Text Book for

INTERMEDIATE

Second Year

Botany

Permission and Support by

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Telugu and Sanskrit Akademi, Andhra Pradesh

Intermediate

Second Year Text Book

Botany

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Y.S. JAGAN MOHAN REDDY

CHIEF MINISTER
ANDHRA PRADESH

AMARAVATI

MESSAGE

I congratulate Akademi for starting its activities with printing of textbooks from the academic year 2021 – 22.

Education is a real asset which cannot be stolen by anyone and it is the foundation on which children build their future. As the world has become a global village, children will have to compete with the world as they grow up. For this there is every need for good books and good education.

Our government has brought in many changes in the education system and more are to come. The government has been taking care to provide education to the poor and needy through various measures, like developing infrastructure, upgrading the skills of teachers, providing incentives to the children and parents to pursue education. Nutritious mid-day meal and converting Anganwadis into pre-primary schools with English as medium of instruction are the steps taken to initiate children into education from a young age. Besides introducing CBSE syllabus and Telugu as a compulsory subject, the government has taken up numerous innovative programmes.

The revival of the Akademi also took place during the tenure of our government as it was neglected after the State was bifurcated. The Akademi, which was started on August 6, 1968 in the undivided state of Andhra Pradesh, was printing text books, works of popular writers and books for competitive exams and personality development.

Our government has decided to make available all kinds of books required for students and employees through Akademi, with headquarters at Tirupati.

I extend my best wishes to the Akademi and hope it will regain its past glory.

Y.S. Jagan Mohan Reddy

Dr. Nandamuri Lakshmiparvathi
M.A., M.Phil., Ph.D.
Chairperson, (Cabinet Minister Rank)
Telugu and Sanskrit Akademi, A.P.

Message of Chairperson, Telugu and Sanskrit Akademi, A.P.

In accordance with the syllabus developed by the Board of Intermediate, State Council for Higher Education, SCERT etc., we design high quality Text books by recruiting efficient Professors, department heads and faculty members from various Universities and Colleges as writers and editors. We are taking steps to print the required number of these books in a timely manner and distribute through the Akademi's Regional Centers present across the Andhra Pradesh.

In addition to text books, we strive to keep monographs, dictionaries, dialect texts, question banks, contact texts, popular texts, essays, linguistics texts, school level dictionaries, glossaries, etc., updated and printed and made available to students from time to time.

For competitive examinations conducted by the Andhra Pradesh Public Service Commission and for Entrance examinations conducted by various Universities, the contents of the Akademi publications are taken as standard. So, I want all the students and Employees to make use of Akademi books of high standards for their golden future.

Congratulations and best wishes to all of you.

Nandamuri Lakshmiparvathi
Chairperson
Telugu and Sanskrit Academi, A.P

J. SYAMALA RAO, I.A.S.,
Principal Secretary to Government

Higher Education Department
Government of Andhra Pradesh

MESSAGE

I Congratulate Telugu and Sanskrit Akademi for taking up the initiative of printing and distributing textbooks in both Telugu and English media within a short span of establishing Telugu and Sanskrit Akademi.

Number of students of Andhra Pradesh are competing of National Level for admissions into Medicine and Engineering courses. In order to help these students Telugu and Sanskrit Akademi consultation with NCERT redesigned their Textbooks to suit the requirement of National Level Examinations in a lucid language.

As the content in Telugu and Sanskrit Akademi books is highly informative and authentic, printed in multi-color on high quality paper and will be made available to the students in a time bound manner. I hope all the students in Andhra Pradesh will utilize the Akademi textbooks for better understanding of the subjects to compete of state and national levels.

(J. SYAMALA RAO)

THE CONSTITUTION OF INDIA

PREAMBLE

WE, THE PEOPLE OF INDIA, having solemnly resolved to constitute India into a [SOVEREIGN SOCIALIST SECULAR DEMOCRATIC REPUBLIC] and to secure to all its citizens:

JUSTICE, social, economic and political;

LIBERTY of thought, expression, belief, faith and worship;

EQUALITY of status and of opportunity; and to promote among them all

FRATERNITY assuring the dignity of the individual and the [unity and integrity of the Nation];

IN OUR CONSTITUENT ASSEMBLY this twenty-sixth day of November, 1949 do HEREBY ADOPT, ENACT AND GIVE TO OURSELVES THIS CONSTITUTION.

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Foreword

The Government of India vowed to remove the educational disparities and adopt a common core curriculum across the country especially at the Intermediate level. Ever since the Government of Andhra Pradesh and the Board of Intermediate Education (BIE) swung into action with the task of evolving a revised syllabus in all the Science subjects on par with that of COBSE, approved by NCERT, its chief intention being enabling the students from Andhra Pradesh to prepare for the National Level Common Entrance tests like NEET, ISEET etc for admission into Institutions of professional courses in our Country.

For the first time BIE AP has decided to prepare the Science textbooks. Accordingly an Academic Review Committee was constituted with the Commissioner of Intermediate Education, AP as Chairman and the Secretary, BIE AP; the Director SCERT and the Director Telugu Akademi as members. The National and State Level Educational luminaries were involved in the textbook preparation, who did it with meticulous care. The textbooks are printed on the lines of NCERT maintaining National Level Standards.

The Education Department of Government of Andhra Pradesh has taken a decision to publish and to supply all the text books with free of cost for the students of all Government and Aided Junior Colleges of newly formed state of Andhra Pradesh.

We express our sincere gratitude to the Director, NCERT for according permission to adopt its syllabi and curriculum of Science textbooks. We have been permitted to make use of their textbooks which will be of great advantage to our student community. I also express my gratitude to the Chairman, BIE and the honorable Minister for HRD and Vice Chairman, BIE and Secretary (SE) for their dedicated sincere guidance and help.

I sincerely hope that the assorted methods of innovation that are adopted in the preparation of these textbooks will be of great help and guidance to the students.

I wholeheartedly appreciate the sincere endeavors of the Textbook Development Committee which has accomplished this noble task.

Constructive suggestions are solicited for the improvement of this textbook from the students, teachers and general public in the subjects concerned so that next edition will be revised duly incorporating these suggestions.

It is very much commendable that Intermediate text books are being printed for the first time by the Akademi from the 2021-22 academic year.

Sri. V. Ramakrishna I.R.S.
Director
Telugu and Sanskrit Akademi,
Andhra Pradesh

Preface

By virtue of having studied the diversity in plant life and its organization in the first volume of the Textbook of Botany (First Year of Intermediate course), one cannot remain without wondering about the functional aspects of these various structures of plants. An equally intriguing question that arises is about the significance of all this knowledge in the context of its practical utility in human life. These two are the aspects that are essentially addressed in this volume of the Botany Text Book (for the Second year of Intermediate course) that has been prepared as per the prescribed syllabus by adopting NCERT Biology Text Books of Class XI and XII and after effecting necessary changes in the contents as per local requirements.

The contents of this Text Books are organized into six Units. The first Unit contains six chapters which deal with the basic physiological processes essential to plant life. The mechanism of absorption of water and mineral elements and their transportation within the plant body are discussed in Chapter 1. The role of the essential elements in plant life forms the contents of Chapter 2. Information on biological catalysts, the enzymes, is dealt with in Chapter 3. This serves as a prelude to understand better the various catabolic and anabolic reactions described in the next two chapters. Chapter 4 deals with the process of autotrophic nutrition, a characteristic feature of the plant kingdom. Cellular respiration, common to most biological entities, has been discussed in Chapter 5. The first Unit ends with Chapter 6 that addresses the growth, differentiation and development in Plants.

