grow in water (marine or fresh) or that inhabit soils containing a quantity of water that would prove supraoptimal for the average plant. The non-vascular aquatics constitute the phytoplanktons. The vascular hydrophytes are divided into three natural groups : 1. Submerged 2. Floating, and 3. Amphibious plants.

Submerged plants: These are of two types, a. Submerged floating hydrophytes that possess long stems usually with small leaves, e.g. *Ceratophyllum, Utricularia*, and b. Submerged rooted hydrophytes which are completely submerged in water in shallow regions, where the plants can get adequate supply of light, e.g. *Vallisnaria, Hydrilla, Potamogeton* (Fig.26.1).

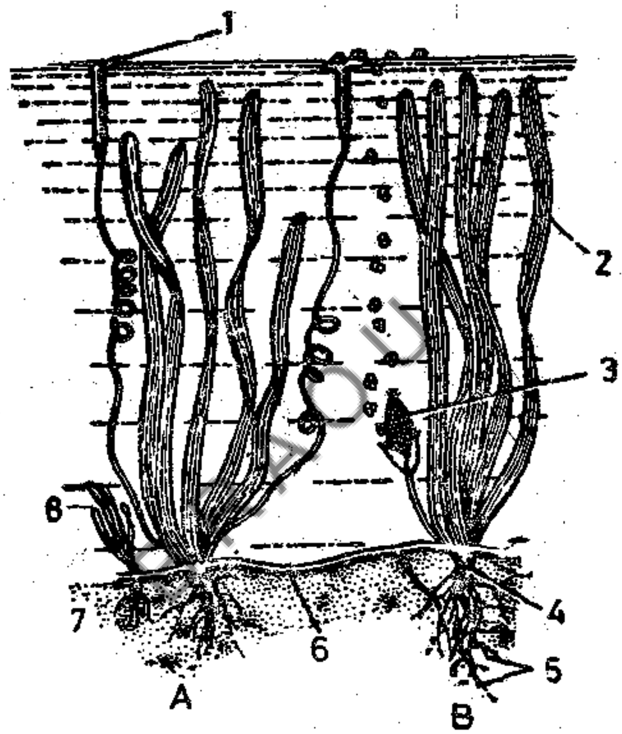

Fig.26.1. Rooted submerged hydrophytes - *Vallisneria*. A. Female plant. B. Male plant. 1. Female flower. 2. Thin submerged leaves. 3. Male flower. 4. Compact rhizome. 5. Roots. 6. Stolon. 7. Mud. 8. Water.

Floating plants: These are of two types. (a) free floating forms which are in contact with water and air but not with soil, e.g. *Lemna, Wolffia, Azolla, Jussieua, Salvinia, Pistia, Eichhornia* (water hyacynth), *Trapa* (water chestnut) (Fig.26.2) (b) Free floating but rooted forms with roots anchored in the mud and leaves floating on the surface of water, e.g. *Nymphaea stellata, Nelumbium speciosum, Victoria regia* (Fig.26.3A).

Amphibious plants: They grow in shallow waters. The basal part of the plant is usually submerged in water and the shoots extend well above the water surface. They exhibit both hydrophytic and mesophytic characters, e.g. *Ranunculus, Typha, Sagittaria*, and *Hygrophila* (Fig.26.3B).

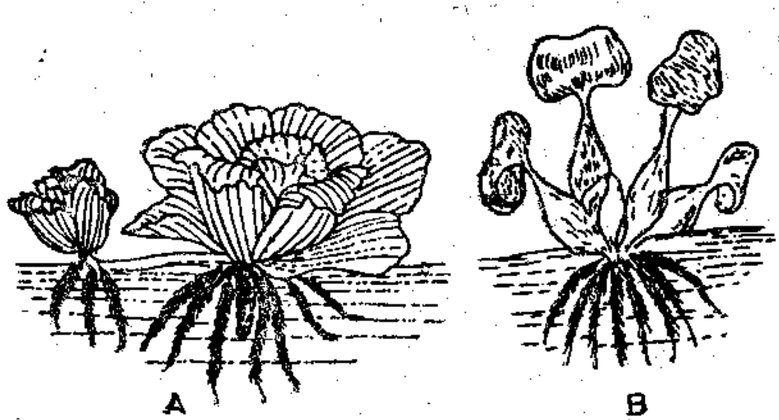

Fig.26.2. Free floating hydropnytes. A. Pistia B. Eichhornia

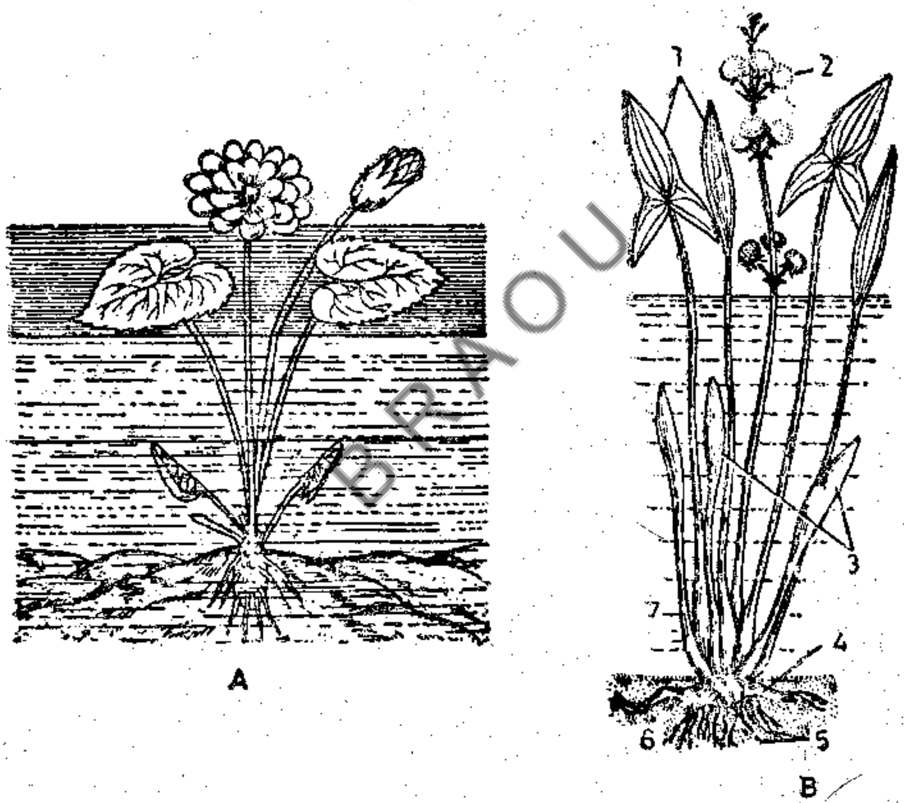

Fig.26.3. Rooted hydrophytes. A. Rooted hydrophyte with floating leaves - *Nymphaea*. B. Rooted emergent hydrophyte - *Sagittaria*. 1. Emergent leaves. 2. Flower. 3. Submerged leaves. 4. Stolon. 5. Roots. 6. Mud. 7. Water

26.5.1. Ecological Adaptations of Hydrophytes

Morphological adaptations - 1. Roots: They are poorly developed as in *Hydrilla* or totally absent as in *Wolffia*, *Salvinia*, and *Ceratophyllum*. 2. Root hairs are absent or poorly developed. 3. Root caps are generally absent and, in some cases as in *pistia* and *Eichhornia* they are replaced by root pockets. (Fig.26.4). 4. Floating aerial roots are seen in addition to the normal adventitious roots. 5. In some emergent forms which grow in mud, roots are well developed with distinct root caps. 6. in *Lemna*, roots act simply as balancing and anchoring organs.

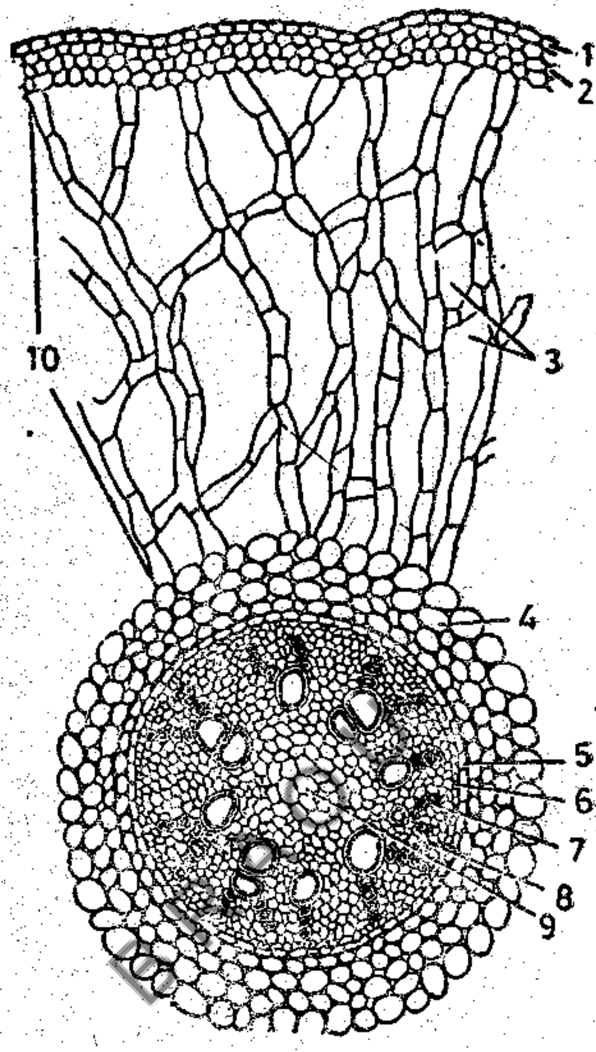

Fig.26.4. T.S. of the root of Eichhornia 1. Epidermis. 2. Outer cortex. 3. Air chambers. 4. Inner cortex. 5. Endodermis. 6. Pericycle. 7. Xylem. 8. Phloem. 9. Pith. 10. Middle aerenchyma.

II. Stems: The stem is thin, long, slender, spongy and flexible in *Hydrilla* and *Ceratophyllum* (Fig.26.5). 2. It is reduced in *Wolffia* and *Spirodela* or it is modified into a rhizome as in *Vallisneria* and *Typha*.

III. Leaves: The leaves are small in *Azolla*, long and ribbon shaped in *Vallisneria*, long and linear in *Potamogaton*, finally dissected into hairy structures in *Ceratophyllum*. In *Nymphaea* and *Nelumbium*, the leaves are large, flat and entire, floating, and their upper surfaces are coated with wax, their petioles are long and slender. *Ranunculus, Limnophila* and *Sagittaria* exhibit heterophylly with submerged, floating and aerial leaves.

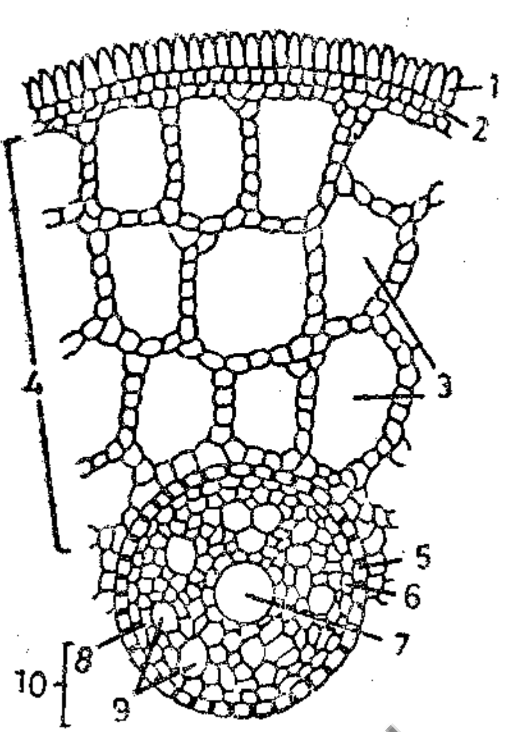

Fig.26.5. T.S. of the stem of *Hydrilla* . 1. Epidermis. 2. Hypodermis. 3. Air chambers. 4. Cortex. 5. Endodermis. 6. Pericycle. 7. Xylem cavity. 8. Parenchyma. 9. Sieve tubes. 10. Phloem.

Anatomical adaptations - I. Roots: 1. Cuticle, in a majority of the cases, is completely absent. 2. Epidermis is uniseriate with thin walled parenchymatous cells 3. Cortex is large, parenchymatous, with well developed air cavities, this aerenchyma offers resistance to bending stress; increases buoyancy and, facilitates exchange of gases. 4. Vascular tissues much reduced except in case of emergent forms. The vessels in xylem are less common. However, in *Ranunculus* and *Typha* vascular elements are comparatively much distinct and well developed. 5. Mechanical tissues are usually absent but in some emergent forms sclerenchymatous pith is present as in *Typha*.

II Stem: 1. Cuctili is usually absent, or it may be in the form of a thin film. 2. Epidermis is uniseriate and consists of thin welled parenchymatous cells; 3. Cortex is much distributed, parenchymatous and extensively traversed by air cavities as in roots. Contrical cells are filled with chloroplasts. Vascular bundles are numerous and scattered in the cortex as in *Nymphaea*. 4. Endodermis is usually distinct and is uniseriate. 5. Vascular tissues are feebly developed. But in some emergent forms as the *Typha* cuticle, epidermis, and vascular tissue are well de veloped and differentiated.

III. Leaves: 1. Cuticle is completely absent in submerged forms. In floating and emergent forms a thin film of cuticle is present on the upper surface of leaf; 2. Epidermis is single layered and the cells are filled with chloroplasts. Stomata are completely absent in submerged leaves, but they are present only on upper surface in floating forms. In emergent form the leaves show stomata. On both surfaces; 3. Mesophyll is usually undifferentiated. In *Nymphaea* (Fig. 26.6 & 26.7) it is differentiated into palisade and spongy tissues with well developed air cavities; 4. Vascular tissues are very much reduced and are not differentiated. However, in *Nymphaea* the xylem elements are thin walled and phloem is well developed; 5. Mechanical tissues are absent.

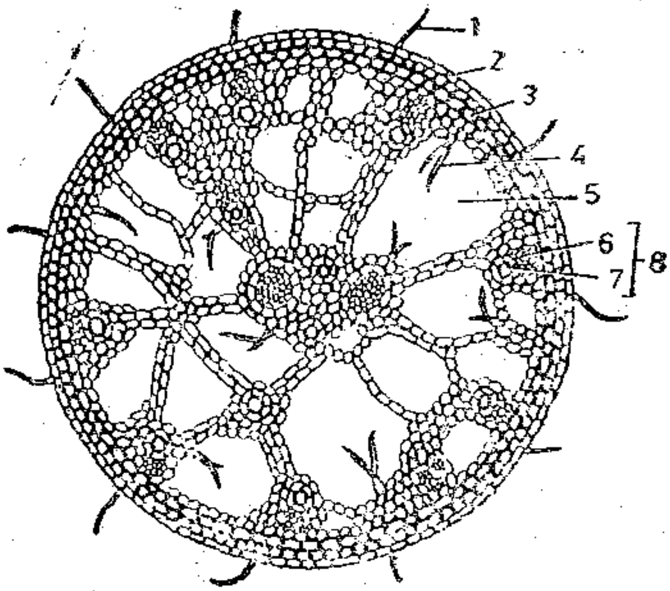

Fig.26.6 T.S. of petiole of *Nymphaea*. 1. Hair. 2. Epidermis 3. Hypodermis. 4. Sclereid. 5. Air chamber, 6. Phloem. 7. Xylem . 8. Vascular bundle.

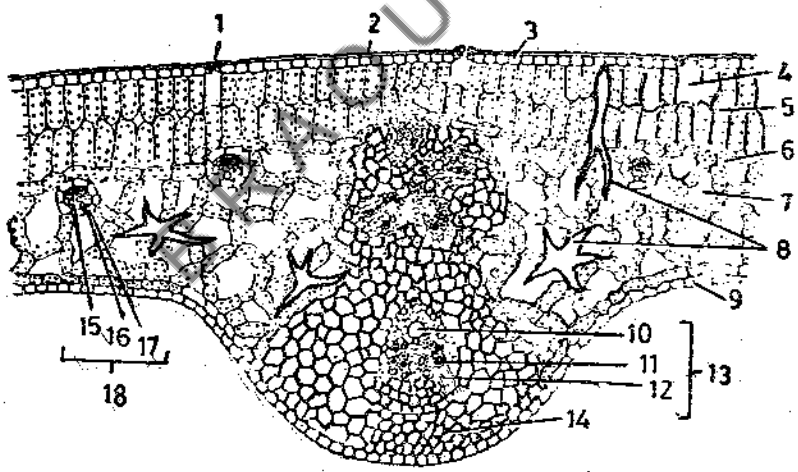

Fig.26.7 T.S. of *Nymphaea* leaf 1. Stoma 2. Cuticle 3. Upper epidermis 4. Substomatal chamber 5. Palisade 6. Spongy parenchyma 7. Air chamber 8. Trichosclereid and astero scleride 9. Lower epidermis 10.Protoxylem lacuna 11. Metaxylem 12. Phloem 13. Vascular bundle of midrib 14. Collenchyma 15. Xylem 16. Phloem 17. Bundle sheath 18. Vascular bundle of vein.

Check Your Progress - 1

Write about the anatomical adaptations found in the roots of hydrophytes.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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26.5.6. Physiological Adaptations

Water and nutrients are absorbed throughout the plant body. 2. Osmotic concentration of the cell sap is equal to or slightly higher than water medium; 3. The CO_2 released during respiration is stored to some extent in the air cavities and it is utilised during photosynthesis, and the released O_2 is used in respiration.

26.6. MESOPHYTES

Mesophytes are the plants which grow in normal habitats, generally moist and well aerated soils. They form the intermediate category between hydrophytes and xerophytes, e.g. most plants around us, common garden plants and cultivated plants.

The general feature of mesophytes are as follows:

1. Root system is well developed, root hairs and root caps are present;
2. Stems generally aerial, rigid and stout;
3. Leaves are generally large, thin and are rich in chlorophyll. Hairy and waxy coatings usually absent, palisade and spongy tissues are well developed, in the latter large intercellular spaces are present. Cuticle is moderately developed. Stomata are present on both surfaces;
4. Mechanical and vascular tissues are well developed;
5. They may exhibit temporary wilting during noon hours.