Unit II provides an introductory account of microbiology with emphasis on bacteria (Chapter 7) and viruses (Chapter 8). This is required to understand the use of microbes described in the later chapters. The next two units, Unit III and Unit IV, contain one chapter each. Unit III (Chapter 9) explains the fundamental concepts underlying the mechanism of Inheritance, a feature common to all living organisms but first worked out in pea plants. The Molecular Basis of Inheritance has been dealt with in Chapter 10 of Unit IV.

A branch of biology that has evolved in the last three decades, Biotechnology, has been dealt with in Unit V. While Chapter 11 of this Unit describes the principles, tools and mechanisms related to Biotechnology, Chapter 12 mentions its

applications. Finally Unit VI focuses on the importance of botanical studies in human life. This Unit comprises two chapters, Chapter 13 deals with the application of plant science in crop improvement and food production. The last chapter (Chapter 14) consists of information on the use of microbes for human welfare.

Each unit begins with an overview of the contents dealt within its constituent chapters. Interdependency of chapters has been indicated at appropriate places to maintain continuity in the learning process. The text is adequately illustrated with figures to make the material easily comprehensible and to create an interest in the subject. Each chapter is provided with a summary of its content, followed by a glossary of critical / new terms used to facilitate a quick review and clear understanding of the topic. Revision questions have been given at the end of each chapter to serve as models for self assessment by students and to test their learning ability. We hope that the scientific scope and style of preparation of this text book will stimulate in the students a spirit of inquiry, power of critical observation and ability to understand and perceive board concepts.

One important word of advice to students is that they should read this book thoroughly and critically. Straight forward/ ready answers to some of the model questions and exercises may not be available in the material of the book. Answers / solutions to such questions can be obtained only by a thorough reading of the matter together with analytical thinking and with the help of the teacher. Such model questions and exercises are included in this book with the intention of inculcating the habit of analytical thinking and practical application of knowledge

It has been a pleasure working with the members of the Text Book Development Committee and the authors who are not only experts in their fields but also dedicated and cooperative. We thank all of them for their diligence and valuable contributions in preparing this Text Book.

On behalf of the Text Book Development Committee, I would like to place on record our deep appreciation and gratitude to late Dr. G. Rajendrudu, Professor in Botany (Retired), Sri Venkateswara University, Tirupati who was actively involved in the preparation and editing of this book. Unfortunately he expired in December 2012 and could not see the fruits of his labour. His death is an irreparable loss to the Academic fraternity.

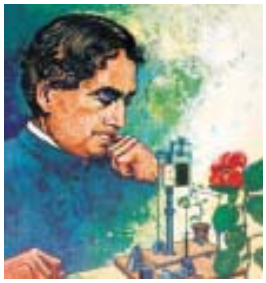
Dr. M.V. Subba Rao

Contents

UNIT I

PLANT PHYSIOLOGY

The description of structure and variation of living organisms over a period of time, ended up as two, apparently irreconcilable perspectives on biology. The two perspectives essentially rested on two levels of organisation of life forms and the related phenomena. One described at organismic and above level of organisation while the second described at cellular and molecular level of organization. The first resulted in ecology and related disciplines. The second resulted in physiology and biochemistry. Description of physiological processes in flowering plants as an example, is what given in the chapters in this unit. The processes of mineral nutrition of plants, photosynthesis, transport, respiration and ultimately plant growth and development are described in molecular terms but in the context of cellular activities and even at organism level. Wherever appropriate, the relation of the physiological processes to the environment is also discussed.



Sir J.C. Bose
1858-1937

In the 19th century when people considered plants as non-living 'things', it was because of **Sir Jagadish Chandra Bose** that the scientific world came to know that plants too have life and feelings. Sir J. C. Bose, born on November 30, 1858 in traditional Bengali family was the first Indian to become a **Fellow of Royal Society**. Intrestingly he began his career as a demonstrator in Physics and contributed in generating electromagnetic waves of minute wavelength and designed an instrument called *'Coherer'* for detecting radio waves. His proficiency in Physics won him the first US patent by any Indian. However, his passion for Nature and plant life made him apply his proficiency in Physics to the understanding of plant life. Through his ***Pulsating Theory***, he explained the various bio-electrical responses shown by plants. Sir J.C. Bose designed a very sophisticated instrument called 'Crescograph' which was so sensitive that it could record even a minute growth of a plant upto a millionth part of a millimeter. Apart from establishing the *Bose Research Institute* at Kolkata, he also authored several books like *Researches on irritability of plants* and *Nervous Mechanism in Plants*.

Chapter 1

Transport in Plants

Have you ever wondered how water reaches the top of tall trees, or for that matter how and why substances move from one cell to the other, whether all substances move in a similar way, in the same direction and whether metabolic energy is required for moving substances? Plants need to move molecules over very long distances, much more than animals do. Besides, they also do not have a circulatory system in place. Water taken up by the roots has to reach all parts of the plant, up to the very tip of the growing stem. The photosynthates or food synthesised by the leaves have also to be moved to all parts including the root tips embedded deep inside the soil. Movement across short distances, say within the cell, across the membranes and from cell to cell within the tissue has also to take place. To understand some of the transport processes that take place in plants, one would have to recollect one's basic knowledge of plant cell structure and the anatomy of the plant body. We also need to revisit our understanding of diffusion, besides gaining some knowledge about chemical potential and ions.

When we talk of the movement of substances, we first need to define what kind of movement we are talking about, and also what substances we are looking at. In a flowering plant the substances that would need to be transported are water, mineral nutrients, organic nutrients and plant growth regulators.

Over small distances substances move by diffusion and by cytoplasmic streaming supplemented by active transport. Transport over longer distances proceeds through the vascular system (the xylem and the phloem) and is called **translocation**.

An important aspect that needs to be considered is the direction of transport. In rooted plants, transport in xylem (of water and minerals) is essentially unidirectional, from roots to the stems. Organic and mineral nutrients however, undergo multidirectional transport. Organic compounds synthesised in the photosynthetic leaves are exported to all other parts of the plant including storage organs. From the storage organs they are later re-exported. The mineral nutrients are taken up by the roots and transported upwards into the stem, leaves and the growing regions. When any plant part undergoes senescence, nutrients may be withdrawn from such regions and moved to the growing parts. Hormones or plant growth regulators and other chemical stimuli are also transported, though in very small amounts, sometimes in a strictly polarised or unidirectional manner from where they are synthesised to other parts. Hence, in a flowering plant there is a complex traffic of compounds (but probably very orderly) moving in different directions, each organ receiving some substances and giving out some others.

1.1 Means of Transport

1.1.1 Diffusion

Movement by **diffusion** is passive, and may be from one part of the cell to the other, or from cell to cell, or over short distances, say, from the inter-cellular spaces of the leaf to the outside. No energy expenditure takes place. In diffusion, molecules move in a random fashion, the net result being substances moving from regions of higher concentration to regions of lower concentration. Diffusion is a slow process and is not dependent on a 'living system'. Diffusion is obvious in gases and liquids, but diffusion in solids rather than of solids is more likely. Diffusion is very important to plants since it is the only means for gaseous movement within the plant body.

Diffusion rates are affected by the gradient of concentration, the permeability of the membrane separating them, temperature and pressure.

1.1.2 Facilitated Diffusion

As pointed out earlier, a gradient must already be present for diffusion to occur. The diffusion rate depends on the size of the substances; obviously smaller substances diffuse faster. The diffusion of any substance across a membrane also depends on its solubility in lipids, the major constituent of

the membrane. Substances soluble in lipids diffuse through the membrane faster. Substances that have a hydrophilic moiety find it difficult to pass through the membrane; their movement has to be facilitated. Membrane proteins provide sites at which such molecules cross the membrane. They do not set up a concentration gradient: a concentration gradient must already be present for molecules to diffuse even if facilitated by the proteins. This process is called **facilitated diffusion**.

In facilitated diffusion, special proteins help move substances across membranes without expenditure of ATP energy. Facilitated diffusion cannot cause net transport of molecules from a low to a high concentration – this would require input of energy. Transport rate reaches a maximum when all of the protein transporters are being used (saturation). Facilitated diffusion is very specific: it allows cell to select substances for uptake. It is sensitive to inhibitors which react with protein side chains.

The proteins form channels in the membrane for molecules to pass through. Some channels are always open; others can be controlled. Some are large, allowing a variety of molecules to cross. **Porins** are proteins that form huge pores in the outer membranes of the plastids, mitochondria and some bacteria, allowing molecules up to the size of small proteins to pass through.

Figure 1.1 shows an extracellular molecule bound to the transport protein; the transport protein then rotates and releases the molecule inside the cell, e.g., water channels – made up of eight different types of **aquaporins**.

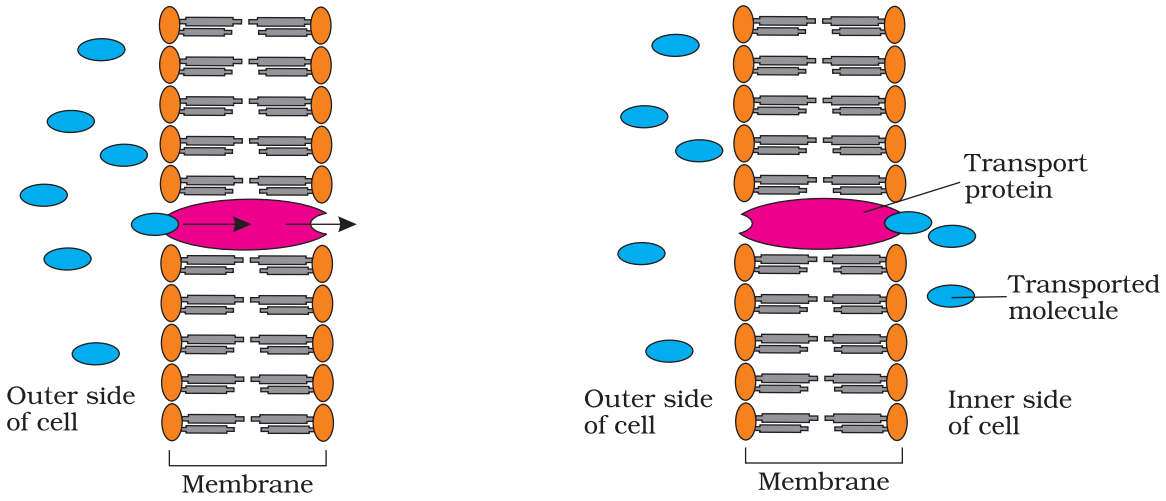


Figure 1.1 Facilitated diffusion

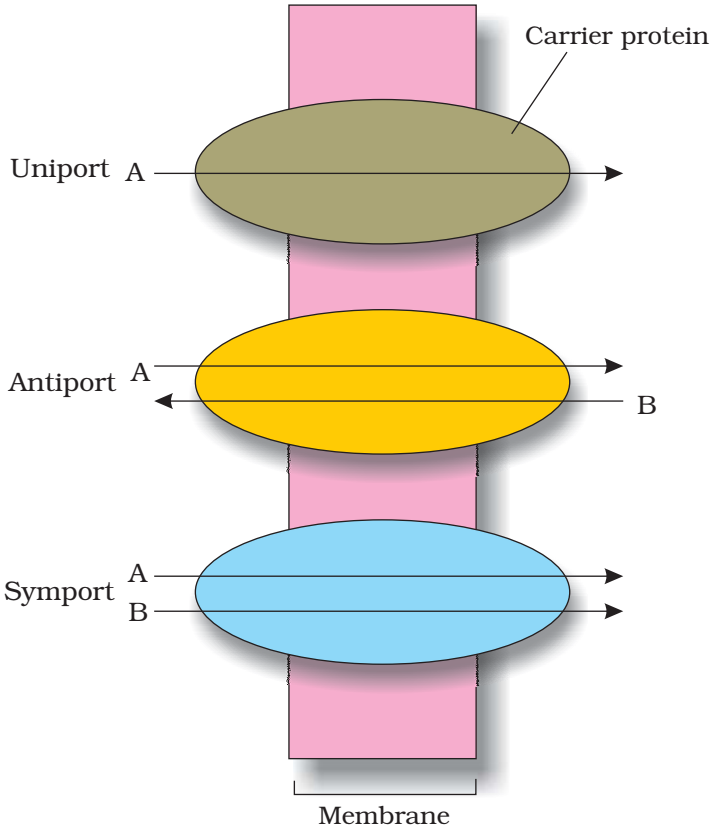


Figure 1.2 Facilitated diffusion

1.1.2.1 Passive symports and antiports

Some carrier or transport proteins allow diffusion only if two types of molecules move together. In a **symport**, both molecules cross the membrane in the same direction; in an **antiport**, they move in opposite directions (Figure 1.2). When a molecule moves across a membrane independent of other molecules, the process is called **uniport**.

1.1.3 Active Transport

Active transport uses energy to pump molecules against a concentration gradient. Active transport is carried out by membrane-proteins. Hence different proteins in the membrane play a major role in both active as well as passive transport. **Pumps** are proteins that use energy to carry substances across the cell membrane. These pumps can transport substances from a low concentration to a high concentration ('uphill' transport). Transport rate reaches a maximum when all the protein transporters are being used or are saturated. Like enzymes the carrier protein is very specific in what it carries across the membrane. These proteins are sensitive to inhibitors that react with protein side chains.

1.1.4 Comparison of Different Transport Processes

Table 1.1 gives a comparison of the different transport mechanisms. Proteins in the membrane are responsible for facilitated diffusion and active transport and hence show common characterstics of being highly selective; they are liable to saturate, respond to inhibitors and are under hormonal regulation. But diffusion whether facilitated or not, takes place only along a gradient and does not use energy.