26.6. XEROPHYTES

Plants growing in extreme dry and arid habitats are known as xerophytes. The term 'xerophyte' has been defined and interpreted variously. There is no hard and fast line between the water status of habitats for mesophytes and xerophytes. Some people regard only those plants which actually suffer from the deficiency of growth water as xerophytes. Others believe that all species that grow in normally dry or arid habitats whether or not they suffer from deficiency of growth water in their habitat should be placed under xerophytes. Dubenmire (1959) defined xerophytes as plants which grow on substrata that usually become depleted of growth water to a depth of atleast 2 decimeters during a normal season. It is difficult to decide whether xerophyte is really xerophylous, and occurs only in dry habitats and deserts, or it is merely drought resistant.

Based on their capability to withstand drought conditions xerophytes are classified into four groups. 1 Drought escaping(ephemerals) 2. Drought evading 3. Drought enduring (non-succulents) 4. Drought resisting (succulents).

Drought escaping: These are annuals which complete their life cycles within a very short period (6-8 weeks time), before the advent of unfavorable dry season, and thus they escape dryness. They are mainly founa growing in semi-arid regions, etc. *Argemone mexicana, Solanum xanthocarpum, Cassia tora.*

Drought evading: These plants are of very small size with restricted growth and require low amount of water. Due to smallness of their size, they conserve whatever little moisture they get and thus evade drought.

Drought enduring: These are the true xerophytes, which include slow growing shrubs that occur sparsely in deserts. They are small and show stunted growth. They possess a number of morphological, anatomical and physiological characteristics which enable them to withstand critical dry conditions. These are the plants that suffer from dryness, both, in their internal as well as external environments. The leaves wilt and fall down during drought period and resume growth when water is again available e.g. *Capparis aphylla, Prosopis, Casuarina, Nerium, Calotropis procera and Zizyphus jujuba, Acacia nelotica.*

Drought resisting: These plants suffer dryness in external environment only. Their succulent, fleshy organs serve as water storage organs which accumulate large amounts of water during brief rainy seasons. The stored water enables them to continue growth and development e.g. *Aloe, Agave, Cactus, Opuntia, Muehelenbeckia*

Based on the nature of their habitat, xerophytes are classified into following types.

1. **Psammophytes:** They grow on sand or small pebbles in dry habitats, e.g. *Acacia senegal*,
2. **Lithophytes:** These are the plants that grow on rocks where water does not stay, e.g. some species of lichens, *Salaginella* sp.
3. **Psychrophytes:** They grow in cold soils where the availability of water is limited on account of frequent freezing, e.g. some plants growing in Himalayas.
4. **Halophytes:** These are plants that grow on highly saline soils, where water is poorly available on account of high osmatic concentration in such habitats. e.g.*Pseuda-maritima, Bruguiera* sp. *Avicennia* sp. *Rhizophora* sp. *Salicornia*. The latter three are also called mangrove plants.

26.7.1. Ecological Adaptations of Xerophytes

Morphological Adaptations - Roots: 1. In xerophytes, the root systems is extensively developed, in some cases it is several times longer than the shoot. Roots are long, with extensive branching spreadover wide areas; 2. Root hairs and root caps are well developed.

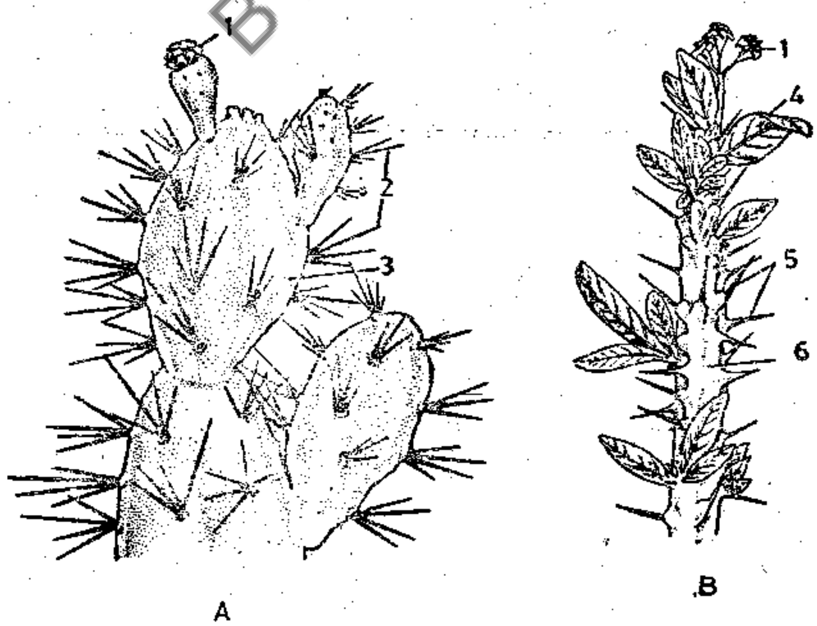


Fig.26.8 Succulent xerophytes. A. *Opuntia*. B. *Euphorbia splendens*. 1.Flower. 2. Spines. 3. Phylloclade. 4. Leaf. 5. Stipular spines. 6. Stem.

Stems: 1. These plants are mostly stunted, woody, dry, hard, ridged and covered with thick bark. 2. In some cases, the stem is underground, it is fleshy, green and leaf-like in *Opuntia* (Fig. 26.8A) In *Euphorbia splendens* (Fig 26.8B), the stem is fleshy and green. 3. The stems and leaves are usually provided with hairs or waxy coatings.

Leaves: 1. The leaves are generally very much reduced to small scale like structures some times modified into spines, to check the rate of transpiration. Lamina may be long, narrow or needle-like as in *Pinus* or divided into many leaflets; 2. Foliage leaves when present, may become thick, fleshy and succulent or tough and coriaceous in texture; 3. Leaf surfaces are usually shiny and glazed to reflect light and heat; 4. In some monocots, *Poa* and *Agropyron* the leaves are folded and rolled in such a manner so that sunken stomata are hidden to reduce the rate of transpiration; 5. In some forms as *Zizypus jujuba*, *Capparis aphylla*, *Acacia nelotica* the stipules are modified into spines; 6. Some xerophytes shed their leaves during dry period.

Anatomical adaptations Roots: 1. Root hairs and root caps are well developed; 2. The roots may become fleshy to store water, e.g., *Asparagus* 3. In *Calotropis* and *Pinus edulis* roots possess rigid and thickened walls.

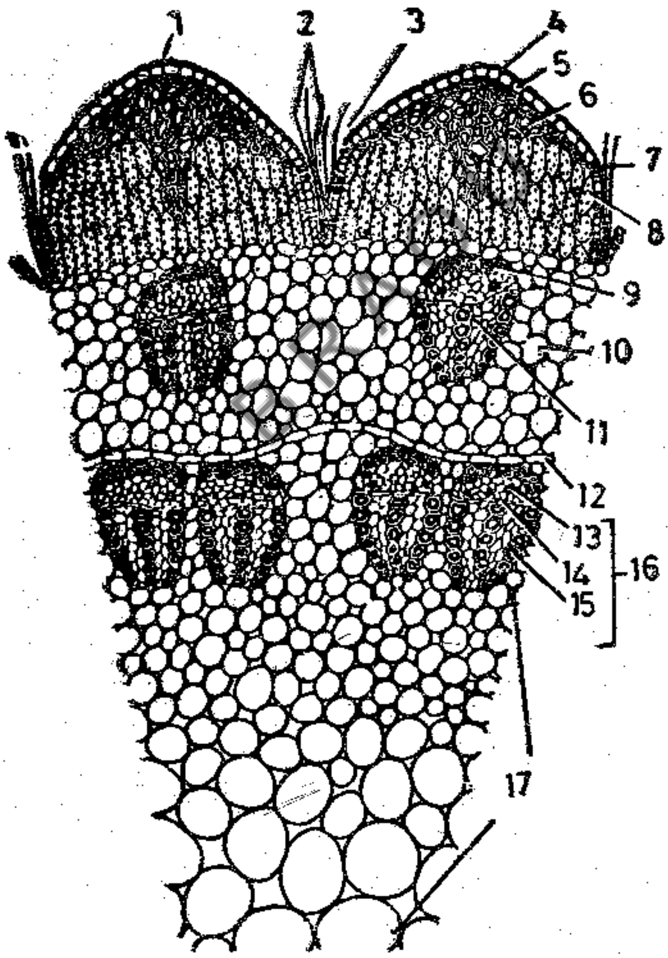


Fig.26.9 T.S. of stem of *Casuarina* 1. Ridge. 2. Hairs. 3. Groove. 4. Cuticle. 5.Epidermis. 6. Hypodermis. 7. Sunken stoma. 8. Palisade parenchyma; 9. Sclerenchyma. 10. Cortex (parenchymatous). 11. Cortical vascular bundle. 12. Starchy sheath. 13. Pholem. 14. Cambium. 15. Xylem. 16. Stelar vascular bundle 17. pith

Stem: 1. Epidermis is well developed with heavily thickened walls and covered with thick cuticle; 2 Hypodermis is multilayered and sclerenchymatous; 3. Sunken stomata are present; 4.Vascular bundles are p rovided with multilayered bundle sheaths; 5. Vascular and mechanical tissues are well developed; 6. Water storage regions are present in succulent fleshy xerophytes; 7. Oils and resins are often present; 8. Stems are covered usually with thick bark, e.g. *Casuarina* (Fig.26.9).

Leaves: 1. In many xerophytes the leaves show thick cuticle on both surfaces to reduce the rate of transpiration; 2. In some succulent xerophytes as *Peperomia*, the epidermal cells serve as water storage organs; 3. In several cases multiple epidermis is present; 4. The stomata are less in number and are of sunken type; 5. Sclerenchymatous hypodermis is seen in several members *(Pinus)*. 6. Mesophyll is well differentiated into palisade and spongy parenchyma and palisade tissue is present on both sides; 7. Vascular tissues are very well developed and differentiated; several vascular bundles are present in the midrib region as in *Nerium* (Fig.26.10). 8. Several kinds of sclereids are present. A. well developed complex transfusion tissue is present in *Pinus*.

26.8. HALOPHYTES

Halophytes are a special type of xerophilous plants, that inhabit saline soils, with high concentration of salts such as sodium chloride, magnesium chloride and magnesium sulphate. The osmotic concentrations of these soils is very high, so they are physiologically dry. The concentration of the cell sap of halophytes is much more than that of mesophytes. The osmotic pressure of *Salicornia* and *Avicennia* exceed 30 atm, in certain cases it is upto 150 atm. The halophytes are divided into 5 groups.

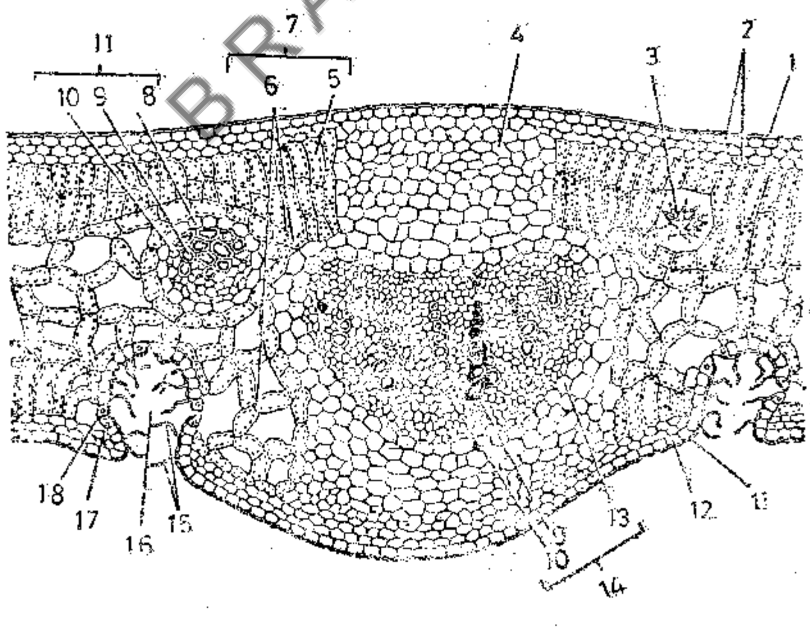


Fig.26.10 T.S. of *Nerium* leaf. 1. Cuticle. 2. Upper epidermis. 3. Calcium oxalate crystals. 4.Parenchyma. 5. Palisade parenchyma. 6. Spongy parenchyma. 7. Mesophyll. 8. Bundle sheath. 9. Xylem. 10. Pholem. 11. Vascular bundle of vein. 12. Lower epidermis. 13. Starchy bundle sheath. 14. Vascular bundle of midrib. 15. Hairs. 16. Stomatal pit. 17. Guard cell. 18. Stoma.

1. **Lithophilous halophytes:** They grow on rocks near the sea shore.

2. **Psammophilous halophytes:** They grow in sandy regions.

3. **Pelophilous halophytes:** They grow in loamy saline soils with a predominance of clay.

4. **Salt swamp or Salt desert:** They grow in calm waters and muddy spots.

5. **Littoral swamp forests or Mangrove vegetation:** They occur in all tropical and subtropical sea-shores, river mouths, especially on flat, muddy shores.

26.8.1. Adaptations in halophytes

Halophytes usually exhibit the xerophytic characters. They are, 1. most of the halophytes are succulents; 2. Leaves are evergreen, thin, small and coriaceous with water storage tissues; 3. Thick cuticle is present, palisade cells are prominent; 4. Most of them produce special type of branched, negatively geotrophic roots which come out of the mud surface to absorb atmospheric oxygen; These roots are called pneumatophores or breathing roots; 5. Some halophytes show vivipary i.e., seeds germinate before the fruits fall off from the plant; e.g. *Rhizophora* (Fig 26.11 26.12).

Fig. 26.11. Pneumetaphorus of *Rhizophora*

Mangroves: The halophytes in tropical and subtropical regions form a typical sea shore vegetation known as mangroves or mangrove formations. These are also called littoral swamp forests. In our country mangroves are distributed in Bengal(delta systems of Ganges). Andhra Pradesh (mouth of Godavari and of Krishna), Andaman and Nicobar islands, (Madras Caveri delta), Orissa (mouth of Mahanandi), Bombay region(Ele phanta caves), Saurashtra and Kutch (Kathiawar). These areas are frequently water logged. The habitat is characterised by sandy, swampy and saline soils, high precipitation, high atmospheric humidity and almost no or little fluctuation in temperature throughout the year. Mangrove formations have a characteristic vegetation with a dense forest of small evergreen trees. Mangroves show several characteristic adaptive features, generally the xerophytic features, given below.

Roots: The internal structure of the normal root shows several layered cork. Cortex is made up of stellate cells connected with each other by lateral arms. Some cortical cells are filled with oil and tannin.

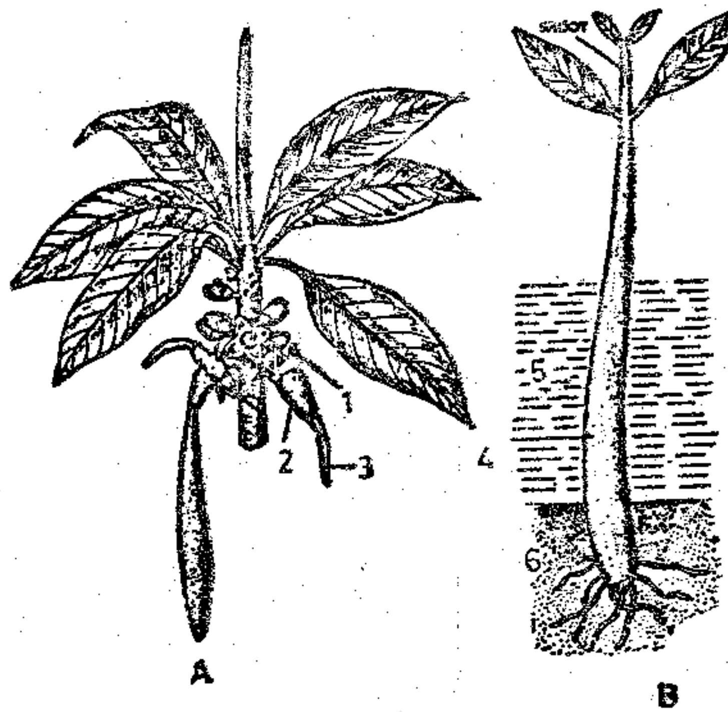


Fig.26.12. *Rhizophora*. A. Twig showing Vivi parous germination. B. A seeding attached to soil. 1. Calyx. 2. Fruit 3. Radicle. 4. Hypocotyl 5. Water. 6. Soil.

Stem: 1. The internal structure of an young stem shows very thick cuticle, 2. Epidermal cells are heavily thickened and filled with oil and tannin, 3. Hypodermis is multilayered, with heavily thickened cells. 4. Numerous lacunae are present in cortex and the cells of which are thickened, and filled with tannin and oil. Calcium oxalate crystals are present in some corticle cells. H-shaped heavily thickened spicules are present in cortex and they provide mechanical strength; 5. Inner cortex possesses numerous thick-walled, branched sclereids that provide mechanical strength; 6. Pericycle is sclerenchymatous and multilayered; 7. Pith possesses lacunae and H-shaped spicules, and the cells are filled with tannin; 8. Vascular tissues are well developed.

Leaf: 1. The leaves are small, thin and coriaceous; 2. Internal structure of leaf shows well developed cuticle; 3. Epidermal cells are much thickened and may contain calcium oxalate crys tals; 4. Stomata are of sunken type and present only on lower surface; 5. Several layers of thin walled cells that are usually filled with water are present below the upper epidermis. Some of these cells are also filled with tannin, oil and mucilage; 6. Mesophyll is well differentiated.

Mangroves are highly productive and are important in several ways. They exhibit a good example of ecological succession of plants. A clear zonation of species from sea water to inland can be seen on the sea coasts.

26.9. EPIPHYTES

Some plants grow on other plants but do not derive their food from supporting plants. These plants are known as 'epiphytes' and the phenomenon is called 'epiphytism'. Mostly they are to be found on the branches of higher plants. They are commonly seen in warm and humid tropical rain forests and coniferous forests of the mountains. Epiphytes produce two types of roots, clinging and long aerial roots. The clinging roots enter the crevices present in the bark of support plant and fix the epiphyte. The aerial roots are long and hand in air. They may also be branched. These roots are coated with a special type of absorbing tissue called velamen. Most of them exhibit the following xerophytic adaptations to check transpiration, e.g. *Vanda* (Fig 26.13).