TABLE 1.1 Comparison of Different Transport Mechanisms

Property	Simple Diffusion	Facilitated Transport	Active Transport
Requires special membrane proteins	No	Yes	Yes
Highly selective	No	Yes	Yes
Transport saturates	No	Yes	Yes
Uphill transport	No	No	Yes
Requires ATP energy	No	No	Yes

1.2 Plant-Water Relations

Water is essential for all physiological activities of the plant and plays a very important role in all living organisms. It provides the medium in which most substances are dissolved. The protoplasm of the cells is nothing but water in which different molecules are dissolved and several particles are suspended. A watermelon contains over 92 per cent water; most herbaceous plants have only about 10 to 15 per cent of their fresh weight as dry matter. Of course, distribution of water within a plant varies – woody parts have relatively very little water, while soft parts mostly contain water. A seed may appear dry but it still has water – otherwise it would not be alive and respiring!

Terrestrial plants take up huge amount of water daily but most of it is lost to the air through evaporation from the leaves, i.e., **transpiration**. A mature corn plant absorbs almost three litres of water in a day, while a mustard plant absorbs water equal to its own weight in about 5 hours. Because of this high demand for water, it is not surprising that water is often the limiting factor for plant growth and productivity in both agricultural and natural environments.

1.2.1 Water Potential

To comprehend plant-water relations, an understanding of certain standard terms is necessary. **Water potential** (ψ_w) is a concept fundamental to understanding water movement. **Solute potential** (ψ_s) and **pressure potential** (ψ_p) are the two main components that determine water potential.

Water molecules possess kinetic energy. In liquid and gaseous form they are in random motion that is both rapid and constant. The greater the concentration of water in a system, the greater is its kinetic energy or 'water potential'. Hence, it is obvious that pure water will have the greatest water potential. If two systems containing water are in contact, random movement of water molecules will result in net movement of water molecules from the system

with higher energy to the one with lower energy. Thus water will move from the system containing water at higher water potential to the one having low water potential. This process of movement of substances down a gradient of free energy is called diffusion. Water potential is denoted by the Greek symbol Psi or ψ and is expressed in pressure units such as pascals (Pa). By convention, the water potential of pure water at standard temperatures, which is not under any pressure, is taken to be zero.

If some solute is dissolved in pure water, the solution has fewer free water molecules and the concentration of water decreases, reducing its water potential. Hence, all solutions have a lower water potential than pure water; the magnitude of this lowering due to dissolution of a solute is called **solute potential** or ψ_s. ψ_s is always negative. The more the solute molecules, the lower (more negative) is ψ_s. For a solution at atmospheric pressure (water potential) ψ_w = (solute potential) ψ_s.

If a pressure greater than atmospheric pressure is applied to pure water or a solution, its water potential increases. It is equivalent to pumping water from one place to another. Can you think of any system in our body where pressure is built up? Pressure can build up in a plant system when water enters a plant cell due to diffusion, causing a pressure to build-up against the cell wall. This makes the cell **turgid** (see section 1.2.2). The magnitude of increment in water potential in such turgid cell is called Pressure potential. It is usually positive, though in plants negative potential or tension in the water column in the xylem plays a major role in water transport up a stem. Pressure potential is denoted as ψ_p.

Water potential of a cell is affected by both solute and pressure potential. The relationship between them is as follows:

$$\psi_w = \psi_s + \psi_p$$

1.2.2 Osmosis

The plant cell is surrounded by a cell membrane and a cell wall. The cell wall is freely permeable to water and substances in solution, hence is not a barrier to movement. In plants the cells usually contain a large central vacuole, whose contents, the vacuolar sap, contribute to the solute potential of the cell. In plant cells, the cell membrane and the membrane of the vacuole (the tonoplast) together are important determinants of movement of molecules in or out of the cell.

Osmosis is the term used to refer specifically to the diffusion of water across a differentially- or semi-permeable membrane. Osmosis occurs spontaneously in response to a driving force. The net direction and rate of osmosis depends on both the **pressure gradient** and **concentration gradient**. Most of the water is absorbed by plants through osmotic mode that occurs due to concentration gradient and hence does not require any metabolic energy (passive absorption).

Water moves from its region of higher chemical potential (or concentration) to its region of lower chemical potential until equilibrium is reached. At equilibrium the two chambers will have the same water potential.

You may have conducted the potato osmometer experiment while in school. If the tuber is placed in water, the cavity in the potato tuber containing a concentrated solution of sugar collects water due to osmosis.

Study Figure 1.3 in which the two chambers, A and B, containing solutions are separated by a semi-permeable membrane.

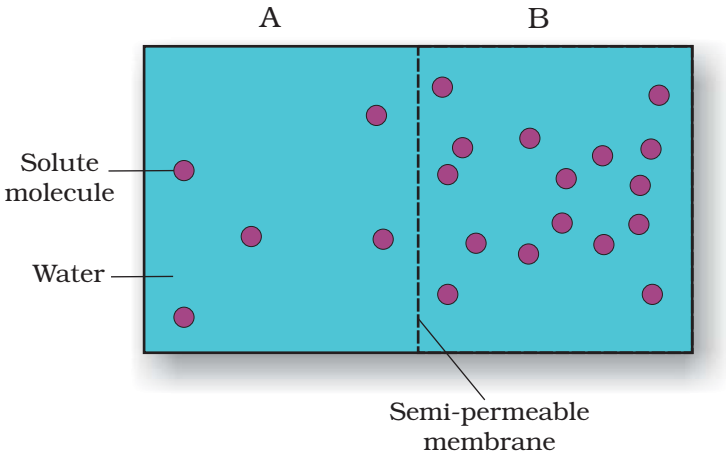


Figure 1.3

(a) The solution of which chamber has a lower water potential?

(b) The solution of which chamber has a lower solute potential?

(c) In which direction thus osmosis occur?

(d) Which solution has a higher solute potential?

(e) At equilibrium which chamber will have lower water potential?

(f) If one chamber has a ψ of – 2000 kPa and the other – 1000 kPa, which is the chamber that has the higher ψ?

Let us discuss another experiment where a solution of sucrose in water taken in a funnel is separated from pure water in a beaker through a semi-permeable membrane (Figure 1.4). You can get this kind of a membrane in an egg. Remove the yolk and albumin through a small hole at one end of the egg, and place the shell in a dilute solution of hydrochloric acid for a few hours. The egg shell dissolves, leaving the membrane intact. Water will move into the funnel, resulting in increasing the level of the solution in the funnel. This will continue till the equilibrium is reached. In case sucrose does diffuse out through the membrane, will this equilibrium be ever reached?

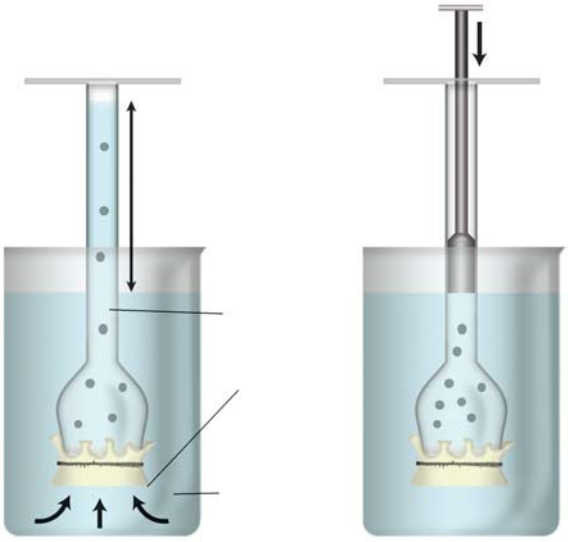


Figure 1.4 A demonstration of osmosis. A thistle funnel is filled with sucrose solution and kept inverted in a beaker containing water. (a) Water will diffuse across the membrane (as shown by arrows) to raise the level of the solution in the funnel (b) Pressure can be applied as shown to stop the water movement into the funnel

External pressure can be applied from the upper part of the funnel such that no water diffuses into the funnel through the membrane. This pressure required to prevent water from diffusing is, in fact, the osmotic pressure and this is the function of the solute concentration; the more solute concentration, the greater will be the pressure required to prevent water from diffusing in. Numerically osmotic pressure is equivalent to the osmotic potential, but the sign is opposite.Osmotic pressure is the positive pressure applied, while osmotic potential is negative.

1.2.3 Plasmolysis

The behaviour of the plant cells (or tissues) with regard to water movement depends on the surrounding solution. If the external solution balances the osmotic pressure of the cytoplasm, it is said to be **isotonic**. If the external solution is more dilute than the cytoplasm, it is **hypotonic** and if the external solution is more concentrated, it is **hypertonic**. Cells swell in hypotonic solutions and shrink in hypertonic ones.

Plasmolysis occurs when water moves out of the cell and the cell membrane of a plant cell shrinks away from its cell wall. This occurs when the cell (or tissue) is placed in a solution that is hypertonic (has more solutes) to the protoplasm. Water moves out; it is first lost from the cytoplasm and then from the vacuole. The water when drawn out of the cell through diffusion into the extracellular (outside cell) fluid causes the protoplast to shrink away from the walls. Initially, this phenomenon is indicated by shrinkage of protoplast, leading to the separation of plasma membrane from the cell wall in the corners. This stage is called **incipient plasmolysis**. When the cell is left in the hypertonic solution for more time, the protoplasm completely shrinks, wherein the cell is said to be *plasmolysed*. The movement of water occurs across the membrane, moving from an area of higher water potential (i.e., the cell) to an area of lower water potential (i.e.,outside the cell) (Figure 1.5). Thus normal living

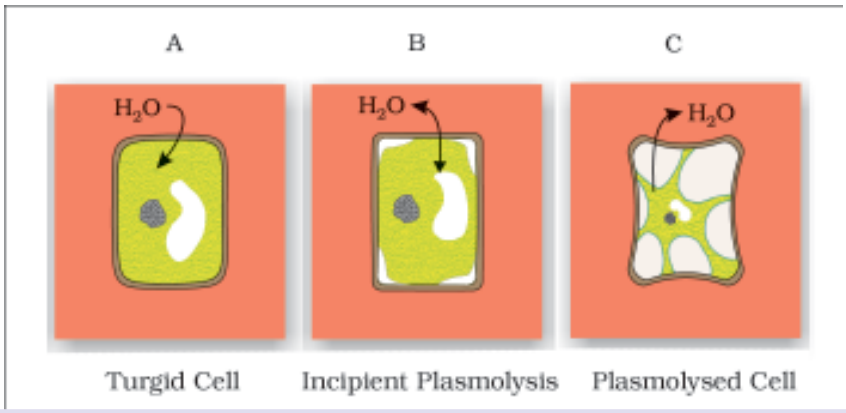


Figure 1.5 Plant cell undergoing plasmolysis

cells when kept in hypertonic solution become flaccid. In such cells the pressure potential becomes zero, hence the water potential becomes equal to the solute potential.

$$\psi_w = \psi_s$$

What occupies the space between the cell wall and the shrunken protoplast in the plasmolysed cell?

Though plasmolysis is an interesting phenomenon, it does not normally occur in nature - with the possible exception of extreme water stress or saline environments. The salting of pickles and preserving of fish and meat in salt are good examples of practical applications of this phenomenon.

When the cell (or tissue) is placed in an isotonic solution, there is no net flow of water towards the inside or outside. The external solution balances the osmotic pressure of the cytoplasm. Hence, it is said to be in isotonic condition and as both are in a state of equilibrium, the net movement of water is zero.

The process of plasmolysis is usually reversible. When the cells are placed in a hypotonic solution (higher water potential or dilute solution as compared to the cytoplasm), water diffuses into the cell causing the cytoplasm to build up pressure against the wall. This is called **turgor pressure**. The pressure exerted by the protoplasts due to entry of water against the rigid walls is called **pressure potential** ψ_p. Because of the rigidity of the cell wall, the cell does not rupture.

Turgor pressure is ultimately responsible for the enlargement and extension or growth of cells.

When animal cells are kept in a hypotonic solution what will happen? Do they burst and Why?

What would be the ψ_p of a flaccid cell? Which organisms other than plants, possess cell wall?

1.2.4 Imbibition

Imbibition is a special type of diffusion when water is absorbed by solids – colloids – causing them to enormously increase in volume. The classical examples of imbibition are absorption of water by seeds and dry wood. The pressure that is produced by the swelling of wood was used by prehistoric man to split rocks and boulders. If it were not for the pressure due to imbibition, seedlings would not be able to emerge out of the soil into the open; they probably would not have been able to establish!

Different types of organic substances have different imbibing capacities. Proteins have very high imbibing capacities compared to carbohydrates. That is why proteinaceous pea seeds swell more on imbibition than starchy wheat seeds.

Imbibition is also a type of diffusion since water movement is along a concentration gradient; the seeds and other such materials have almost no water, hence they absorb water easily. Water potential gradient between the adsorbent and the liquid imbibed is essential for imbibition. In addition, for any substance to imbibe any liquid, affinity between the adsorbent and the liquid is also a pre-requisite.

1.3 Long Distance Transport of Water

At an earlier stage you might have carried out an experiment in which you placed a twig bearing white flowers in coloured water and watched it turn colour. On examining the cut end of the twig after a few hours you had noted the region through which the coloured water moved. That experiment very easily demonstrates that the path of water movement is through the vascular bundles, more specifically, the xylem. Now we have to go further and try and understand the mechanism of movement of water and other substances up a plant.

Long distance transport of substances within a plant cannot take place by diffusion alone. Diffusion is a slow process. It can account for only short distance movement of molecules. For example, the movement of a molecule across a typical plant cell (about 50 µm) takes approximately 2.5s. At this rate, can you calculate how many years it would take for the movement of molecules over a distance of 1m within a plant by diffusion alone?

In large and complex organisms, substances often have to be moved across very large distances. Sometimes the sites of production or absorption and sites of storage are too far from each other; diffusion or active transport would not suffice. Special long distance transport systems become necessary so as to move substances across long distances and at a much faster rate. Water and minerals, and food are generally moved by a **mass** or **bulk** flow system. Mass flow is the movement of substances in bulk or *en masse* from one point to another as a result of pressure differences between the two points. It is a characteristic of mass flow that substances, whether in solution or in suspension, are swept along at the same pace, as in a flowing river. This is unlike diffusion where different substances move independently depending on their concentration gradients. Bulk flow can be achieved either through a positive hydrostatic pressure gradient (e.g., a garden hose) or a negative hydrostatic pressure gradient (e.g., suction through a straw).

The bulk movement of substances through the conducting or vascular tissues of plants is called **translocation**.

Do you remember studying cross sections of roots, stems and leaves of higher plants and studying the vascular system? The higher plants have highly specialised vascular tissues–xylem and phloem. Xylem is associated with the translocation of mainly water, mineral salts, some organic nitrogen and hormones from the roots to the aerial parts of the plants. Phloem translocates a variety of organic and inorganic solutes, mainly from the leaves to other parts of the plants.