1. The leaves are few in number, coriaceous and succulent. 2. Cuticle is thick. 3. Stomata are of sunken type.

In *Dischidia rafflesiana* (Fig.26.14) an interesting type of adaptation for meeting water requirement is seen. In this plant leaf is modified into a pitcher. The pitcher has a basal opening but no lid. A root enters the cavity of the pitcher and becomes much branched. Inside the pitcher debris is collected by ants. All this water is then absorbed by the root.

Schimper has classified epiphytes into four groups.

1. **Protoepiphytes:** They derive their nutrition from support plant and atmosphere, e.g., *Peperomia* and few ferns.

2. **Hemi-epiphytes:**These are the epiphytes of peculiar type and their epiphytism is limited to their early developmental stages. Later on, they establish contact with soil and grow with the help of their own roots and completely become independent terrestrial plants, e.g., *Ficus*, Some Aroids, *Scindapsus officinalis*.

3. **Nest epiphytes:** Many orchids form nest like structures to collect large quantities of humus and water. They produce a thick net work of roots and when wind blows over these roots, the dust accumulates and provide the necessary edaphic environment.

Tank epiphytes: The leaves of these epiphytes are variously modified into pocket, funnel

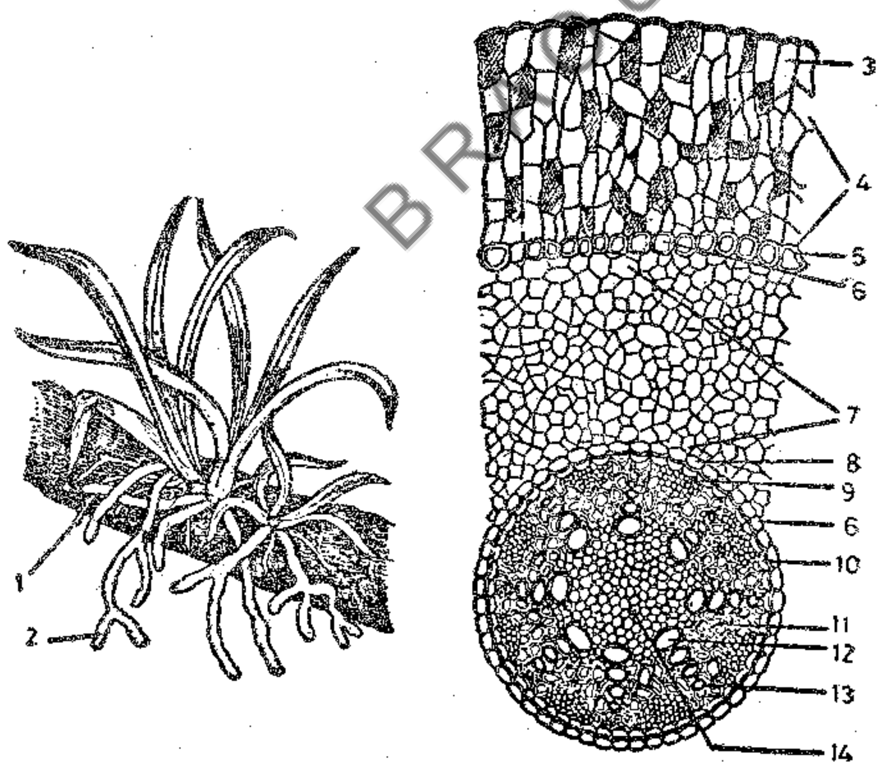
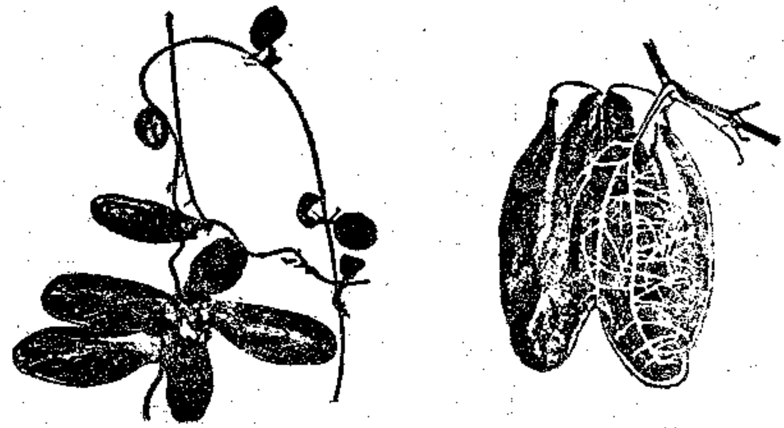


Fig.26.13. *Vanda*. A. An epiphytic plant showing aerial roots. B. T.S. of root showing Velamen. 1.Clinging root. 2. Aerial root. 3. Limiting layer. 4. Velamen. 5. Exodermis. 16. Passege cell. 17.Cortex. 8.Endodermis. 9. Phloem. 10. Pericycle. 11. Sclerenchyma. 112. Metaxylem. 13. Protoxylem. 14. Pith.

or spoon-like structures which collect humus and absorb mineral nutrients which accumulate in the crevices of bark of support plant. Peltate scaly hairs present on the base of the leaves absorb nutrients. The roots are useful only to fix the epiphyte on the support plant, e.g., Epiphytic species of Bromeliaceae.



A B

Fig.26.14. *Dischidia rfflesiana.* A. Several pitchers. B. A pitcher opened.

Check Your Progress - 2

What are velamen roots?

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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26.10.SUMMARY

Ecology is the study of interrelationships between organisms and their environment. Odum defines ecology as the study of the structure and function of nature. A typical ecosystem has six components. They are inorganic substances, organic compounds, climatic regime, producers, macroconsumers and microconsumers. The former three form the biotic component and the latter three the abiotic component. The different types of ecosystems found in nature are terrestrial(forest, desert grassland ecosystem), fresh water (river, pond and lake ecosystem), marine, and agricultural ecosystems. These are named on the basis of habitat, size and nature. Nutrient and energy transfer occur from one system to another, hence they are inter-dependent. All types of ecosystems present in the world constitute the biosphere. Plants exhibit various modifications in order to withstand the extreme conditions of environment, such modifications are called plant adaptations. Based on their water requirements, the plants are broadly classified into three groups : 1. Hydrophytes 2. Mesophytes and 3. Xerophytes.Halophytes are a special type of xerophytes which inhabit saline soils. Mangroves are the halophytes that inhabit the sandy, swampy and saline soils especially on sea shores and river mouths and form a dense forest of small ever green trees.Epiphytes are the plants which lodge on other plants. They produce clinging roots, which are useful in fixing the plant to the support plants and long aerial roots, the velamen roots, which absorb rain water and atmospheric moisture.

26.11. CHECK YOUR PROGRESS : MODEL ANSWERS

1. (a) Cuticle absent.
   (b) Epidermis uniseriate, thin walled parenchymatous.
   (c) Cortex large, parenchymatous with well developed air cavities.
   (d) vascular tissues much recued except in case of emergent forms.
   (e) Mechanical tissues are usually absent.
2. The aerial roots of epiphytes which are coated by a special type of absorbing tissue,— velamen are called as "Velamen roots".

26.12. MODEL EXAMINATION QUESTIONS.

I. Answer the following questions in about 30 lines each.

1. Explain in detail the various components of an ecosystem. Add a note on the food chain.
2. What are the various modifications that are exhibited by the hydrophytes and xerophytes.

II. Answer the following questions in about 10 lines each.

1. Write briefly about bio-geo chemical cycles.
2. What are different types of hydrophytes? Briefly explain about each one of them.
3. Write briefly about the various modifications which are met within xerophytes.
4. Write in detail about halophytes.

UNIT - 27: ECOLOGICAL FACTORS

Contents

27.1. OBJECTIVES

At the end of this unit you will be able to:

1. describe various ecological factors viz., climatic, edaphic, topographic and biotic,
2. describe the influence of ecological factors on plant life.

27.2. INTRODUCTION

The environment is a complex whole of so many interacting factors that influence every organism, the geographical distribution of plants or a group of plants. We find different kinds of plants in different places under different sets of environmental conditions. The various conditions which affect the form and function of the plants directly or indirectly are termed as environmental factors or ecological factors. The life of the plants is so intimately related with the environmental factors of a place that every species of a plant seems to be fitted to a particular set of environmental conditions within the limits of which it is able to grow and reproduce its own kind. All the factors as a matter of fact interact with each other to influence the organisms.

Ecological factors are customarily classified as : 1. Climatic, 2. Edaphic, 3. Topographic and 4. Biotic factors.

27.3. CLIMATIC FACTORS

The climate of a particular region is fairly uniform over a wide area and variations may occur in small areas due to topographical or other factors. The atmospheric factors which affect the plants are called climatic factors and thus are classified into a.Water, b.Atmospheric moisture, c.Temperature, d.Light, e.Wind velocity and f.Atmospheric gases.

27.3.1. Water

The role of water in growth and development of a plant is of great importance. It being a universal solvent, dissolves the minerals present is the soil. It is an electron donor in photosynthesis. It is the medium for the movement of gases, minerals etc. Enzymes function in the medium of water.

Precipitation: It is closely related to the amount of water available to the plant. Water is most important factor for controlling the type of distribution of plant communities. The water vapour content present in the air is called relative humidity. Warm air can hold more water vapour than cold air. Precipitation takes place when warm air full of water vapour from oceans, rivers, lakes, streams, ponds and plants etc. moves up to the colder belts of the atmosphere. The water vapour cools off at these heights and condenses in the form of water drops.

The yearly precipitation greatly effects the natural vegetation and cultivated vegetation. If the rainfall is moderate and uniform for a number of days, plants will be highly benefited. Little rainfall results in poor growth of plants. Rainfall and temperature are the chief climatic factors and they together determine the pattern of vegetation of a place., Very low rain fall or complete absence accompanied by high temperature will result in drought. The rain water which enters the soil, as soil moisture, depends on the rate and kind of precipitation and also on the nature of vegetation. Rainfall is measured by rain guage.

27.3.2 Atmospheric Moisture

Water is always present in the atmosphere which is invisible and called as 'humidity' of the air. The capacity of the atmosphere to hold moisture in vapour is dependent on the temperature of the atmosphere. It increases with the increase in temperature. The relative humidity is the quantity of moisture as a percentage or maximum quantity that the air can hold at the prevailing temperature. The relative humidity of a place, besides being affected by temperature and pressure which change with altitude, is also affected by wind, exposure, vegetation and water content of the soil.

Effect of atmospheric moisture on plants: a. epiphytes, mosses, lichens and other plants can directly absorb atmospheric moisture. Dew drops are absorbed by leaf surfaces of the plant.

b. Atmospheric moisture reduces the intensity of light falling on the earth surface. Thus plants receive excessive sunlight in dry conditions and exhibit several xeromorphic conditions.

c. Atmospheric moisture in the form of fog and mist are absorbed by the soil particles and are thus sources of soil moisture.

d. The evaporation of water from plants or a body of water is directly dependent on the relative humidity of the atmosphere.

The relative humidity has a direct control on the rate of loss of water by the plants (i.e.transpiration). Many cryptogamic plants grow in habitats, where relative humidity is normally very high. They are called hygrophytes. Certain fungi and lichens show luxuriant growth in the presence of high humidity. Epiphytes also grow in such conditions.

27.3.3. Temperature

Temperature is the measurement of degree of hotness. Temperature variations are the most important of ecological factors and together with moisture, determine to a large degree the distribution of plants over large areas of the earth. Summer and winter seasons are expression of rhythmic change in temperature. Temperature denotes the heat energy available from solar radiations. The radiation of heat from the ground is called terrestrial radiation. The difference between solar (incoming) radiation and terrestrial (out going) radiation is called net radiation. It is generally positive during the day and negative during the night. Relating to variation in temperature, it is maximum at the equator and gradually decreases as one goes to the north or south. The vegetation of the earth can be grouped in relation to different temperature zones, e.g. equatorial forests, tropical forests, coniferous forests, alipne vegetation.

Thermoperiodism: The temperature of any place changes constantly from hour to hour. Many plants have in course of time, got adjusted to the rhytmic diarnal cycle in temperature changes for several phenological events. This regulation of phenology to periodic changes is called thermoperiodism.

The temperature and the length of the vegetative season affect the physiognomy of the individual plant and whole of the vegetation. In the worm tropics the plants continue to grow luxuriantly throughout the year while in cold arctics and the mountain tops the plants have a very short vegetative season and, therefore they have condensed, short and small leaves.

Effect of temperature on plants: Temperature effects the rate of metabolic activities of plants. The rate of photosynthesis and respiration increases with increase in temperature. High temperature during day increases the rate of phytosynsthesis and low temperature during night reduces the rate of respiration considerably, so that the photosynthetic products are conserved and stored. Temperature influences the ratio of cuticular to stomatal respiration. High temperature increases the rate of cuticular transpiration even if the stomata are closed as at night.

High temperature accompanied by heavy rainfall results in very luxuriant vegetation and great complexity and diversity-tropical rain forests. In the temperate region the plants adopted for low temperature and the vegetation consists of coniferous trees like pine, and dicots. In cold polar and alpine regions of tropical or temperate mountains, the plants grow sparsely and remain dwarf.

Cold injury and cold resistance: The degree of injury that plants suffer from low temperature depends (a)on the degree and duration of temperature (b) the suddenness of change (c) on the mineral and moisture content of tissue and also (d) on adaptational characteristics.

Desiccation: In winter the transpiration rate is high and rate of absorption is slow due to low temperature, the tissues are desiccated and injured.

Chilling injury: Plants growing in hot climate if exposed to low temperature (above freezing point) they are found to be killed and injured. This is called chilling injury.

Freezing injury: Due to low temperature water is frozen into ice crystals in the inter-cellular spaces. Protoplasm of the cell also get dehydrated. Proteins are precipitated due to an increase in the concentration of cell sap.

Heat injury: Very high temperature stops growth. The plant becomes stunted and if such a condition persists for a long period the plant dies.

Heat resistance: Plant develop some devices for heat resistance. Dry seeds and spores which have low water contents are most resistant to heat injury. High sugar content also increases the heat resistance of the plants. Plants with bark can endure high temperature.

27.3.4. Light

Light is one of the most important climatic factors for many vital processes of the plant. It is essential for the synthesis of pigment of plants. i.e., chlorophyll. Only the chlorophyll is capable of absorbing radiant energy and converting it into potential chemical energy of carbohydrates. The effect of light on plants is the regulations of the rate of transpiration by controlling the opening and closing of stomata.

The effect of light in nature is uniform over wide areas and therefore, it is not likely that it would affect the general features of the plant communities. The majority of plants growing in direct sunlight are called 'heliophytes' (sun loving), whereas small plant communities growing in deeply shaded areas are called 'sciophytes' (shade-loving).

Light intensity: Atmosphere influences the intensity of light to a very large extent. Moisture of the atmosphere absorbs a high proportion of longer light wave. Dust and smoke can reduce the light by 90% in metropolitan cities. The intensity of light is normally reduced to about 20% by a canopy of vegetation. In thick forests it can be reduced to 1% of the total sunlight and therefore, the ground of such forests does not have good vegetation. In land plants the variations in light intensity is always accompanied with a change in temperature and relative humidity.

Effects of light intensity: Light intensity falling at a particular place is normally enough for the plants and their physiological phenomena as photosynthesis etc. In photosynthesis 1% of the light energy is converted into potential chemical energy. Sometimes low intensity results in closing of the stomata. Very high light intensity increases the rate of respiration and thus disturbs the photosynthesis-respiration balance. The most harmful effect of light intensity is the phenomenon of solarization in which all the cell contents are oxidised by the atmospheric oxygen. This oxidation is different from respiration and is called as photo-oxidation. The light intensity is measured by luxmeter.

Light duration (light meter): The duration of total light period in a year and the duration of daily light period (photoperiod) at a particular place show effects on the vegetative and reproductive structures (i.e. flowering) of plants. The daily light period has a marked effect on the flowering of a plant and they are classified as (a) short-day plants (b) long-day plants (c) Day-neutral plants. Most of the plants belong to the first category. Most of the plants irrespective of their category, show more luxuriant vegetative growth under long days. The amount of vegetative growth is proportional to the duration of day light. Morphological features like the formation of bulb, tuber and other storage products are affected by the length of the day.

27.3.5. Wind Velocity

The velocity of wind has very important effects on the vegetation of a place.

Breakage: A very high wind velocity (gale, hurricane) breaks dead and living branches of the trees and even uproots them completely.

Soil erosion: In bare deserts, high velocity of wind causes constant soil erosion, which makes the plants difficult to grow there. Wind is also responsible for causing rainfall to a very large extent. In India rainfall is determined by wind movement.

Deformation: Continuous strong winds in a particular direction cause bending of trees. This can be seen in coastal or mountain side plants.

Lodging: Plants like rice, wheat, maize, sugar-cane fall flat on the ground due to injury caused by strong winds. This phenomenon is called lodging. Wind is a medium of transport of pollen of anemophilous plants, anemochorous seeds, fruits, and microorganisms.

Desiccation: Wind removes accumulated water vapour from above the plants and thus increases the rate of transpiration. Strong winds during winter increase the rate of evaporation whereas the low temperature reduces the rate of absorption of water. Plants exposed to continuous winds show desiccating effects to transpiration. Wind velocity is measured by anemometer.

27.3.6. Atmospheric Gases

The atmosphere surrounding the earth contains three important gases viz. CO_2, O_2, and N_2.

Carbon dioxide: CO_2 is the main raw material for the synthesis of carbohydrates through photosynthesis. Aquatics get CO_2 dissolved in water. The CO_2 content in the soil is also important in effecting the plants. Excess of CO_2 in the soil cause poor aeration.

Oxygen: Atmospheric oxygen is vital to its life and normal growth. The amount of oxygen is normally constant in the air because plants give off oxygen during photosynthesis which replaces the oxygen consumed in respiration.

Nitrogen: Plants utilise nitrogen in the form of nitrates only. Free nitrogen of the atmosphere can be fixed by certain bacteria and blue-green algae. Proteins in the dead plants and animals are decomposed by micro-organisms, enriching the nitrogen pool of the soil. The nitrogen content of the plant is largely consumed by animals including man as food.

27.4. EDAPHIC FACTORS

Soil: It is the natural thin layer covering the surface of earth in which life is supported. It is the environment for the complex root system of plants and other underground parts such as rhizomes, corms, bulbs, and also soil organisms. It provides the plants with constant supplies of water and mineral salts.

Soil profile: Soil has certain definite structural and positional characteristics which form what is known as soil profile, which can be distinguished into three layers: top soil, sub-soil, and weathered rock. The top soil that is the weathered product of parent material constitutes the first two layers. The semi- broken region of the parent rock is the third layer.

Soil colour: Tropical soils vary greatly in colour. They are black, gray, red or brown. The most important factor which determine the soil colour are proportion of silicon, iron and humus which a particular soil contains. The black soils are formed in anaerobic condition where drainage is available.

Composition of Soil: The main components of soil are mineral matter (about 40%), organic matter (about 10%),5%), and soil air (25%).

Soil texture: The texture of the soil is determined by the relative proportion of rock particles of different sizes present in that. On the basis of texture, soils may be classified as (a) Sandy soils (b) Clayey soils (c) Loam soils (d) Sandy-loam soils (e) Silt-loam soils (f) Clayey-loam soils.

Loam soils are the best for the growth of plants since they contain coarse sand as well as fine silt and clay particles. Thus texture allows aeration and also retaining of water.

Organic matter: Organic matter is added to the mineral matter of the soil year after year. The organic increment of the soil is largely derived from the fallen leaves and twigs, dead and decaying plants and animals.

Soil Water: The water content of the soil is more important than any other ecological factor causing differences between plant mmunities. Soil water is directly taken up by the plants for their life and growth. It acts as a medium for the transport of salts.

Soil air: Aeration of the soil is absolutely essential for the absorption of water by roots. Absorption of water will be good in well aerated soils.

Soil temperature: Soil temperature vary significantly between day and night only on the top layers. In tropical climate the high temperature of the soil by day results in rapid loss of water by evaporation from the heated surface layers. This is only partly replaced by deposition at night as dew. Dry and porous soil is quickly affected by change in temperature. Wet and heavy soil show a very slow change in temperature. The temperature of the soil besides affecting the physical and chemical process going on in the soil also influences the rate of absorption of water and solutes. Soil temperature is measured by soil thermometer.

Soil reaction: Soil pH (pH = Hydrogen ion concentration) has a direct effect on the viability of plants and on the nutrient supply. The protoplasm of root cells is badly damaged below pH_3 and beyond pH_9. Soils may be neutral, acidic or alkaline. The chemical changes in the mineral components, the decomposition of organic matter and the biological activities of the microorganisms are also responsible for contributing to the acidity of the soil. Soil acidity beyond a particular limit is injurious to plant. High acidity may be toxic to plants, interfers with the absorption of several nutrients, and reduces decomposition of organic matter by microorganisms.

Saline and alkali soils: Saline soils are soils which are highly charged with soluble salts. Saline soils restrict the types of plants that can grow on them. Alkalinity is a solution found with soils that are comparatively high in their content of both calcium and sodium. Excess of salts in the soils is injurious to plants. In fact the salt does not favour vegetation at all. In such cases few species have been found to survive. They are called halophytes. The halophytes have adopted for several xeromorphic characters to check water loss due to transpiration.

Soil cover: Soil cover is an important edaphic factor in as much as it protects the soil from loss of water and from rapid temperature changes. The soil cover may be non-living (snow, dead leaves etc.) or living (living plants).

In exterm cold places, snow cover is very effective in protecting soil and also herbaceous plants growing there. Another example of non-living cover is that of dead leaves and withered remains of plants. A living cover consisting of vegetation protects soil moisture and temperature.

Check Your Progress - 1

Name various types of soils.

Note: (a) Write your answer in the space given below.
(b) Compare your answer with the one given at the end.

27.5. TOPOGRAPHIC FACTORS

If the surface of the earth is even over wide areas, the vegetation is uniform. If the surface is irregular or uneven, the vegetation is modified considerably. The irregular factors of the earth's surface are known as topographic or physiographic factors. The topographic factors bring about local variation in climatic condition and modifies the edaphic factors of the habitat. The topographic factors are 1. altitude of the place 2. Steepness of the slop 3. exposure of the slop to light and wind 4. direction of the mountain chain.

1. Altitude: The continuous increase in altitude and its effect on the vegetation can best be observed on the mountains. Increase in the altitude results decrease in temperature, increase in precipitation, increase in wind velocity, increase in humidity. The general decrease in temperature with altitude is about 6-7^{o}C per Km. In addition, the amount of total solar energy absorbed at higher altitudes is higher than at low levels because of the thin atmosphere. The increase rainfall and low temperature greatly influences the development of soil and vegetation.

Steepness of slope: The slope of rock or a mountain is another feature of topography which influences the nature of vegetation. Steep alone causes swift-run-off of water after a rainfall. This decreases the moisture content of the soil which becomes dry. The swift running streams of water carry away the superficial layers of the soil. The humus is not allowed to accumulate. To counteract the harmful effect of steep slopes, the farmers employ terrace-farming on the hill. The small terrace-like flat fields reduce the force of the flowing water.

Exposure of the slope to light and wind: A mountain slope exposed to weak intensity of light and strong dry winds has xerophilous vegetation, e.g. the vegetation of northern slopes of the Himalayas. Usually the north facing slope consists of shade loving species. The slope exposed to direct sunlight and humid wind has luxuriant mesophilous vegetation, e.g. the vegetation of the southern slopes of the Himalayas. Soil of a steep slope is usually eroded and therefore, higher plants may be completely absent.

Direction of mountain chains: If the mountain chains lie in the path of wind which is full of the mountain as in Cherrapunji in India which receives the world's highest rainfall. This results in very luxiriant vegetation in the area. Mountains are the great sources of water for the nearby areas. Large rivers originate in the mountains when after being fed by rainfall they flow down into valleys below and the plains beyond.

27.6. BIOTIC FACTORS

The phenomena of close biotic relationships between organisms are often denoted by symbiosis, mutualism and commensalism. Symbiosis and mutualism denote relationship between two organisms and commensalism is of relationship between animal partners.

Symbiosis may be classified as under:

1. Disjunctive symbiosis: The organisms involved are not in constant contact

a. **Atrophic** (not related to food): Includes the effects of one plant on another with respect to shade, air movement, soil moisture etc. There is no direct nutritional relationship.

b. **Trophic (related to food):** Animals effecting pollination or dissemination incidental to their activities in obtaining food from plants.

2. Conjunctive symbiosis: Organisms involved live in contact with each other.

a. **Atrophic:** Lianas and epiphytes using other plants for support.

b. **Trophic:** Lichens, mycorrhizae, nitrogen fixing bacteria living in the root nodules.

Herbivorous animals: Grazing is one of the most important biotic factors. The domestic as well as wild animals graze vast areas of grassland every day in all parts of the world. These animals like cattle, goat, sheep, deer, rabbit cause heavy damage to plant life. The damage is by complete destruction of photosynthetic organs. The different kinds of animals have their preferences for different species. Goats and sheep like broad-leaved herbs, cattle and horses usually prefer grasses, and the deer and mouse browse on many kinds of shrubs. Rodents and birds consume a large number of nutritive seeds and fruits of small plants. Such plants, therefore, fail to establish themselves, but some animals are useful in dispersal of the species.

Insectivorous plants: These are also known a carnivorous plants. They are partly autotrophic and partly heterotrophic. They can manufacture carbohydrates themselves but are incapable of synthesizing proteins. They get their requirements of nitrogenious food by capturing insects and other small animals, and digesting them extracellularily. e.g. Pitcher plant *(Nepenthes)*,Bladder wort *(Utricularia)*,sun dew *(Drocera)*, Sarracenia, Venus's fly trap (*Dionaea*)..

Lianas: In most tropics there are divers forest with a thick canopy of leaves at the tops of the very tall plants. Very little sun light can pass through the canopy of leaves to the lower levels of the forest. Under such conditions some woody plants climb up with the support of other trees. Such rooted climbing vascular plants are called Lianas, e.g.*Bauhinia, Tinospora*.

Epiphytes: Epiphytes, air plants grow up on other plants usually trees. They are not parasitic but manufacture their own food. They are entirely dependent on rain and heavy dew for their water supply. Most of them obtain their essential elements from decaying organic matter that collects around their roots. many kinds of plants may be ephiphytic. Among them are ferns, lichens, cactus, and pineapple families.

Parasites: Parasites obtain nutrition from living plants or animals, which are called host. The parasites produce parasitic roots or haustoria which penetrate into host's root tissue to make connection with the conducting elements of the host. Parasitism is common among fungi and bacteria. The angiosperms living as parasites are classified as total stem parasite (e.g. *Cuscuta)* partial stem parasite (e.g. *Loranthus*), total root parasite (e.g. *Orobanche*), and partial root parasite (e.g.*Santalum*).

Lichens: Algae and fungi are sometimes associated together in a form of compound organism called lichens. The algae manufacture food material which is also shared by fungi. The fungi absorb water and minerals and protect the algae from drying up.

Mycorrhiza: In plants like pine, active portions of the roots are invaded by specific soil fungi. The resulting association is intimate and complex, and the combination of root and fungus is termed mycorrhiza (literally, fungus-root). The hyphae, like root hairs absorb water and mineral salts which are passed on to the plant. In general, mycorrhizae appear to enable plants under unfavourable soil conditions. Then fungus, in turn, is believed to obtain from the root certain growth-promoting and food substances in which it may be deffcient.

Check Your Progress - 2

Define lianas.

Note: (a) Write your answer in the space given below.

(b) compare your answer with the one given at the end.

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27.7. FIRE

Fire is a major ecological factor which is, and has been for centuries, almost a part of the normal "climate" in most of the terrestrial environments of the world. The biotic communities adapt and compensate for the factor just as they do for other factors like temperature and water. Fire forms an ecological tool of great value when it is properly used. Fire was thus an extremely important limiting factor long before modern man began to drastically modify his environment in an attempt to improve his status.

Long before the advent of man, periodic fires started by lightening of volcanic action swept the vegetation of various areas except in the very wet-rain-forest. Man is known to have used fire since paleolithic times. He has utilized fire to clear forest for agriculture, to improve grazing land for domestic animals, to attract game, to deprive game of cover, to drive game from cover in hunting to kill and drive away predatory animals, to repel the attacks of enemies, for cooking and other purposes.

The question whether fire is a friend or foe, will depend on intelligent knowledge and control. One must ascertain how important man-made fires are in comparison with natural fires especially caused by lightening activity. There are several types of fire in nature which are different in effect. Crown fires and surface fires are the two extreme types with entirely different effect. The former is limiting to most organism and the latter one exerts selective effect and limiting to some organisms. regular light surface fires greatly reduce the danger of severe crown fires in regions subjected to fire.

Fire has many consequences on the individual plants in the vegetation and also on the soil. It played a great role in the formation of various major types of vegetation e.g.some savannas, mild-latitude grasslands and shrublands. It may assist in seed germination. There is a well known notion that fire stimulates the germination of certain pasture species. The pasture seeds which are not killed by fire may be stimulated to germinate earlier. West (1965) observed that the germination of fresh seeds of *Themeda triandra*,an African grass, was not only favoured by fire, but was significantly increased when treated with dry heat. In tropical Australia, shaw (1959) observed that seedling of *Heteropogon contortus* appeared in great numbers only burned areas. Some seeds of chaparsal species require scarification by fire. The better germination of those not requiring scarification may be related to removal by fire of competition litter, and some substances in soils which are toxic to plants.

Fire alters seeds beds. If litter and humus removal are substantial, large areas of this ash, bare soil, or thin humus may be created. Fire shows varied effects on the subsequent size of vegetative organs of herbs. On the coastal plain of Georgia, fire was reported to have reduced the size of the vegetative organs of both *Paspalum notatum* and *Cynodon dactylon* (Burton 1944) although associated species were not affected. Fire sometimes triggers the release of seeds (as with the Jack pine), and seems to stimulate the vegetative reproduction of many woody and herbaceous species. Fire can control forest insects, parasites and fungi and the process is called sanitization. Fire also seems to stimulate the flowering and fruiting of many shrubs and herbs and to modify the physico-chemical environment of the plants. Areas subject to fire often show greater species diversity.

The effects of burning on soil humus have been of much interest. Moore (1960) found that 30 years of annual burning in Nigcria led to an increase in the humus content of top soil by 17% where the burning was light and was applied early in the dry season. On the other hand, the humus was reduced by 12% where the fires were kindled late in the dry season. Moore's findings evidently show that early burning must stimulate productivity resulting in the higher humus levels in the top soil, whereas late burning does not. Soil moisture is also affected by burning grasslands. In central and southern Africa various workers have shown that fire reduces the water-retaining capacity of the soil, so that the grass becomes more susceptible to drought injury. The burning of plant tissue is known to result in the volatilization of nitrogen and sulphur. Other nutrients are converted into simple salts, which are more readily available to the soil and plants. The main effect of fire, then is bulk release of nutrients to the soil surface instead of the gradual release that normally takes place as a result of decay by decomposers.

It is a well-known observation that new grass following recent burns is attractive to hoofed grazing animals probably because of increased palatability. The cattle are found to make great gains in weight when they graze on recently burned grassland. Many full grown hoofed animals are able to escape fire, but their young ones are often destroyed. Fire causes much harm to ground nesting birds. Their insect food sources may also be eliminated. Mice and other rodents suffer loss of their essential food and grass cover, but they are better protected by virtue of the fact that they rear their young in burrows. Insects are also destroyed by fire which may be said to exert some measure of control over insect pests in the field.

27.8. SUMMARY

Environment is a net work of so many factors that interact and influence the living organisms. Ecological factors are climatic, edaphic, topographic and biotic. These effect the form and function of the plant communities. Climatic factors are the atmospheric factors that include water, atmospheric moisture, temperature, light, wind velocity and atmospheric gases. Soil, the natural thin layer covering the surface of earth in which life is supported is delt under adaphic factors. Topographic factors include altitude, slope-steepness, slope exposure to light and wind and direction of the mountain chains. Biotic factors include biotic relationships related to symbiosis, mutualism and commensalism, insectivorous plants, lianas, epiphytes, parasites, lichens and mycorrhizae.

27.9. CHECK YOUR PROGRESS : MODEL ANSWERS

1. On the basis of texture, soils may be classified as (a) Sandy soils (b) Clayey soils (c) Loam soils (d) Sandy loam soils (e) Slit loam soils (f) Clayey loam soils.
2. In most of tropical forests some woody plants gowing at the bottom, under thick shade climb up with the support of other trees as to get light. Such rooted climbing vascular plants are called lianas.

27.10. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in 30 lines each.

1. Write briefly about the climatic factors that influence plant life.
2. Explain briefly about the edaphic factors that influence plant life.
3. What are physiographic or topographic factors? How do they influence plant life ?
4. What are biotic factors? Explain with examples?

II. Answer the following questions in 10 lines each.

1. How does the yearly precipitation affect the natural and cultivated vegetation?
2. How does temperature determine the distribution of plants?
3. Write briefly about light as an ecological factor.
4. "Wind has an important effect on the vegetation of a place". Explain.

UNIT-28 ENERGY AND TROPHIC LEVELS

Contents

28.1 OBJECTIVES

After going through this unit you will be able to:

1. recognize the importance of two functional events i.e., flow of energy and material of an ecosystem and
2. describe the steps involved in the flow of energy through the ecosystem.

28.2. INTRODUCTION

Two functional events of importance to ecosystems are the energy relations within it, and the cyclical passage of materials through it. Energy and materials flow through the community together, one cannot be well separated from the other. The flow energy is one way, once used by the ecosystem, it is lost. Material, on the other hand, recirculates.

28.3. ENERGY SOURCE

The sun supplies the basic source of energy to the earth's ecosystems. In the high temperature of the sun a fusion reaction converts hydrogen to helium releasing radiant energy in the form of electromagnetic waves. The energy that reaches the earth from the sun constitutes a small region in the spectrum of electromagnetic radiation. It includes radiation with wavelengths between 400 and 700 nm. This is the familiar visible solar light spectrum, where energy is transmitted through space. At the earth's outer atmosphere the average rate of energy transfer is about 2 gram calories per square centimeter of surface per minute. This value is called the solar constant. However, not all this energy is actually available for distribution throughout the biosphere. About one-third is readily returned into space as a function of the reflective properties of the earth's atmosphere and surface. This property, ability to reflect sun's radiation is called the earth's albedo. The radiant energy entering the atmosphere is diminished further by about 20% through absorption by gases and particulates, so that a little less than one half of the radiation found in the outer atmosphere reaches the earth itself.

The amount of solar energy actually received in the biosphere varied widely. Regions in the tropics receive the most sunshine. With increasing distance from the sun, energy values per unit of surface area are less. The northern most climates receive only about 40% as much radiation as the tropics.

The quantity of solar radiation that reach the earth at any one place also varies considerably with daily and seasonal fluctuations. In the northern hemisphere there is more sunshine in summer than in winter.

28.4. ENERGY AND THE LAWS OF THERMODYNAMICS

Energy exists in two forms, potential and kinetic. Potential energy is energy at rest, that is, capable of and available for performing work. Kinetic energy is energy of motion and results in work, performed at the expense of potential energy. Work, which results from the expenditure of energy, is of at least two kinds: the storage of energy and the arranging or ordering of matter with no storage. The behaviour of energy is described by the two laws of thermodynamics.

The first law of thermodynamics holds that energy may be transformed from one type to another but that it can be neither created nor destroyed, and regardless of transformations no gain or loss in total energy takes place.

The second law of thermodynamics says that an energy transformation is never entirely efficient. When energy is changed from one form to another, some of the energy is dispersed into unstable heat. For example when coal is burned in a boiler to produce steam, some of the energy creates steam and rest is dispersed as heat to the surrounding air. The same thing happens to the energy in the ecosystem.

28.5. ENERGY FLOW AND THE ECOSYSTEM

This can be studied under four subheadings. viz., (a) Primary production, (b) Food chains and food webs, (c)Trophic levels and (d) Ecological Pyramids.

28.5.1. Primary Production

The flow of energy through the community starts with the fixation of solar energy as chemical energy by plants through the process of photosynthesis wherein carbon dioxide and water are used as the raw materials. Once started the green plants begin to accumulate energy. Energy accumulated by plants is called production or, more specifically primary production, since it is the first and basic form of energy storage. The rate at which energy accumulates per unit of area per unit time is known as primary productivity. The part of sun's energy that is converted to chemical energy in photosynthesis is gross primary production. The energy remaining after respiration and stored as organic matter is not primary production. Thus net production for a plant or plant community can be described as:

$$\text{Solar energy} \xrightarrow{\text{Photosynthesis}} \text{Gross primary productivity} - \text{Respiration} = \text{Net production}$$

Net primary production accumulates over time as plant biomass. The amount of biomass found on a given area at a given time is the standing crop biomass.