1.3.1 How do Plants Absorb Water?

We know that the roots absorb most of the water that goes into plants; obviously that is why we pour water on the soil and not on the leaves. The responsibility of absorption of water and minerals is more specifically the function of the root hairs that are present in millions at the tips of the roots. Root hairs are thin-walled slender extensions of root epidermal cells that greatly increase the surface area for absorption. Water is absorbed along with mineral solutes, by the root hairs, purely by diffusion. Once water is absorbed by the root hairs, it can move deeper into root layers by two distinct pathways:

- apoplast pathway
- symplast pathway

The **apoplast** is the system of adjacent cell walls that is continuous throughout the plant, except at the **casparian** strips of the endodermis in the roots (Figure 1.6). The apoplastic movement of water occurs exclusively through the

intercellular spaces and the walls of the cells. Movement through the apoplast does not involve crossing the cell membrane. This movement is dependent on the gradient. The apoplast does not provide any barrier to the water movement and water movement is through mass flow. As water evaporates into the intercellular spaces or the atmosphere, tension develops in the continuous stream of water in the apoplast, hence mass flow of water occurs due to the adhesive and cohesive properties of water.

The **symplastic** system is the system of interconnected protoplasts. Neighbouring cells are connected through cytoplasmic strands that extend through **plasmodesmata**. During symplastic movement, water travels through the cells – their cytoplasm; intercellular movement is through the plasmodesmata. Water has to enter the cells through the cell membrane, hence the movement is relatively slower. Movement is again down a potential gradient. Symplastic movement may be aided by cytoplasmic streaming. You may have observed cytoplasmic streaming in the cells of the *Hydrilla* leaf; the movement of chloroplast due to streaming is easily visible.

Most of the water flow in the roots occurs via the apoplast since the cortical cells are loosely packed, and hence offer no resistance to water movement. However, the inner boundary of the cortex, the **endodermis**, is impervious to water because of a band of suberised matrix called the casparian strip. Water molecules are unable to penetrate the layer, so they are directed to wall regions that are not suberised, into the cells proper through the membranes. The

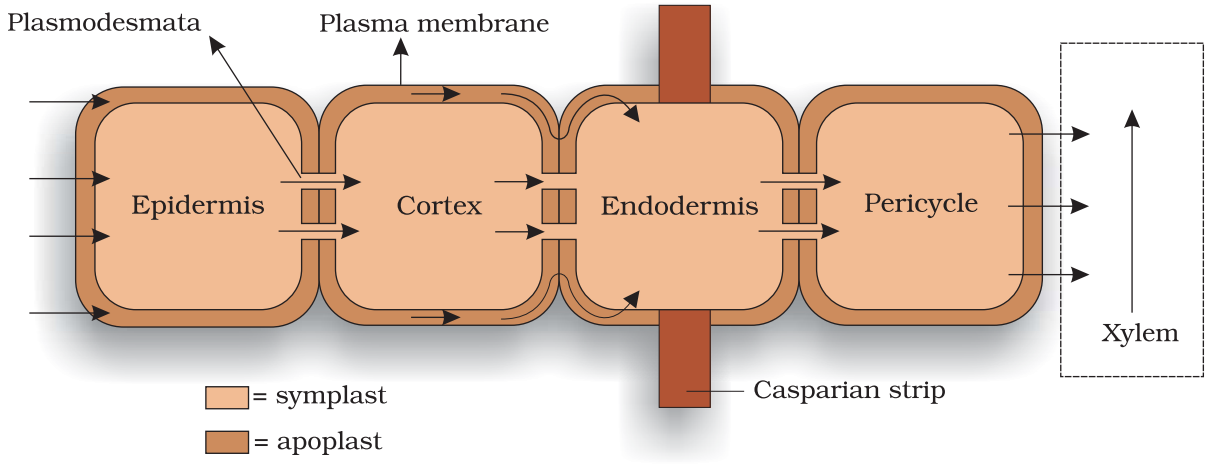


Figure 1.6 Pathway of water movement in the root

water then moves through the symplast and again crosses a membrane to reach the cells of the xylem. The movement of water through the root layers is ultimately symplastic in the endodermis. This is the only way water and other solutes can enter the vascular cylinder.

Once inside the xylem, water is again free to move between cells as well as through them. In young roots, water enters directly into the xylem vessels and/or tracheids. These are non-living conduits and so are parts of the apoplast. The path of water and mineral ions into the root vascular system is summarised in Figure 1.7.

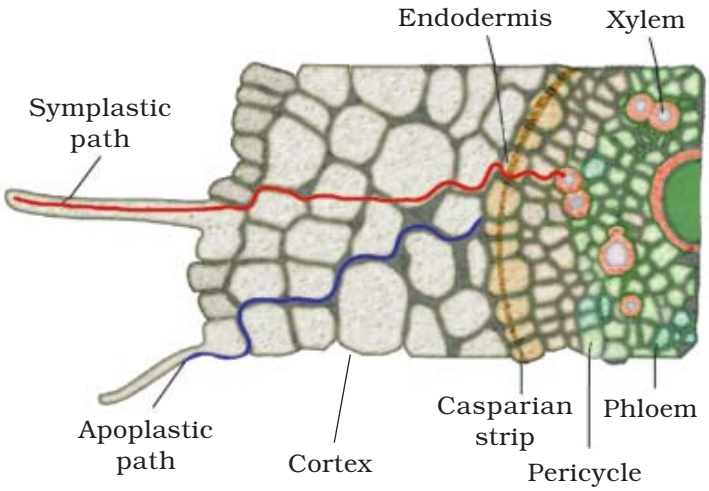


Figure 1.7 Symplastic and apoplastic pathways of water and ion absorption and movement in roots

Some plants have additional structures associated with them that help in water (and mineral) absorption. A **mycorrhiza** is a symbiotic association of a fungus with a root system. The fungal filaments either form a network around the young root or penetrate into the root cells. The hyphae have a very large surface area that absorbs mineral ions and water from the soil, from a much larger volume of soil, that perhaps a root cannot do. The fungus provides minerals and water to the roots, in turn the roots provide sugars and N-containing compounds to the mycorrhizae. Some plants have an obligate association with the mycorrhizae. For example, *Pinus* seeds cannot germinate and establish without the presence of mycorrhizae.

1.3.2 Water Movement up a Plant

We looked at how plants absorb water from the soil, and move it into the vascular tissues. We now have to try and understand how this water is transported to various parts of the plant. Is the water movement active, or is it still passive? Since the water has to be moved up a stem against gravity, what provides the energy for this?

1.3.2.1 Root Pressure

As various ions from the soil are actively transported into the vascular tissues of the roots, water follows its potential gradient and increases the pressure inside the xylem. This positive pressure is called root pressure, and is responsible for pushing up water to small heights in the stem. How can we see that root pressure exists? Choose a small soft-stemmed plant and on a day when there is plenty of atmospheric moisture, cut the stem horizontally near the base with a sharp blade early in the morning. You will soon see drops of solution ooze out of the cut stem; this happens due to the positive root pressure. If you fix a rubber tube to the cut stem as a sleeve, you can actually collect and measure the rate of exudation, and also determine the composition of the exudates. The effect of root pressure is also observable at night and early morning when evaporation is low, and excess water collects in the form of droplets around special openings of veins near the tips of grass blades and leaves of many herbaceous parts. Such water loss in its liquid phase is known as **guttation**.