The basic unit or energy used in ecological work is the calorie, usually expressed as kilocalories per square meter per year (k cal/m^2/year). Production may also be expressed by dry matter in grams per square meter per year (g/m^2/year). Gross and net productivities vary widely among different plant communities. Variations in gross production for different communities are indicated in Fig.28.1. Communities like estuaries, springs, marshes, and eutrophic lakes have relatively high rate of productivity, but they constitute a relatively small proportion of the earth compared with deserts, deep oceans, or other area of low productivity.

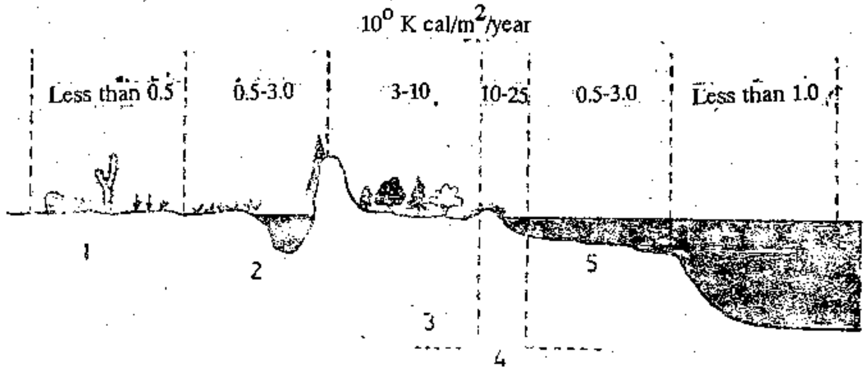

Fig. 28.1. Primary productivity in terms of annual gross production in some world ecosystems. Notice that the highest productivity occurs in some estuaries, springs, coral reefs, and terrestrial communities, which constitute a very small part of the total world area. Much of the world, including the deep oceans and deserts, has very low productivity. 1. Deserts. 2. Grasslands, deep lakes, mountain forests, some Agriculture. 3. Moist forests and secondary communities, shallow lakes, moist grasslands most Agriculture. 4. Some estuaries; springs; coral reefs; terrestrial communities on alluvial plains; Energy subsidized agriculture. 5. Continental shelf waters. 6. Deep oceans.

28.5.2. Food Chains And Food Webs

The energy stored by plants is passed along through the ecosystem in a series of steps of consumption(being eaten), is known as a food chain. Food is the means that energy uses to move from one organism to another. In a grassland community the grass is eaten by grasshopper, the grasshopper is consumed by the frog, the frog by the snake in that order. The food chain here may be represented as: Grass - Grasshoppers - Frogs - Snakes. Numbers of links in such chains are variable, but three to five links are common. Positions of links in the chains are named; 1. Producer: the Photosynthetic plant or first organism of the sequence. 2. Herbivore or primary consumer: the first animal, which feeds on plant food. 3. First carnivore or secondary consumer: an animal feeding (as predator, parasite and scavenger) or a plant-eating animal. 4. Secondary carnivore or tertiary consumer: feeding on the preceding and 5. Tertiary carnivores. Apart from the two major groups of organisms i.e., producers and consumers, there is a third group of organisms - reducers or transformers or decomposers - the saprobes or organisms of decomposition and decay.

But in nature, on one organism lives wholly on another, the resources are shared, especially at the beginning of the food chain. The grass may be eaten by a variety of herbivores, and some of the animals may be consumed by a variety of predators. Thus food chains become interlinked to form a food web, the complexity of which varies within and between ecosystem.

Within any community two major food chains are recognised, the grazing food chain and the detritus food chain. Detritus is the dead organic matter in ecosystem. Because of high standing crop and the relatively low harvest of primary production, the most important food chain in most terrestrial and shallow water ecosystem is the detritus chain. In deep water aquatic systems, with their low biomass, rapid turnover of organisms, and high rate of harvest, the grazing food chain is the dominant one. The grazing food chain involves moving energy and minerals from green plans to herbivores to carnivores. The decomposition or detritus food chain becomes operative when organisms die. Millions of decomposer organisms break up the dead bodies, using energy and releasing nutrients from plant and animal matter back into nutrient cycles. Organisms such as earthworms and beetles, called macrodecomposers, begin the process by removing large pieces of the dead organisms. Microdecomposers such as bacteria and fungi then finish the process. Phytoplankton form the base of the grazing food chain in aquatic systems. They are eaten by other animals. Decomposers including crabs, worms, and bacteria tend to operate rapidly in the aquatic system by beginning to break up the organic matter immediately after death. There are a number of supplementary food chains in the community such as parasitic food chain, saprophagic food chain etc.

Check Your Progress - 1

Write briefly about detritus food chain.

Note: (a) Write your answer in the space given below

(b) Compare your answer with the one given at the end of this unit.

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28.5.3. Trophic Levels.

Each level of consumption in a food chain is called trophic level, such as the grasshopper may occupy a single trophic level, but most animals at the higher levels such as hawk, owl participate simultaneously in several trophic levels. The first trophic level belongs to the producers, the second trophic level to the harbivores (primary consumers), the third level to the first order carnivores(secondary consumers)and so on. The decomposers and parasites are their herbivores or carnivores depending on their nature of food source. Decomposers feeding on dead

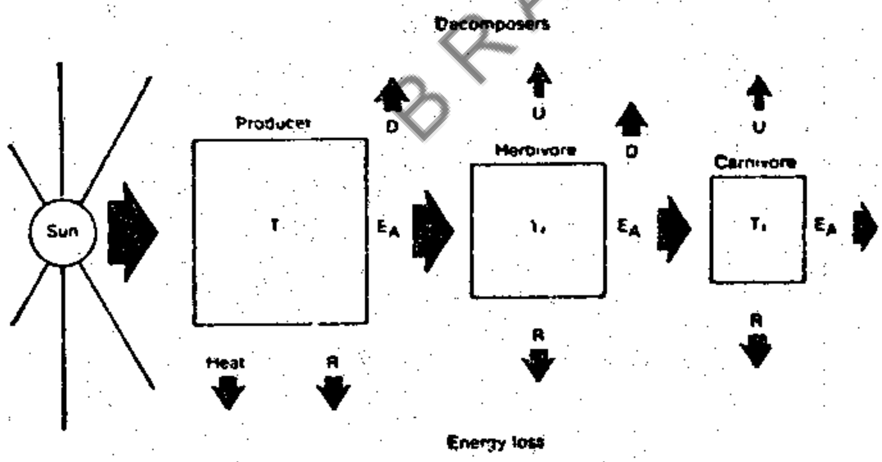

Fig.28.2. Input and loss of energy at each trophic level. The sun's energy provides the fuel for the operation of a food chain. During photosynthesis this heat is lost. At each trophic level (T_1, T_2, T_3), fuel is lost because of respiration (R). Some of the energy available to the next trophic level (EA) is removed by decomposers (D), and some energy taken in by the next trophic level is not used (U).

plant material as well as bacteria occupying the rumen of ungulate animals are the functional herbivores, those feeding on the bodies of animals are carnivores. In this manner all the various steps in energy transfer in an ecosystem can be placed in some trophic level. The transfer of energy from one trophic level to the next is not 100 per cent efficient because the organisms in each trophic level require energy is also lost as organisms consume one another. Not all the organisms at one trophic level are eaten by others; some die and decay, transferring this energy to the detritus food chain. Producers use energy for respiration and lose energy as heat in the photosynthetic reaction. Energy uptake by herbivores respresents the total amount of energy available not only to herbivores but to all animals. In essence, three things can happen to energy assimilated at each trophic level. 1. It can be used for respiration of organisms on that trophic level and lost as heat. 2.It can become part of the detritus food chain either when the organisms of that level die and decay or as it passes through the bodies of other animals without being assimilated. 3. It can be passed on to the next trophic level when animals are consumed and assimilated (Fig 28.2).

28.5.4. Ecological Pyramids

The interaction of food chain phenomena in an ecosystem has a definite trophic structure, which is measured as standing crop per unit area or as energy fixed per unit area per unit time at each trophic level. Trophic structure of an ecosystem could be indicated graphically by means of pyramids in which green plants, the primary producers form the base and successive trophic levels from the tiers which make up the apex. These are called ecological pyramids. They are of three types: 1. pyramid of numbers. 2. pyramid of biomass and 3. pyramid of energy.

Pyramid of Numbers: In large ecosystems, such as grassland and cropland ecosystem, the number of primary producers is the largest, the number of primary consumers is less than that of the producers and those of consumers of different orders decrease further in that order.If these numbers are represented in a diagrammatic way, an upright pyramid is formed (Fig. 28.3A). In such ecosystems as tree ecosystem, the number of primary producers is the least and that of the ultimate consumers is maximum. A single tree (primary producer) supports a large number of fruit eating herbivore birds(primary consumers)which in turn support still higher number of parasites like bugs and lice (secondary consumers). Here an inverted pyramid is formed (Fig 28.3B).

Pyramid of Biomass: The pyramid of biomass indicates by weight or other means of measuring living material i.e. the total bulk of organisms or fixed energy present at any one time - standing crop. In general the biomass of the producers must be greater than that of the herbivores they support, and the biomass of the herbivores must be greater than that of the carnivores. This usually results in an upright pyramid for most ecosystem, particularly the terrestrial(forest ecosystem, grassland ecosystem etc.) (Fig.28.3C) and shallow water ones, where the producers are large, and characterised by an accumulation of organic matter, life cycles are long and there is low harvesting rate.

In such aquatic systems as lake and ocean the situation is different. The primary producers are microscopic algae(phytoplankton) which are characterised by short life cycle, rapid multiplication, little accumulation of organic matter, and heavy grazing by zooplankton. At any point in time the standing crop biomass of the producers is low. As a result, the pyramid of biomass for these aquatic ecosystem is inverted (Fig.28.3 D).

Pyramid of Energy: Here the pyramid is based on the amount of energy produced per unit area(often per sq. cm) by different trophic levels over a given period of time(often per year). Such pyramids are always upright(Fig 28.3E). Since less energy is transferred from each level than was paid into it. This is in accord with the second law of thermodynamics.

Some organism may have a small biomass, but the total energy they assimilate and pass may be considerably greater than of organisms with a much larger biomass. On a pyramid of biomass these organisms would appear less important in the community than they really are. Thus numbers overemphasize the importance of small organism, and biomass overemphasizes the importance of large organisms. Energy flow provides a more suitable index for comparing any and all components of an ecosystem.

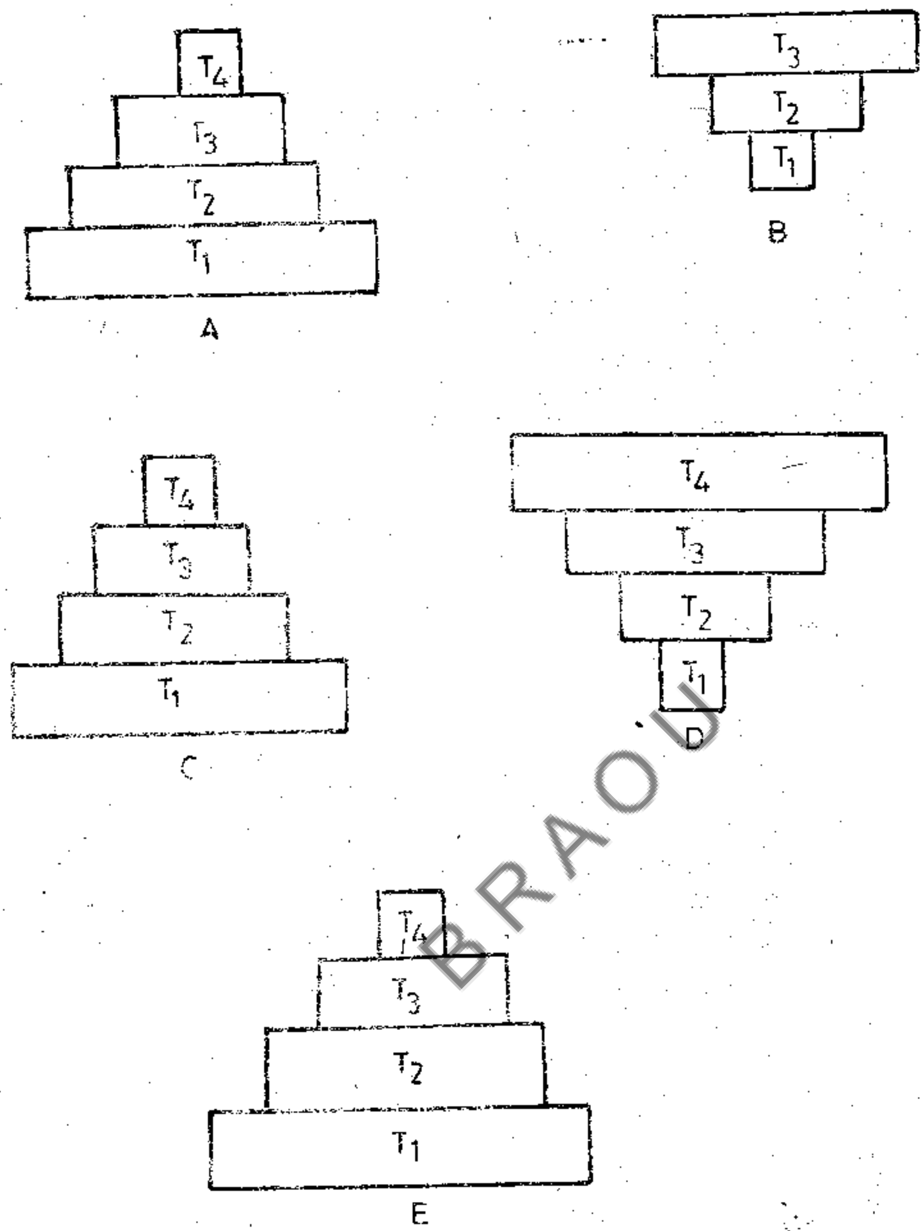

Fig.283 Ecological pyramids: (A-B) Pyramids of number. (C-D) Pyramids of biomass. (E) Pyramid of energy. T_1, T_2, T_3 and T_4 are green plants, herbivores, carnivores and top carnivores respectively.

Check Your Progress - 2

Write briefly about pyramid of energy.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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28.6. SUMMARY

Flow of energy and material are the most important functional events of an ecosystem and they are inseparable;the former being a cyclic and unidirectional and the latter cyclic. The primary source of energy for life on earth is sun and is available as electromagnetic radiation. Energy exists in potential and Kinetic forms. The basic unit of energy in ecological work is the calorie. The steps involved in the flow of energy through the ecosystem are primary production, food chains and food webs, trophic levels and ecological pyramids. Green plants accumulate energy and the energy accumulated per unit area per unit time is called primary productivity. The energy transfer from primary producers to top level consumers in a series of steps along through the ecosystem is 'food chain'. Trophic level is the consumption of energy at each level in a food chain. Ecological pyramid is a trophic structure of an ecosystem. There are three kinds of pyramids:pyramid of numbers, pyramid of biomass and pyramid of energy.

28.7 CHECK YOUR PROGRESS: MODEL ANSWERS

1. Detritus is the dead organic matter in ecosystem. By the decomposition or detritus food chain becomes operative when organisms die. Millions of decomposer organisms break up the dead bodies, using energy and releasing nutrients from plant and animal matter back into nutrient cycle.

2. Pyramid of energy is represented on the basis of the amount of energy produced per unit area(per sq.cm) by different trophic levels over a given period of time(Per year). Since less energy is transferred from each level than was paid into it, these pyramids are always upright.

28.8. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines.

1. How does the energy flow through the ecosystem.
2. What is a food chain? Explain the series of steps that are involved in a food chain?
3. What is ecological pyramid? Explain different types of ecological pyramids.

II. Answer the following questions in about 10 lines each.

1. Write briefly about food chain.
2. Write about the pyramid of numbers.
3. Write briefly about the pyramid of biomass.
4. Write about the pyramid of energy.

UNIT-29: ENVIRONMENTAL POLLUTION

Contents

29.1. OBJECTIVES

At the end of this unit you will be able to:

1. list out the major pollutants of air, water, soil and mention their sources,
2. describe the effects of various pollutants on animal and plant systems, and
3. describe the methods of control of environmental pollution.

29.2. INTRODUCTION

Pollution is a major problem of the environment. Pollution can be defined as an unwanted change of environment which is harmful to the living organisms. The deteriorating pollution is causing world wide concern. Pollution can be natural or man made. The agents that pollute are termed "Pollutants" and they are of various kinds. The environmental pollution can be broadly divided into 3 categories: 1. Air pollution 2. Water pollution 3. Soil pollution.

29.3. AIR POLLUTION

The gaseous envelop that surrounds the earth is its atmosphere and the gaseous content is air. The average composition of dry air below 25 Km is Nitrogen 79%, Oxygen 20%, CO_2 0.03% and other gases 1%. The earth's atmosphere in its present form and composition is believed to have developed at least some 350 million years ago when extensive vegetation cover originated on land. Its presence provides an indispensable shield from harmful radiation from the sun and its gaseous content sustains the plant and animal life. The composition of the atmosphere is progressively being changed by man's activities with the result that plant, animal and human life is affected, hence the preservation of its quality is of utmost concern.

29.3.1. Air Pollutants - Sources and Effects

Table 33.1 gives the majc : air pollutants, and their emission sources, and Table 33.2 lists the air pollutants that affect the plant system, nature of symptoms they cause etc.

Carbon Dioxide: It is chiefly produced in the combustion of the fuel in the homes, factories, power stations etc. It is also released in respiration by plants and animals. A balance, or dynamic equilibrium, is maintained primarily by photosynthesis which removes approximately 3% of the world's total CO_2 annually. However, between 1870 and 1970 the total quantity of atmospheric CO_2 is estimated to have increased by 11% (from 294 to 321 ppm) due to the burning of fossil fuels. The actual use of fossil fuel should have produced an increase of about 20% but apparently the excess is taken up by the land biosphere and the oceans. Calculations suggest that there will be an increase of CO_2 concentration from 320 to 370 ppm by AD 2000.

Most of the incoming solar radiation passes through air containing CO_2 and water vapour because most of its electromagnetic energy is in the shorter wave lengths. However, as the earth reradiates heat back into space, the shift to the longer wave lengths puts the radiation in a range readily absorbed by water vapor and CO_2. Therefore, any increase in the CO_2 content of the atmosphere may result in more of the outgoing earth radiation being absorbed in the air and a warming of the earth's atmosphere. This warming effect has become, widely known as the 'green house effect', by analogy with the ability of green house to keep it inside warmer than the outside during the day time.

Table 29.1. Major air pollutants and their emission sources.