Root pressure can, at best, only provide a modest push in the overall process of water transport. It obviously does not play a major role in water movement in tall trees such as *Sequoia sempervirens* (gymnosperm).

The greatest contribution of root pressure may be to re-establish the continuous chains of water molecules in the xylem which often break under the enormous tensions created by transpiration. Root pressure does not account for the majority of water transport; most plants meet their need by transpiratory pull.

1.3.2.2 Transpiration pull

Despite the absence of a heart or a circulatory system in plants, the flow of water upward through the xylem in plants can achieve fairly high rates, up to 15 metres per hour. How is this movement accomplished? A long standing question is, whether water is 'pushed' or 'pulled' through the plant. Most researchers agree that water is mainly 'pulled' through the plant, and that the driving force for this process is transpiration from the leaves. This is referred to as the **cohesion-tension-transpiration pull model** of water transport (proposed by Dixon, 1914).

But, what generates this transpirational pull?

Water is transient in plants. Less than 1 per cent of the water reaching the leaves is used in photosynthesis and plant growth. Most of it is lost through

the **stomata** in the leaves. The water loss from the plant surface in the form of vapour is known as **transpiration**.

The transpiration-driven ascent of xylem sap depends mainly on the following physical properties of water:

- Cohesion – mutual attraction between water molecules.
- Adhesion – attraction of water molecules to polar surfaces (such as the surface of tracheary elements).
- Transpiration pull - driving force for upward movement of water.

These properties give water high **tensile strength**, i.e., an ability to resist a pulling force, and high **capillarity**, i.e., the ability to rise in thin tubes. In plants capillarity is aided by the small diameter of the tracheary elements – the tracheids and vessels.

The process of photosynthesis requires water. The system of xylem vessels from the root to the leaf vein can supply the needed water. But what force does a plant use to move water molecules into the leaf parenchyma cells where they are needed? As water evaporates through the stomata, since the thin film of water over the cells is continuous, it results in pulling of water, molecule by molecule, into the leaf from the xylem. Also, because of lower concentration of water vapour in the atmosphere as compared to the substomatal cavity and intercellular spaces, water diffuses into the surrounding air. This creates 'transpiration pull' (Figure 1.8).

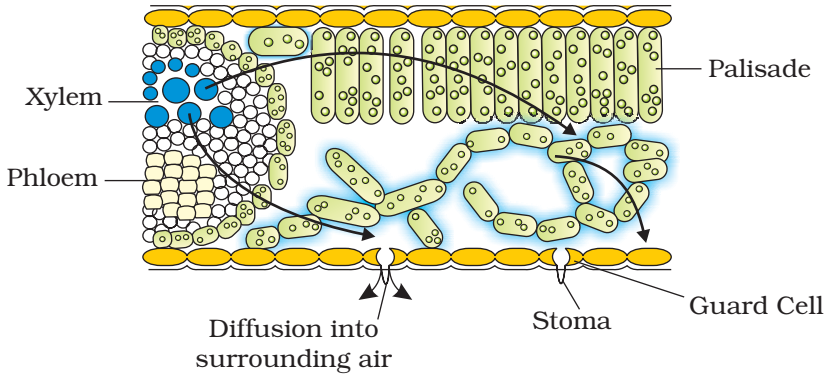


Figure1.8 Water movement in the leaf. Evaporation from the leaf sets up a pressure gradient between the outside air and the air spaces of the leaf. The gradient is transmitted into the photosynthetic cells and on the water-filled xylem in the leaf vein.

Measurements reveal that the forces generated by transpiration can create pressures sufficient to lift a xylem sized column of water over 130 metres high.

You have studied transpiration in an earlier class by enclosing a healthy plant in a polythene bag and observing the droplets of water formed inside the bag. You could also study water loss from a leaf using cobalt chloride paper, which changes colour on absorbing water.

1.4 Transpiration

Transpiration is defined as the loss of water in the form of vapour from the living tissues of aerial parts of plants. It plays a significant role in the concept of SPAC (Soil-Plant Atmosphere Continuum). The water absorbed by the plant from the soil through the root system is lost into the atmosphere through the shoot system which in turn gets back to the soil thorough rains.

The phenomenon of transpiration occurs mostly through stomata (singular-stoma) located on leaves (Figure 1.9). It also occurs through cuticle and lenticels in insignificant quantities. You have studied the basic structural details of stomatal apparatus in your previous class (Chapter 12). Stomata in most plants are **Photoactive stomata**. They open during the day and close during the night. But in succulent plants (e.g. Cacti, *Bryophyllum*) it is noted that transpiration occurs at night through **Scotoactive stomata** that open during the night and remain closed during the day time.

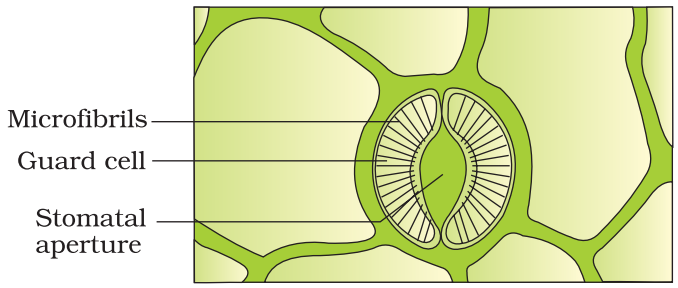


Figure1.9 A stomatal aperture with guard cells

Can you guess why?

Besides transpiration, exchange of oxygen and carbon dioxide also occurs through stomata. Usually the lower surface of a dorsiventral (often dicotyledonous) leaf has a greater number of stomata while in an isobilateral (often monocotyledonous) leaf they are nearly equal on both surfaces.

Transpiration is affected by several external factors: temperature, light, humidity and wind speed. Plant factors that affect transpiration include number and distribution of stomata, per cent of opened stomata, water status of the plant, canopy structure, available soil water, root / shoot ratio etc.

Opening and Closing of Stomata

The immediate cause of opening or closing of the stomata is a change in the turgidity of the guard cells. The inner wall of each guard cell, towards the pore or stomatal aperture, is thick and elastic. When turgidity increases within the two guard cells flanking each stomatal aperture or pore, the thin outer walls bulge out and force the inner walls into a crescent shape. The opening of the stoma is also aided by the orientation of the microfibrils in the cell walls of the guard cells. Cellulose microfibrils are oriented radially rather than longitudinally, making it easier for the stoma to open. When the guard cells lose turgor due to water loss (or water stress) the elastic inner walls regain their original shape, the guard cells become flaccid and the stoma closes.

Levitt (1974) proposed **K^+ pump theory** to explain the mechanism of opening and closing of photoactive stomata. According to this theory, accumulation of K^+ ions into the guard cells from the subsidiary cells occurs in the presence of light. This coupled with efflux of protons leads to increase in pH of the guard cells. Accumulation of K^+ ions into the guard cells is associated with passive influx of Cl^- ions thereby decreasing the water potential of the guard cells. Water thereby enters the guard cells, making them turgid. As the outer walls are thin and elastic, the guard cells expand outwardly, leaving a minute pore at the centre open.