Pollutants	Where They Come From
Aldehydes	Thermal decomposition of fats, oil or glycerol
Ammonias	Chemical process-dye-making ; explosives; lacquer ; fertilizer
Arsines	Processes involving metals or acids containing arsenic soldering
Carbon monoxides	Gasoline motor exhausts
Chlorines	Bleaching cotton and flour; many other chemical processes.
Hydrogen cyanides	Fumigation ; blast fornaces; chemical manufacturing ; metal plating
Hydrogen fluorides	Petroleum refining ; glasses etching ; aluminium and metal plating
Hydrogen sulphides	Refineries and chemical industries ; bituminous fuels,
Nitrogen oxides	Motor vehicle exhaust ; soft coal
Phosgenes (carbonylchloride)	Chemical and dye manufacturing.
Sulphur dioxides	Coal and oil combustion
Suspended particles (ash, soot, smoke)	Incinerators; almost any manufacturing

Table. 29.2 Significant pollutants toxic to plants.

Pollutants	Markings	Level (parts per million)
Sulful dioxide	White to brown, bleaching, blotching between veins	0.1 to 3.0
Fluoride	Necrosis on tips and edges of leaves, sharply demarcated by dark brown-red band	0.0001
Ozone	Red-brown flecks	0.15
Oxidant smog	Silver or bronzelike on underside of leaf; banded pattern	0.2
Chlorine	Bleaching, necrosis on margins and between veins, scattered spotting	About 1.5
Ethylene	Withering and drying of flowers, growth retardation, loss of flower buds	0.01

* Cut roses are sensitive to fluoride.

Table.29.3. Specific effect of air pollutants on humans.

Groups	Agent	Principally Affected Organs
Respiratory Pollutants		
Pulmonary irritants	Sulfur oxides	Lining of respiratory tract
	Nitrogen oxides	
	Ozone	
	Chlorine	
	Ammonia	
Dust	Quartz	Pulmonary interstitial tissue
	Silica	
	Carbon	
	Asbestos	
	Cobalt	
	Iron oxides	
Granuloma-producing agents	Beryllium	Lungs
	Hair sprays	
	Talcum powder	
Fever-Causing agents	Zinc	Alveoli
	Manganese	
	Hemp, cotton	
Asphyxiating pollutants	Carbon monoxide	Hemoglobin
	Hydrogen sulfide	Respiratory centre
Systemic Pollutants	Lead	Nerve tissue
	Mercury	Brain, bowels
	Fluoride	Bones, teeth
	Cadmium	Blood vessels, kidneys
	Chlorinated-hydrocarbons	Fat tissue, liver
	Organophosphates	Nerve muscle synapses
Host-Specific Agents Allergenic	Epoxy resins	Skin
	Thiocyanate	Respiratory tract
	Formaldehyde	Skin,lungs
	Pollen	Respiratory tract
	Fungi	
	House dust	
Carcinogens	Strontium-90	Bones
	Iodine-121	Thyroid
	Nickle carbonyl	lungs,sinuses
	Chromium	Nose
	Asbestos	Pleura
	Selenium	Testicular tissue
	Arsenic	Skin
	Polyvinyl	
	Benzo [a] pyrene	
Mutagens	Most systemic pollutants; organic mercury, lead, chlorinated hydrocarbons, arsenic, fluoride, cadmium	chromosomes and genes

An increased warming of the earth has consequences. The glaciers would recede, polar ice caps would disappear, and ocean levels would rise. It is estimated that if all the ice on earth should melt 200 feet of water would be added to the surfaces of all oceans, and coastal cities would be inundated.

Carbon Monoxide: It is the most dangerous and abundant air pollutant and is produced mainly from the incomplete combustion of carbon in vehicle fuels, and consequently is very plentiful in urban air. It also results from steel industry, in solid waste disposal. CO is absorbed through the lungs and reacts with the haemoglobin of the blood to form carboxyhaemoglobin. Haemoglobin has an affinity for CO that is 200 times stronger than for Oxygen, preventing oxygen from reacing body tissues. Thus it is highly hazardous to human health, and the general effect is strain on the heart and respiratory system.

Cigarette smokers have a carboxyhaemoglobin level of about 5 per cent, contrasted with non-smokers at 0.5 per cent. Exposure to high levels of CO produces death quickly. CO is a special problem for street traffic; better the traffic police wear gas masks and have oxygen suppliers.

Oxides of Sulphur: The important sulphur oxides are SO_2 and SO_3. The former is considered to be one of the most serious pollutants of the atmosphere and is associated with major air pollution disastrous on record. These oxides of sulphur are emitted by smelters, oil refineries and paper mills, but mostly from burning sulphur containing coal and petroleum. SO_3 is mostly formed in the atmosphere by the oxidation of SO_2 under the influence of sunlight. The moisture in the air reacts rapidly with SO_3 to form a mist of sulphuric acid. In such a case SO_2 is called the primary pollutant and the other secondary pollutants.

SO_2 is quite harmful to humans causing diseases of eyes, throat and lungs. In plants, the gas causes bleaching of leaf (Chlorosis) thereby affecting photosynthesis; breakdown of cells. It may however be noted that the sensitive to SO_2 to toxicity is different in different plants. Lichens are extremely sensitive and could be regarded as infallible indicators of SO_2 pollution.

H_2SO_4 formed as secondary pollutant corrodes metals, destroys living tissue, and deteriorates various building materials. Marble is particularly vulnerable to such attack; as a result, many buildings and monuments that have endured for centuries are now being rapidly eroded. The danger to the Taj Mahal of Agra has been anticipated from the oil refinery at a nearby town, Mathura and appropriate anticontrol measures are taken.

Acid rain collected in lakes affects the eggs of amphibian and fish, especially trout. As lakes became more acidic, bacteria disappear, frogs die, and leaves and other litter pile up on the lake bottom. Below 5.5 pH, traditional plants are replaced by masses, fungi and algae.

Fluorides: Fluoride containing rocks, soils, minerals on heating release the hydrogen fluoride into the atmosphere. It is also emitted from various specific industrial operations such as the production of aluminium. It is extremely toxic to plants, and causes widespread damage to vegetation. It enters the leaves through stomata and gets accumulated in their tips and margin's resulting in necrosis and chlorosis.

Oxides of Nitrogen, Hydrocarbons and Ozone: Nitrogen oxide (NO) and nitrogen dioxide (NO_2) are the important oxides of Nitrogen that occur in the air as pollutants. Both are produced by any combustion process, but, again it is the locally high concentrations that are responsible for adverse effects on living organisms and on materials. The effects of NO_2 on man range from unpleasant odour to serious lung congestion to death, depending on the concentration of NO_2 and the duration of exposure. Hydrocarbons which are a variety of chemicals, are emitted in large quantity by the burning of petrol in the automobiles. These are quite harmful Ozone (O_3 occurs to some extent in 'normal' air, but in higher concentrations (15 to 20 ppm) it is toxic.

It is abundantly produced from oxygen- containing molecules. High energy causes splitting of NO_2 to NO and atomic oxygen which in turn reacts with molecular oxygen to form ozone.

The oxides of nitrogen and hydrocarbons combine in the presence of sunlight producing still more harmful 'photochemical smog'. Ozone and PAN (Peroxyacetyl nitrate) are the chief constituents of smog, and are extremely toxic to plants, but also cause eye- watering and respiratory distress in man. Smog is now a serious problem of sunny urban areas with heavy automobile traffic, such as Los Angeles, New York and London in the west.

Lead: Lead which is mixed in petrol to minimise "knocking" comes out from the automobiles and very badly affects human health. Further more, it accumulates in the automobile engine and increases the hydrocarbon emissions, whose effects have already been discussed.

Ethylene: It is released from automobile engines, from incomplete combustion of almost any organic substance. It causes withering and dying of flowers, growth retardation, loss of flower buds.

Particulate Matter: Not all air pollutants are gases. There are particles (solid or liquid) that include sea salt, dust, organic matter, smoke. Dusts are produced from such manufacturing processes involving grinding and crushing or handing of dusty materials, cement plants, asphalt plants, foundries and so on. Construction and demolition also make dust. Burning of coal produces unburned or incompletely burned particulates of carbon (smoke and soot) and solid residue (fly ash). Incomplete burning of oil will produce soot.

Particles discharged from natural (volcanoes) or from man made sources can reduce visibility and restrict the amount of sunlight reaching the earth, possibility leading to a cooling of the earth's atmosphere.

There are many kinds of materials of biological origin in the air like bacteria, fungal spores, pollen grains etc., which quite often cause atopic allergy and bronchial troubles like asthma.

29.3.2. Air Pollution Control

There are various devices now developed to check particulate materials and abnoxious gases from being dischatged into the atmosphere. The use of simple air cleaning equipment such as Cyclones, scrubbers, bag houses and electrostatic precipitators enable to capture most of the particles of combustion, and dusts from manufacturing and processing units. If gaseous waste is passed through a porous chamber particles are held up. The gases could then be passed through a water tower before releasing into the general atmosphere so that most of the water soluble pollutants are absorbed before hand. Substituting natural gas for coal and oil would eliminate SO_2 and particulate emission problems from combustion, because it is scare it is no longer particle for power plants. As far as possible steps must be taken to avoid too much use of fossil fuels like coal and petroleum, and instead hydroelectric and solar power should be harnessed more and more. Nuclear plants do not have these air pollution problems but people have other concerns about using nuclear fuel. The social and economic costs and benefits including environmental impacts, need to be evaluated in order to choose the best fuel system.

Check Your Progress - 1

"Carbon monoxide is highly hazardous to human health" - Explain.

Note: a) Write your answer in the space given below.

b) Compare your answer with the one given at the end.

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29.4. WATER AND SOIL POLLUTION

The principal waste disposal practice employed almost every where is based on the philosophy that solution to pollution is dilution. Therefore effluents from human settlements and industry are being increasingly let out at such a rate that many great rivers and lakes have turned murky. The industrial wastes, radioactive materials dumped in the sea and oil spills have been affecting the marine life. The indiscriminate and excessive use of pesticides in agriculture greatly damaged the soil as well as aquatic systems.

These pollutants are classified in a general ways as:

1. Sewage and other oxygen demanding waste;
2. Disease-causing or infectious wastes;
3. Plant nutrients such as nitrogen and phosphorous compounds;
4. Sediments or silt i.e. particles of soil, sand and minerals;
5. Toxic substances such as synthetic organic compounds, detergents, insecticides;
6. Chlorinated hydrocarbons;
7. Radioactive wastes and
8. Heat

Biodegradable and nondegradable pollutants: From the ecosystem point of view, two basic types of pollutants are recognised. They are nondegradable pollutants and biodegradable pollutants. The first one includes the materials and poisons, such as aluminium cans, mercurial salts, long-chain phenolic chemicals and DDT, that either do not degrade or degrade only very slowly and accumulate in the natural environment. By contrast, biodegradable pollutants such as domestic waste can be rapidly decomposed by natural processes involving microorganism. Heat or thermal pollution is dispersible by natural means, and hence can be considered in this category. However, problems arise with the biodegradable type of pollutants when the input into the environment exceeds the decomposition or dispersal capacity.

The effects of the two basic kinds of pollution on systems energetics are different. Degradable pollutants that provide energy (organic matter) or nutrients (phosphates, carbonates etc.) will increase the productivity of the ecosystem by providing a subsidy when the rate of input is moderate (upper graph). At high rates of input a critical range is reached that is frequently characterised by severe oscillations. Additional input above this level becomes a stress and the system essentially becomes poisoned by 'too much of a good thing'. Toxic materials are stressful from the beginning. They increasingly depress productivity as the amounts increase. But the effect may be hard to detect at low levels.

29.4.1. Biochemical Oxygen Demand (B.O.D)

Sewage and other oxygen demanding wastes constitute one of the largest pollutants of our waters. This group includes those materials usually derived from domestic sewage and animal or food processing plants. Normally, if the quantity of sewage deposited in natural bodies of water is low, the organic materials are acted upon by aerobic bacteria and fungi, and turned into minerals in a short time. But if the sewage quantity is higher than the purifying capacity of the water body, the population of bacteria tremendously increase. The aerobic bacteria, as their name indicates, require a great deal of oxygen. As a result, any oxygen found in the body of water in which the pollutants occur is in great demand and often rapidly reduced. Such oxygen reduction is used as an indicator of the degree of pollution and is measured as the biochemical oxygen demand or BOD. In a poorly oxygenated condition with increased CO_2, fishes, other animals and plants die, and the clean water body is turned into stinking one.

29.4.2. Water Borne Human Diseases

Human diseases carried by water include typhoid, cholera, amoebiasis, salmonellosis, shigellosis, infectious hepatitis (Jaundice), amoebic dysentry. They are caused by certain microorganisms-bacteria, viruses, protozoa and helminths that can be found in the feaces and urine of a person ill with the disease. Intestinal and kidney discharges from ill persons, if not properly disposed of, can contaminate water subsequently used for drinking, cooking, washing dishes or bathing. A person consuming food or water containing these organisms will contract the disease. The drinking water obtained from such water bodies polluted with intestinal discharges is to be chlorinated to kill the disease causing microorganisms.

29.4.3. Eutrophication

The rain water from the land runs from the surface or percolates through the soil to enter the streams and eventually the lakes, estuaries and oceans. The water carries with it silt and nutrients in solution, all of which enrich aquatic ecosystems. Man has added through agriculture a heavy load of silt, nutrients especially of nitrogenous and phosphate fertilizers and other organic matter to the water bodies. One of the outcomes of this activity is the excessive nutrient enrichment of our aquatic systems - which become highly productive or eutrophic. Eutrophication is another form of pollution. The mat of algal blooms became quite thick; on their death they become medium for bacterial decomposition which in turn leads to oxygen depletion and the associated kinds of pollution. In such anaerobic situations many useful plants and fishes die and the water begins to stink.

29.4.4. Biological Magnification of Insecticides:

Organochloride insecticides like DDT, though helped to control mosquitoes and agricultural pests, have become serious pollutants of soil and water. They are highly persistent, and their concentrations continued to increase with successful applications. The long term effects of insecticides in soils are not known. Available evidence shows that they bring about significant changes in the population of soil organisms thereby affecting soil mineralisation. They inhibit nitrification by soil bacteria. Termites have not been able to survive in soils sprayed with Toxaphene. Aldenine depleted the earthworm population.

A large fraction of soil-based insecticides gets carried into the world's surface waters with the result all major rivers and the oceans are contaminated with these pesticides. The effect of a given spray application is found to be more severe on carnivorous insects than on the original pest. The chemical was linked with the death of various birds. The egg shells of birds that have ingested DDT contain less calcium and are thinner. In these cases, a series of steps along food chains was involved; leaves of sprayed trees dropped, decayed, and tainted the soil; the birds ate the worms. Fish-eating birds and animals were also affected by DDT. Carried into lakes lakes and oceans, the chemical was ingested by small organisms which in turn were eaten by fish or clams. The DDT is more concentrated in their bodies through the process called biological magnification. Stored in fats and oils DDT is concentrated when these materials are eaten and digested. Clams may show a DDT body concentration of only 0.09 ppm, for example, whereas a sea gull may contain 25 ppm in its body fat. It is alarming that DDT is present in human breast milk, obviously reaching there through food chains.

29.4.5. Mercury Pollution

Mercury in an organic form is highly harmful. The organic mercurials-ethyl or methyl mercury are produced when inorganic mercury compounds are dumped into a body of water and settle on a muddy bottom. There bacteria convert the inorganic mercury into organic mercury, commonly the methyl form. The highly toxic methyl mercury then enters the food chain, where it is concentrated in fish it may further be concentrated in humans who eat such fish. The toxic effects are mainly on the central nervous system. Mercury poison can be fatal. The famous minimata disease is a clear example of many poisons. For years, Industrial mercury was dumped into Minimata bay in Japan. The fisherman and their families who lived on the fish they caught, began to show the crippling effects of mercury poisoning. By 1970 the effects of the Minimata dumping had reached epidemic proportions.

Mercury also enters our environment in other ways; from burning of fossil fuels and from the use of methyl mercury as a fungicide.

29.4.6. Radioactive Wastes

A serious threat to our environment is from radioactive wastes- the deadly, long-lived by products of nuclear explosions, nuclear reactors, nuclear power stations etc. When a nuclear bomb explodes high in the atmosphere or at the earth's surface the explosion releases fine radioactive dust particles and gases, which are then spread around the globe by winds. As these particles settle to earth of their own weight or are brought down in rain, they become radioactive "fallout" that may be breathed or ingested by living organisms.

The radioactive wastes can enter the food chain, and be taken up by plants, animals and man. Radio-iodine gets concentrated along food chain from low levels in the environment to relatively high levels in the thyroid gland. Radiostrontium is similar in chemical behaviour to calcium. Strontium-90 is concentrated from low levels in the environment to higher levels in human bones; the concentration ratios are highest in children who are adding calcium to bone more rapidly than adults and who receive strontium in milk as well as in other foods. Cesium-137 is taken up by plants along with potassium. One of the least expected hazards to human beings involved the food-chain concentration of Cesium from dust from atomic explosions, to soil and lichens in the arctic tundra, to reindeer feeding on these lichens, to Eskimos eating the reindeer.

Life is damaged genetically or somatically by radiation. Genetic damage might not appear for many generations, but somatic effects appear immediately or later in life. Cancer, cataracts or a shorter life span are some somatic effects of chronic, or long- term exposure. In Japan the after-effects of the atomic blasts of 1945 at Hiroshima and Nagasaki are still visible. Hundreds of people died due to malignant growth, leukemia and cancer. Strontium-90 entering and accumulating in bones through the food chain: Plant-herbivore (cow) - and human, is the potential cause for bone cancer and leukemia.

29.4.7. Thermal Pollution:

Many industrial processes including nuclear and fossil fuel power plants produce great amounts of heat which ends up in the air and water. This waste heat, called thermal pollution, can cause severe problems in aquatic environments. Many aquatic species are very sensitive to changes in temperature and oxygen levels. Oxygen demand (BOD) go us as temperature goes up. On the other hand, oxygen is less soluble in warm water than in cold. Water at $20^{o}C$ will hold roughly 17% more oxygen than the one at $30^{o}C$. The entire ecological balance of a stream can be affected by thermal pollution. Increased water temperatures cause fish eggs to hatch so early in the spring when natural food organisms needed by the young ones would not be available. Trout eggs commonly fail to hatch in water that is too warm, and samon often fails to spawn in such conditions. Increased temperature may even affect CO_2 assimilation and consequently the primary production of water bodies is considerably depressed.

Check Your Progress - 2

What is B.O.D?

Note: a) Write your answer in the space given below.

b) Compare your answer with the one given at the end.

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29.5. SUMMARY

Air is being polluted by different activities of man and results in many harmful hazards to all living things. Major air pollutants, are oxides of Carbon, Sulphur and Nitrogen; Fluorides, Hydrocarbons, and Ozone, Lead, Ethylene and particulate matter. CO_2 is chiefly produced in combustion of fuel. Increase in CO_2 content causes warming of earth's atmosphere; this is known as "green house effect". CO is the most dangerous pollutant and accumulates in the atmosphere by incomplete combustion of carbon from vehicle fuels, steel industry and solid waste disposal. CO on inhalation quickly combines with haemoglobin to form carboxy-haemoglobin preventing O_2 to reach body tissue. The result is strain on the heart and respiratory system. SO_2 and SO_3 are also the major pollutants mostly produced from burning sulphur containing coal and petroleum. They cause disease of eyes, throat, lungs and, chlorosis in plants, and affects photosynthesis. Nitrogen Oxide (NO) and Nitrogen dioxide (NO_2) are harmful pollutants produced in any incomplete combustion. The unpleasant odour of NO_2 brings congestion of lungs and leads to death. Hydrocarbons are emitted during the burning of petrol in the automobiles and various chemicals. The oxides of nitrogen and hydrocarbons combine in the presence of sunlight producing harmful effect and it is called photochemical smog. Ozone and PAN (Peroxyacetyl nitrate) are the main constituents of smog and are toxic to plants. Ethylene comes from incomplete combustion of organic substances and is highly harmful to plants.

Different types of particles (Solid and Liquid) act as pollutants and enter into atmosphere from various sources. The chief water and soil polluting sources are human settlements, industrial wastes, radioactive materials, oil spills, and pesticides. Water and soil pollutants are basically of two types: biodegradable and non-degradable. The biodegradable pollutants are poisons, such as aluminium cansmercuria long chain phenolic chemicals and dichloro diphenyl ethylene tetrachloride (DDT). Polluted water causes human diseases. They are Typhoid, Cholera Amoebiosis, Salmonellosis, Shigellosis and Jaundice. Water enriched with nitrogenous and phosphorous fertilizers promotes algal blooms, on death they become a medium for bacterial decomposition, thereby oxygen depletion. It is known as 'Eutrophication'. DDT in high concentration causes water and soil pollution, the issue becomes more serious because of 'biological magnification'. Mercury in organic form is highly harmful. Radioactive wastes are dangerous pollutants liberated into atmosphere from the explosion of nuclear bombs. These particles during rain reaches the earth's surface and through food chain affects the living organisms.

29.6. CHECK YOUR PROGRESS: MODEL ANSWERS

1. The most dangerous and abundant air pollutant, carbon monoxide (CO) is absorbed through the lungs and reacts with the haemoglobin of the blood to form Carboxyhaemoglobin in human beings. Haemoglobin has stronger affinity (200 times) for CO than for oxygen. This results in strain in the heart and respiratory system.
2. In a water body if the seepage quantity is higher than the purifying capacity of the water body, the population of bacteria (Aerobic) tremendously increase. This results in great demand and often rapid reduction of oxygen. Such oxygen reduction is used as an indicator of the degree of pollution and is measured as the biochemical oxygen demand or B.O.D.

29.7. MODEL EXAMINATION QUESTIONS

I. Answer the following question in about 30 lines each.

1. What are the important air pollutants? Write briefly about their sources and their effects on animal and plant life.
2. What are the important water and soil pollutants? Write briefly about their effect on living organisms.

II. Answer the following questions in 10 lines each.

1. "Carbon Monoxide is a dangerous air pollutant" - Explain.
2. What are the dangerous effects of sulphur acids?
3. What are the measures you suggest to control air pollution?
4. Explain briefly about the biodegradable and nondegradable pollutants.
5. Write briefly about the water borne human disease.
6. Write briefly about the effect of radioactive waste on living organisms.

UNIT-30: CONSERVATION AND MANAGEMENT OF NATURAL RESOURCES

Contents

30.1. OBJECTIVES

After growing through this unit you will be able to:

1. describe various natural resources,
2. know the principles of conservation of natural resources,
3. describe the management of natural resources.

30.2. INTRODUCTION

As the human population increases, greater demands are placed upon the available resources. Large areas of the earth are converted to the exclusive use of man. The result is that several changes are occurring in the various components of the biosphere-pollution of seas, rivers, and lakes denudation of forests, disappearance of thousands of plant and animal species, loss of valuable genetic resources especially in the tropics; ecological imbalance resulting from excessive and widespread use of chemicals, degradation of extensive areas of pasture, especially in the semi-rid regions of the world. These changes are well documented in UNEP's 1982 report - The World Environment. Thus in the present day, many valuable natural assets and resources, which were available yesterday are not seen today. The problem then is what will happen to our future generation if the natural resosurces are consumed at rapid rate.

Increased attention is now being paid to understand the ecological principles, and find ways, and means for proper utilization, and management of natural resources so that their continued availability for future needs is ensured. With man's growing awareness of his effects upon nature, he is constantly searching for better control of his industrial waste products, and methods of land use which are harmonious with nature. Conservation programmes are becoming more common practice.

30.3. NATURAL RESOURCES

Land, minerals, timber, and wildlife are all resources. Our lakes, rivers, oceans and underground water constitute our water resources. The resource, then, is anything that satisfies the needs or wants of human society.

Natural resources have been customarily divided into (1) renewable and (2) non-renewable. The former includes plants and animals whose continued harvest is possible upon proper management, the later includes coal, iron, and oil deposits, which, once gone, have very little chances of recovery or resynthesis.

What is conservation?

The word conservation is derived from two Latin words: *con* meaning together and *servare* meaning to keep or guard. The word itself was coined by Pinchot shortly after the 1908 White House Conference.

The aim of conservation as stated by Odum (1971) must be to ensure (1) the preservation of quality environment that considers aesthetic and recreational as well as product needs. (2) continuous yield of useful plants, animals and materials by establishing a balanced cycle of harvest and renewal.

As defined in World Conservation Strategy prepared by IUCN in 1980 conservation is "the management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generations while maintaining its potential to meet the needs and aspirations of future generations. Thus conservations is positive, embracing preservation, maintenance, sustainable utilization, restoration and enhancement of natural environment". The World Conservation Strategy sets out three basic principles for maintaining the balance between man and the environment. Here is a prescription for conservation, which man must take in order to ensure his survival on earth: (1) to maintain essential ecological processes and life-support systems; this primarily requires rational planning and allocation of uses and high quality management of those uses; (2) to preserve genetic diversity; this primarily requires the timely collection of genetic materials and its protection in banks, plantations, and so on, in the case of off site preservation; and ecosystem protection in the case of on site preservation; (3) to ensure the sustainable utilization of species and ecosystems; this requires knowledge of the productive capacities of those resources and measures to ensure that utilization does not exceed those capacities.

Awareness is the first requisite for meaningful analysis of environmental problems and appropriate action. A mutually reinforcing awareness-analysis-action chain can be promoted only by education. An old Chinese proverb says: If you are thinking one year ahead, plant rice; If you are thinking 10 years ahead, plant trees, and, if you are thinking 100 years ahead educate the people.

The UN Conference on Human Environment held in Stockholm on 5th June, 1972, which had the distinction of the Prime Minister of India, late Indira Gandhi addressing it was a major breakthrough in creating awareness. From then on the World Environment day is being observed every year on June 5 with a view of arousing the masses and bringing environmental issues to the forefront of thinking and planing, and also to remember this mother earth the only environment we have, and to take stock of its great capacity to assure all of the lasting prosperity. The recent controversy over the Silent-Valley hydel project to save 'one of the last vestiges' of typical rain forest and the "Chipko-Movement" in Himalayas (Almora Hills) in India is a welcome sign of people's awareness.

There is a great difference between the problems that the developed and the developing contries face with regard to the environment. The developing contries like India face the dual problem of population growth and proverty.

Therefore one of the fundamental problems confronting mankind is how to meet the basic need and requirements of all people without simultaneously destroying the resource base, that is the environment, from which ultimately these needs have to be met. Developmental activities leading to the reduction of poverty and improvement of the quality of life cannot be improved, if the development activities cause serious environmental degradation. The crucial need is therefore to be planned for development that is in harmony with the environment. Thus environment and development are to be treated as two sides of the same coin and we must achieve development without destructio.ı. The implementation of conservation strategies depend on the collective efforts of people, State and Central Governments.

In the following the conservation and management of some important natural resources are dealt with:

Check Your Progress - 1

What are resosurces? Give examples of natural resources.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end.

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30.4 SOIL CONSERVATION

Soil is a crucial life-spport system since the bulk of all food production depends on it. As with other countries, the future of India or at least of its ability to feed its people, will depend on the health of its soils.

Soil has never stayed in one place. Running water and wind have moved soils since they were first formed from the early rocks. Thus, soil erosion is not new, nor is man exclusively the cause of it. Nevertheless, today man's agricultural practices certainly aggravate the problem in a major way. Soils in tropical zones are generally more susceptible to erosion than the temperate zone, due to the topography of the land, and the nature of soil and rainfall. More than half of India, fo example, suffers form some form of soil degradation. Out of her total of 3.3 million km^2, 1.4 million km^2 are subject to increased soil loss, while an additional 270,000 km^2 are being degraded by floods, salinity and alkalinity. An estimated 6,000 million tonnes os soil are lost every year from 800,000 km^2 alone; with them go more than millin tonnes of nutrients-more than the amount that is applied in the form of fertilizers. The world Charter for Nture, which the United Nations General Assembly adopted on 9th November, 1982 recognised soil conservation as fundamental and provides that the productivity of soils shall be maintained or enhanced through measures which safeguard their long term fertility and the process of organic composition and prevent erosion and all other forms of degradation. The United Nations Environment Progress (UNEP) has developed a World Soil Policy to conserve the productivity of soils and is now considering how to implement it.

Soil erosion occurs easily when these are cut or the soil is ploughed. Plant roots bind the soil, and their destruction allows the soil to be readily moved by wind or flowing water. Such destruction, aided by drought, accounted for the formation of the dust bowls. Secondary causes of soil instability include the disturbance of the cycles involving humus and nitrogen. In undisturbed soils, these materials are normally replaced at least at the rate at which they are removed. In disturbed soils, the lack of recycling of organic matter demands that fertilizers be used, often rich in nitrogen, which greatly increases nitrogen content of soil and adjacent waterways. If fertilizers are not used, soil fertility continues to decline, and in time this is reflected in the crop.

Destruction of natural vegetation cover by overfelling and overgrazing is the genesis of soil erosion. Water and wind are the principal causes for the removal of soil from one place to another.

Water erodes or cuts and removes the soil by falling on it as raindrops, as well as by its surface flow action. The chief sheet form of soil erosion by water are 1.Sheet erosion, 2. Rill Erosion, 3. Gully erosion and 4. Riparian erosion.

Sheet Erosion: A thin covering of soil is removed uniformly from all over without leaving any mark behind. The evidence of such a type of erosion can be seen in the heavy quantity of silt that deposits elsewhere.

Rill erosion: The run off water moves rapidly over the soil surface cutting well-defined finger shaped groove-like structures, appearing as stream-like structure,

Gully erosion: Several rills (thin channels formed during rill erosion) coverage towards steep slope, which form together wider channels (grooves) of water known as gullies. These gullies may become still deeper and wider due to further rains.

Riparian erosion: The surface current of fast running rivers cuts the margins of the bank lethally. Once much of the soil from beneath is cut away the top soil of the river banks tumbles down into the river with big splash. Such riparian is more intense during floods.

Soil erosion due to wind is very common in dry arid-rigions where soil is chiefly sandy and the vegetation is very poor or even totally absent. In India erosion by wind affects approximately 50 million hecteres of land, most of which is in Rajastan. As in water erosion, wind erosion is also triggered by the destruction of plant cover of land by overfelling and overgrazing. Once the top soil is laid bare to the fury of strong gales, it gets blown off in the form of dust storm and sand storm. Landsliding or slip erosion sometimes results from heavy pressure caused by heavy rains on rocks at cliffs which come under the gravitational force and finally slip or fall off. Thus an entire field or hillock may slide down.

The principal methods of soil conservation include, 1. minimum tillage, 2. terracing. 3. contour ploughing. 4. strip cropping. 5. basin listing. 6. contruction of dams. 7. controlled gazing. and 8. afforestation.

Minimum tillage attempts to cultivate only to the degree necessary to ensure quick seed germination and satisfactory growth of row crops. The seed bed is only roughened, so water sinks easily into the soil. Thus, little water flows off to erode. Contour tillage is ploughing along the contours of the fields rather up and down the hills. Consequently water cannot run off easily. Strip cropping involves clear-cultivation of crops between strips of grassess or legumes on contoured slopes. This is effective in retarding the run-off and it helps hold the soil. Terracing is widely used on long slopes. The slope is converted into a series of broad, level areas called terraces. Water draining down the incline is diverted into the flat areas. It slows down and reduces its erosion ability. Furthermore, the water is retained for watering crops grown in the terraces.

30.4.1. Dams

Disastrous floods are annual featurs of many rivers. Floods bring havoc to the soil and vegetation of the plains. Soil erosion is severe and widespread. Big dams constructed in the head waters of such rivers control the floods effectively. Soil erosion is prevented and when better irrigational facilities are provided the waste land become fertile and productive. But before planning to construct such dams environmental impact assessment should be made to aviod any unforeseen ill effects.

30.4.2. Controlled Grazing

Excessive grazing makes a land completely bare and the soil particles compact. Rain water cannot percolate in such a soil and there is quick run off. The soil ultimately becomes dry. Such a dry and week top soil is easily eroded. A strong check on overgrazing can only prevent this type of soil erosion.

30.4.3. Afforestation

Probably one of the best ways of preventing erosion is by maintaining a vegetation cover on land. Of course, such a technique cannot be practical on land destined for farming. Yet most likely a great amount of unploughed farm land should or could be reforested or regrassed. It was found that on the banks of the riverse Ganga and Varuna at Varanasi that there is considerable erosion due to the sloping topography, and wherever the slope is under cover of plants the rate of erosion is minimal.

Trees planted in short blocks are called wind breaks and extensive plantation of trees are called shelter belts. Both the types of plantations are aimed at checking the spread of sand dunes or desert conditions or blowing away of the fertile top soil. The plantation is usually being done in two or three belts. Small sized plants are planted on the wind-ward side and tall trees on the leeward side. *Casuarina, Lawsonia, Agave, Thevetia, Calotropis, Acacia, Dalbergia, Zizyphus, Prosopis, Parkinsonia* serve as useful wind breaks and shelter belts.

In conclusion, it can be said that man's existence in the past and today is to a great extent a product of a life- sustaining relationship which he has maintained with the soil. To abuse the soil is to endanger that relationship and thus to seriously threaten man's very existence.

Check Your Progress - 2

Name the methods of soil conservation.

Note: (a) Write your answer in the space given below.

(b) Compare your answer with the one given at the end of this unit.

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30.5. WETLANDS

Wetlands are the areas of submerged or water satuarated lands. They may be natural or man made, fresh water or marine. They are of immense economic, scientific and aesthetik importance. They form excellent habitats for water fowl, and nursery grounds for prawn, fishes and crabs. Some of the important wetlands in the country are Chilka lake of 1,15,150 ha. when full; Kolleru lake (90,100 ha.) and Pulicat lake (35,000 ha.).These lakes are rich in fish, and the annual landings of fish respectively are 36,000; 7500; 1200 tonnes. There is a gradual decline in the fish yields in all the rivers and lakes, and the principal factors responsible for a decline include over- fishing, and sharp degradation of the aqutic environment by industrial and domestic pollution and construction of river-vally projects. While over-fishing calls for effective conservation vis-a-vis judicious management, the coming up of river-valley projects causes obstruction to free movement of migratory fish (hilsa, musheers) rapid industrialisation and urbanisation are serious threats to the fish life of the waters.

It is now increasingly realised, that these wetlands should be properly managed, and exploited to the maximum without causing damage. The Department of Environment, Government of India through the National Committee on Environment Planning (NCEP) has considered the question of Wetland management and gave great importance to gathering the required information for their ecologically sound management.

30.6. GRASSLANDS

The grasssland ecosystems are those in which the vegetation is dominated by such herbaceous species as (i) grasses and grass- like plants (particularly sedges) referred collectively as 'graminoids' and (ii) forbs, which are non-grass-like herbs. Frequently, there are also dwarf shrubs that do not exceed the height of the grasses, while taller shrubs occur in some areas as isoloted individuals or in patches; scattered trees characterise the tropical subtropical savannas (Grassland Ecosystems of the world-Ed.R.T.Coupland, 1979).

Natural and artifical grasslands cover atleast one-third of the land surface of the globe, and produce much of the world's food. These areas provide forage for free-ranging native and domestic animals as well as producing possible sources of wood production, water and wild life. Thus the natural grassland areas of the world have been used primarily either as cropland or as rangeland, because of the increasing degree of intensity of management for crop and meat production, natural grasslands are being changed at an accelerating rate. It is therefore, urgent to obtain more information about grasslands in their present condition before they are further modified.

With just a fortieth of world's land, India supports over half of its buffaloes and over a seventh of its cattle and goats. The result is a fodder famine. Large areas of former grazing lands now come under agriculture, leading to an overstocking of the remaining areas. In Gujarat, permanent pastures and grazing lands have dwindled from 1.04 million hectares in 1960-61 to 8,52,000 hectares in 1976-77 while the bovine population has increased slightly. Rajastan, which ranks third in India in animal wealth has experienced a similar shrinking of grazing lands between 1951-52 and 1977-78 leading to an increase in animal units per 100 hectares of common grazing land from 39 to 105. In Andhra Pradesh, the area of permanent pasture and other grazing lands in 1969-70 was 11,56,817 hectares, and in 1980-81 it was 9,23,648 hectares, and the bovine population slightly increased. Similar decrease in grassland area and increase in livestock might have taken place in other states.

Range management seeks to maintain and improve the range so as to produce highest possible forage yield for the animal feeding on them. This is normally brought about by applying ecological principles. Indian grassland and Fodder Research Institute at Jhansi works to evolve the ecological principles and management practices. Overgrazing has caused depletion of the grass cover in many areas, and is a haphazard to the continued productivity of rangeland. It has caused some grassland landscapes to become desertic in character.

It also leads to the disapperance of palatable species. Trampling for larger periods by grazing animals prevents the growth of young plants. The soil becomes compact, and the seeds cannot sprout. Soil erosion is accelerated.

Many small animals such as rabbits, rodents, and even insects forage on grasses and other range plants. It is, therefore, necessary that people who manage range lands, must know the principles of food chain.

The major management practice in use is control over the numbers of livestock and their distribution on range. in rotational grazing, livestock is moved from one pasture to another according to a schedule, and this reduce grazing intensity and allows time for vegetation to grow. Sometimes, a pasture is prevented from grazing until all seed and grass maturity is assured; this is deferred rotation.

The use of fire forms an important tool, especially in tropical regions. In maintaining herbaceous cover by interference with invasion by unwanted species such as trees or shrubs. However, this tool must be used with some caution. The IBP studies showed that fire seems to be beneficial in some subhumid temperate situations in releasing nutrients from accumulated litter; while in drier areas vigour of the herbaceous vegetation is noticeably reduced for several years after burning. The undesirable plants in the pastures can be removed by use of herbicides or by mechanical means.

Fertilisation is a standard means of increasing productivity in intensively used arable pastures, and in certain semi-natural pastures and meadows. Under extensive rangeland conditions, insufficient moisture and economic feasibility have interfered with widespread use of fertilisers. Periodic seeding of suitable species is an additional practice to increase the grass production.

30.7. FORESTS

Forests provide both goods and services to mankind. The goods include wood for homes, furniture, fuel, and paper; nuts, fruits, honey for food; medicinal plants for health and fodder for livestock.

The services rendered by forests include harbouring of wildlife, rebuilding of soils, protecting soil against erosion, reducing the danger of floods, protection of the catchments and regulating the flow of streams for irrigation and hydroelectric power, prevention of polution etc. Forests regulate local microclimate and provide beauty amenity and recreation. Such a natural resource with a great variety of functions and services, has been thoughtlessly destroyed in the last century. According to Forest Department Statistics, India lost between 1951 and 1972, 3.4 million hectares of forest to dams, new croplands, roads and industries, which means an annual deforestation of about 0.15 million hectares. Besides, recent report prepared by the International Union for the conservation of nature and Natural Resources (IUCN) says that India has lost nearly 2.5 million hectares of mangrove forests over the last 80 years.

The National Policy envisages that 33% of the land should be under Forest cover as against this, according to Forest Department statistics, approximately 23% of the country's total area is the forest land. A report of the National Committee and Environmental Planning clearly states that no more than 12% of the country's total land surface is under adequate tree cover.

The reasons identified for such degradation of forest land are unauthorised felling of trees for wood, over-grazing, shifting or podu or slash and burn cultivation and the expolitation of forests for forest based industries, and for urban markets. Whatever may be the reseason for the rapid denudation of forests, the consequences are increasing floods, soil erosion, heavy siltation of dams and changes in microclimate. Thus there is an urgent need to evolve workable principles of conservation, exploitation and management of such a valuable resource on sound ecological principles.

The National Commission on Agriculture estimated the total wood requirement for the year 1980 and 1990 to be 25.5 and 40.5 million m^3. This demand is likely to rise to 64 million m^3 by 2000 A.D. The estimated requirement of fuel-wood by 1980 and 1985, and 2000 A.D. will be about 184,202 and 225 million m^3 respectively. The production at present is hardly 10% of this estimated demand. This demand for wood by the 2000 A.D. can be met by man-made forestry programmes, concentrated natural regeneration programmes, working of an accessible forests by infrastructural development, timely thinning and final fellings of existing plantations, social forestry or farm forestry. So, during the past three decades steps have been taken to implement these programmes. In fact, social or farm forestry holds the key to ease the scarcity of industrial wood and fuel wood and can only provide the cushion to protect the reserved forests.

Silviculture is the term for forest management which deals through ecological principles with the establishment, growth and reproduction of timber trees.

Many barren areas and lands that are unsuitable for crop cultivation have been and are increasingly brought under the cover of forest trees. The less productivity forests and waste lands can be turned into forests short term tree plantations to meet the demand for firewood by raising high yielding/quick growing plants like *Casuarina, Acacia auriculiformis, Cassia siamea, Caesalpinia coriacea, Pongamia,* hybrid Eucalyptus like *Eucalyptus citrodora,* and *Leucaena leucocephala* (koo-babul or see-babul). Many artificial forests of single dominant species are also maintained eg. teak and sal forests. No doubt such forests are of great commercial importance, but pure stands or monocultures are susceptable to damage by insect attack and disease epidemic. In order to reduce the expenses over plantation of trees a system of forest culture plus agriculture has been evolved. This is called the Tungya-system in India. In this system the landless poor are allotted cleared forest plots. Crops are grown along with the seedlings of the forest trees for 2-3 years and after the seedling grow deep down due to competition with crop plants, the labourers are moved onto a cleared plot. The labourers enjoy the returns from crops.

For successful forest management, a knowledge of the ecology of the habitat and of succession is a must, otherwise the programmes may end in failure. Tree improvement practices such as genetic hybridization, and tisssee culture are to be combined with management practices to reduce the length of growth cycle. To meet the increasing demand for wood by the ever increasing population, undoubtedly the management practices will become more intensive and costs will accelerate accordingly.

30.8. WILDLIFE

The greatest nature lover and architect of modern India, our first Prime Minister Jawaharlal Nehru, referred to wildlife as: "Wildlife" - that is how we refer to the magnificient animals of our jungle and to the beautiful birds that brighten our lives. Inspite of our culture and civilization in many ways man continues to be not only wild but more dangerous than any of the so called animals. Life would be very dull and colourless if we do not have these magnificient animals and birds to look at and to play with. We should therefore, encourage as many sanctuaries as possible for preservtion of what yet remains of our wildlife".

The expression 'wildlife' is extended to include both animals and plants. The former includes not only the bigger and showy animals like the Tiger, the Lion, the Elephant etc. but also the smaller and bright creatures such as snakes, lizards, bats, insects etc. The Indian sub-continent is one of the most fascinating ecological, geographic regions, in the world and sustains a tremendous amount of diverse life forms, that includes about 15,000 species of plants (out of a known world total of 2,50,000) and 75,000 animal species (out of a world total of 1.5 million). Several of these as Sloth Bear, Blackbuck, fourhorned Antelope are peculiarly indigenous.

There is a general lack of awareness of economic value of wildlife. Wildlife can be treated as a national resource that provide many benefits to mankind. Rajesh Gopol (Wildlife Newsletter, July 1979) classifies the uses of wildlife as consumptive and non-consumptive, the former includes sport, hunting, food, hides, horns, bones, musk, ivory, feathers etc., and the later comprise benefits of scientific, cultural, recreational, aesthetic, educational and ecological nature.

It is often realised that wild animals yield more food material than we get from domestic animal husbandry. The diversified natural animal communities acting as a whole can maintain a far greater production of animal flesh than the goat, sheep, cattle and buffaloes which mostly constitute the livestock in India. We have a number of species such as wild pig, chital, sambar, nilgai and blackbuck, which can be exploited by way of wildlife ranching.

Today a significant portion of our wildlife is threatened by the pressure of human activity on land, forests in other words due to habitat destruction. The first comprehensive listing of endangered wildlife species was compiled in wildlife (protection) Act 1972 Schedule 1 of the act identifies 70 mammals, 22 reptiles and amphibians and 41 birds as rare and highly endangered species which are totally protected throughout the land. Such threatened animals include, Lionatailed Macaque, the wolf, the Jackal, the Indian fox and the wild Dog, the Tiger, the Lion, the Leopard etc. The Cheetah, the pink-headed Duck and the Jerdon's Courser have already disappeared from India. The Indian Lion is now confined to a small patch of dry scrub jungles of Gir forests in Gujarat, the Indian Ass to Rann of Cutch and the Indian Rhinoceros to the marshy patches of Assam, West Bengal and Tarai area of Himalayas. The Tiger is confined mostly to the Tiger reserves created under world wildlife Fund Project Tiger in 1973.

Compared to endangered animal life, plant life has not received much attention. An estimate of threatened Plant Committee of IUCN reveals that about 10% of the world's flowering plants are reported to be dangeriously rare. The Botanical Survey of India, in a recently published "State of the Art" report lists 134 most threatened plant species of which 99 are in the Himalayas and north-east, 28 species of orchids are listed as endanagered. The seriousness of the situation is amplified from the observation of Peter H. Raven, Director of Mussouri Botanical Gardens (USA) that "the disappearing plant can take with it 10 to 30 depending species such as insects, higher animals and few other plants".

As rightly remarked by our late Prime Minister Smt.Indira Gandhi, "the environment in which animals and plants become extinct is not safe for the human beings either. Our country has a rich heritage of wildlife and at the same time has long history and tradition of its conservation". Speaking at a function of launching the World Conservation Strategy on March, 1980 at Delhi, Smt.Gandhi reterred to this heritage as: "The interest in conservation is not a sentimental one but the rediscovery of a truth well known to our ancient sages. Indian tradition teaches us that all forms of life: human, animal and plant, are so closely interlinked that disturbance in one gives rise to imbalance in the rest".

Broadly, there are two ways of conservation of wildlife. One to conserve in the wild or on site conservation and the other in captivity. The on site conservation involves the identification and declaration of Biosphere-reserves, National Parks, Sancturies etc. The Biosphere-reserve Programme was launched on a world wide scale by UNESCO in 1973 as the Man and Biosphere (MAB) Programme. It has led to the creation of 200 Biosphere-reserves in about 40 countrieis so far. In India seven areas are being considered for setting up such reserves. They include Nilgiri at the tri-junction of Karnataka, Kerala, and Tamil Nadu. Tura Citrus Gene Sanctuary in Meghalaya, Namdapha in Arunachal Pradesh, Valley of flowers Tunganath-Rudranath in U.P., Nandadevi in U.P., some islands in Andaman-Nicobar group and the Gulf of Mannar on the Tamil Nadu coast. One of the objectives of the international Biosphere reserves is to conserve for present and future use, the diversity and integrity of plant and animal communities within natural ecosystem, and to safeguard the genetic diversity of the species on which their continuing evolution depends. However, the most significant aspect of Indians Biosphere-reserve Programme is the concept of ecodevelopment. It is an attempt to integrate the restoration of degraded habitats, with efforts for water and soil conservation.

30.9. SUMMARY

Natural resources included land, minerals, forests, wildlife, lakes, rivers, oceans and undergrownd water. Several valuable natural resources, which were abundant, are diminishing day by day. Much attention is paid to find out the ways and means for proper utilization and judicious and wise management of nutural resources on sound ecological principles.

Conservation is the management of human use of the biosphere for the continued benefit of the present as well as future generations. Developing countries like India face the duel problem of population growth and poverty. Developmental activities aimed at reducing the poverty and improving the quality of life of people must be in harmony with the environment. Soil is an important life-support system and the future of any country depends upon the health of its soil. Soil abuse, seriously threatens the very existence of man. The principal methods of soil conservation are 1) minimum tillage 2) terracing 3) contour ploughing 4) strip cropping 5) construction of dams 6) controlled grazing and 7) afforestation. Forests are a valuable resource which provide various goods eg: Wood, Food, Fodder, Medicinal plants, and render various services like rebuilding of soils, harbouring of wildlife, reducing the danger of flood, protecting the soil against erosion, prevention of pollution etc. Proper steps are urgently needed to conserve, exploit and manage the forests on sound ecological principles. India is facing fodder famine due to excessive cattle growth and disappearance of permanent pastures and grazing lands. The general rangeland managereal practices include (i) controlling overgrazing (ii) fertilisation and (iii) use of fire. Wetlands are the submerged or water-saturated land; they may be natural or man-made, freshwater or marine. Wetlands should be properly managed and exploited to the maximum extent. Wildlife as a resource, provides many positive values to mankind. India has a rich heritage of wildlife and at the same time has a long history and tradition of its conservation. About 10% of the world's flowering plants are dangerously rare. The major cause identified for the vanishing wildlife has been the habitats destruction. There are two categories of conservation of wildlife: to conserve in the wild and in captivity. The wild of onsite conservation can be achieved by identifying and declaring Biosphere-reserves, National Parks, Sanctuaries etc. The Zoological and Botanical gardens serve to conserve the wildlife in captivity.

30.10. CHECK YOUR PROGRESS: MODEL ANSWERS

1. Anything that satisfies the needs or wants of human society is called a resource. Natural resources are of two types renewable and non-renewable. Forests, grassland, crops and Animals are examples for the former type and minerals, fossils and fuel for the latter.

2. The principal methods of soil conservation include 1. Minimum tillage; 2. Terracing, 3. Contour ploughing, 4. Strip cropping, 5. Basin listing, 6. Construction of dams, 7. Controlled grazing and 8. Afforestation.

30.11. MODEL EXAMINATION QUESTIONS

I. Answer the following questions in about 30 lines each.

1. Write briefly about soil erosion and methods to be taken for soil conservations.
2. Write briefly about wild life conservation.
3. Write briefly about the conservation and management of grasslands and forests.

2. Answer the following questions in 10 lines each.

1. What is soil erosion? Explain briefly about different types of soil erosion.
2. Write briefly about natural resources.
3. "Water and wind are the principal causes of soil erosin" - Explain.
4. What are the natural resources of wet lands? How do you conserve them?

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BOTANY
COURSE - III:PLANT PHYSIOLOGY, GENETICS & ECOLOGY
B.Sc., III YEAR

ASSIGNMENT - 1

Time: 2 hours

Note:

1. Do not copy the answer directly from any of the books.
2. As far as possible try to answer the questions independently in your own words.
3. If it is necessary to quote from any source, give the current reference
4. Use your own foolscap pages for writing the assignment.
5. Leave sufficient margin for the comments of the evaluators.
6. Completion of this assignment normally should not take more than two hours time.

I. Answer the following questions in about 30 lines each.

1. Explain the fluid mosaic model of Plasma membrane structure with a neat diagram.

2. Ennumerate micro-nutrient elements in Plant nutrition. Describe how they are utilised by plants.

3. Describe the structure of enzymes and explain their mode of action.

II. Answer the following questions in about 10 lines each.

1. Describe the structure and function of Golgi body.

2. Explain the Phenomenon of mass flow.

3. What are the main differences in carbon fixation mechanisms between C_3 and C_4 Plants?

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ASSIGNMENT - 2

Time: 2 hours

Note:

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I. Answer the following questions in about 30 lines each.

1. Describe Krebs Cycle and the reactions in order.
2. Give a brief account on nitrogen metabolism in higher plants.
3. What is meant by Photoperiodism? Explain it with reference to short-day plants.

II. Answer the following questions in about 10 lines each.

1. Explain the role of auxins in geotropism.
2. What is fermentation? How is alcohol produced in alcohol fermentation?
3. Write briefly about the role of hormones in flower initiation.

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ASSIGNMENT - 3

Time: 2 hours

Note:

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4. Use your own foolscap pages for writing the assignment.
5. Leave sufficient margin for the comments of the evaluators.
6. Completion of this assignment normally should not take more than two hours time.

I. Answer the following questions in about 30 lines each.

1. Write an essay on gene mutations giving suitable examples.
2. Write briefly about the Mendelian laws of inheritance.
3. Explain ecological pyramid and discuss its different types.

II. Answer the following questions in about 10 lines each.

1. Write briefly about modofying genes or modifiers.
2. Write briefly about crossing over and its significance.
3. Explain briefly about the biodegradabie and non-degradable pollutants.

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B.Sc., III YEAR

SYLLABUS

BLOCK-I	:	**PLANT PHYSIOLOGY**
Unit-1	:	Plant Cell-Structure and Functions
Unit-2	:	Transpiration and Stomatal Movements
Unit-3	:	Macro- and Micro- nutrients
Unit-4	:	Ion Uptake
Unit-5	:	Translocation of Solutes
Unit-6	:	Structure, Nomenclature and Classification of Enzymes
Unit-7	:	Photosynthetic Pigments and Photochemical Reactions
Unit-8	:	C_3, C_4 and CAM Pathways and Factors influencing Photosynthesis
Unit-9	:	Glycolysis and Fermentation
Unit-10	:	Krebs Cycle and Electron Transport System
Unit-11	:	HMP Pathway and Factors Affecting Respiration
Unit-12	:	Photorespiration
Unit-13	:	Nitrogen Fixation
Unit-14	:	Nitrogen Metabolism
Unit-15	:	Plant Growth and Growth Regulators
Unit-16	:	Role of Growth Regulators in Dormancy and Movements
Unit-17	:	Physiology of Flowering
BLOCK-II	:	**GENETICS**
Unit-18	:	Mendelism
Unit-19	:	Modified Mendelian ratios and Polygenes
Unit-20	:	Linkage and Crossing Over
Unit-21	:	Cytoplasmic Inheritance
Unit-22	:	Gene Structure and Function
Unit-23	:	Genetic Code and Protein Synthesis
Unit-24	:	Mutations
Unit-25	:	An Account of Genetic Engineering
BLOCK-III	:	**ECOLOGY**
Unit-26	:	Ecosystem Concept and Plant Adaptation
Unit-27	:	Ecological Factors
Unit-28	:	Energy and Trophic Levels
Unit-29	:	Environmental Pollution
Unit-30	:	Conservation and Management of Natural Resources

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FACULTY OF SCIENCE

B.Sc.III Year (3 Year Degree Courses) Examination, July 1991

BOTANY

COURSE-I Plant Physiology, Genetics and Ecology

MODEL EXAMINATION PAPER

Time 3 Hours] [Max.Marks 75

Min.Marks 27

Section A (Marks 3 X 15 = 45)

Answer any THREE questions.
Each question carries **15** marks.
Answer the following in about **30** lines each.

1. Describe any model explaining the ultra-structure of plasma membrane
2. Explain the differences between active and passive uptake of solutes
3. Explain Mendel's law of inheritance.
4. Describe HMP pathway. What is its importance?
5. Explain how C_4 pathway of carbon fixation is more efficient than C_3 pathway. Name two C_4 crop plants.
6. Describe the ecological adaptations in hydrophytes with examples.

Section B (Marks 5 X 6 = 30)

Answer any FIVE questions.
Each question carries **6** marks.
Answer the following in about **10** lines each.

7. Describe the ultra-structure of mitochondrion and its main functions.
8. Explain Statolith theory of geotropism.
9. What is genetic code? Describe it with examples.
10. Discuss the importance of environmental conservation.
11. Explain Hill reaction.
12. Explain electro-osmosis.
13. What is parthenocarpy? How is it induced? Describe its importance.
14. Describe the role of iron and phosphorus in plants.
15. Explain the pyramid of energy.
16. Discuss the significance of crossing over.