At night, in the absence of light, the K^+ and Cl^- ions move out of the guard cells due to which the water potential of guard cells increases and water starts moving out of them leading to closure of stomata (Figure. 1.10).

Under water stress conditions, abscisic acid (ABA), a natural anti-transpirant drives the K^+ ions out of guard cells making them close.

In succulent plants, the water potential gradient established due to accumulation of organic acids at night makes the guard cells become turgid, hence stomata open at night.

1.4.1 Transpiration and Photosynthesis – a Compromise

Transpiration has more than one purpose; it

- creates transpiration pull for absorption and transportation in plants
- supplies water for photosynthesis
- transports minerals from the soil to all parts of the plant
- cools leaf surfaces, sometimes 10 to 15 degrees, by evaporative cooling and
- maintains the shape and structure of the plants by keeping cells turgid

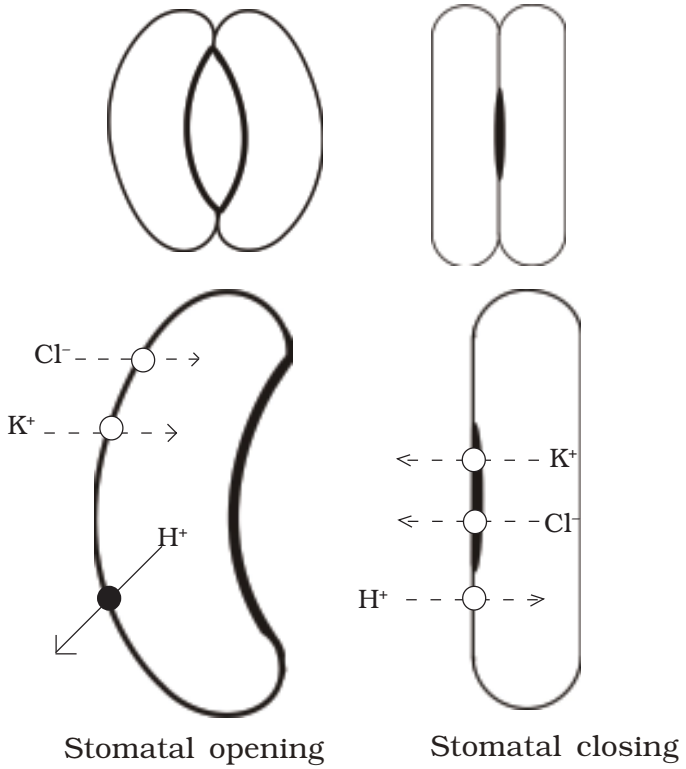


Figure1.10 Stomatal opening and closing : Broken arrows indicate passive movement and continous arrow indicates active movement.

An actively photosynthesising plant has an insatiable need for water. Photosynthesis is limited by available water which can be swiftly depleted by transpiration. The humidity of rainforests is largely due to this vast cycling of water from the root to the leaf to the atmosphere and back to the soil.

Despite making plants loose substantial amount of water, transpiration is beneficial to plants in many ways. Hence it is considered to be a *"necessary evil"*.

The evolution of the C_4 photosynthetic system is probably one of the strategies for maximising the availability of CO_2 while minimising water loss. C_4 plants are twice as efficient as C_3 plants in terms of fixing carbon (making sugar; see Chapter 4). However, a C_4 plant loses only half as much water as a C_3 plant for the same amount of CO_2 fixed.

1.5 Uptake and Transport of Mineral Nutrients

Plants obtain their carbon and most of their oxygen from CO_2 in the atmosphere. However, their remaining nutritional requirements are obtained from minerals and water in the soil.

1.5.1 Uptake of Mineral Ions

Unlike water, all minerals cannot be passively absorbed by the roots. Two factors account for this: (i) minerals are present in the soil as charged particles (ions) which cannot move across cell membranes and (ii) the concentration of minerals in the soil is usually lower than the concentration of minerals in the root. Therefore, most minerals must enter the root by active absorption into the cytoplasm of epidermal cells. This needs energy in the form of ATP. The active uptake of ions is partly responsible for the water potential gradient in roots, and therefore for the uptake of water by osmosis. Some ions also move into the epidermal cells passively.

Ions are absorbed from the soil by both passive and active transport. Specific proteins in the membranes of root hair cells actively pump ions from the soil into the cytoplasms of the epidermal cells. Like all cells, the endodermal cells have many transport proteins embedded in their plasma membrane; they let some solutes cross the membrane, but not others. Transport proteins of endodermal cells are the control points, where a plant adjusts the quantity and types of solutes that reach the xylem. Note that the root endodermis, because of the layer of suberin, has the ability to actively transport ions in one direction only.

1.5.2 Translocation of Mineral Ions

After the ions have reached xylem through active or passive uptake, or a combination of the two, their further transport up the stem to all parts of the plant is through the transpiration stream.

The chief sinks for the mineral elements are the growing regions of the plant, such as the apical and lateral meristems, young leaves, developing flowers, fruits and seeds, and the storage organs. Unloading of mineral ions occurs at the fine vein endings through diffusion and active uptake by these cells.

Mineral ions are frequently remobilised, particularly from older, senescing parts. Older dying leaves export much of their mineral content to younger leaves. Similarly, before leaf fall in decidous plants, minerals are removed to other parts. Elements most readily mobilised are phosphorus, sulphur, nitrogen

and potassium. Some elements that are structural components like calcium are not remobilised.

An analysis of the xylem exudates shows that though some of the nitrogen travels as inorganic ions, much of it is carried in the organic form as amino acids and related compounds. Similarly, small amounts of P and S are carried as organic compounds. In addition, small amount of exchange of materials does take place between xylem and phloem. Hence, it is not that we can clearly make a distinction and say categorically that xylem transports only inorganic nutrients while phloem transports only organic materials, as was traditionally believed.

1.6 Phloem Transport: Flow from Source to Sink

Food, primarily sucrose, is transported by the vascular tissue phloem from a source to a sink. Usually the source is understood to be that part of the plant which synthesises the food, i.e., the leaf, and sink, the part that needs or stores the food. But, the source and sink may be reversed depending on the season or the plant's needs. Sugar stored in roots may be mobilised to become a source of food in the early spring when the buds of trees act as sink; they need energy for growth and development of the photosynthetic apparatus. Since the source-sink relationship is variable, the direction of movement in the phloem can be upwards or downwards, i.e., **bi-directional**. This contrasts with that of the xylem where the movement is always **unidirectional**, i.e., upwards. Hence, unlike one-way flow of water in transpiration, food in phloem sap can be transported in any required direction so long as there is a source of sugar and a sink which is able to use, store or remove the sugar. Phloem sap is mainly water and sucrose, but other sugars, hormones and amino acids are also transported or translocated through phloem. An experimental set-up suggested by Munch as shown in Figure 1.11 helps in understanding bi-directional phloem transport in plants.

1.6.1 The Pressure Flow or Mass Flow Hypothesis

The accepted mechanism used for the translocation of sugars from source to sink is called the pressure flow hypothesis. (see Figure 1.12). As glucose is prepared at the source (by photosynthesis) it is converted to sucrose (a dissacharide). The sugar is then moved in the form of sucrose into the companion cells and then into the living phloem sieve tube cells by active transport. This process of loading at the source produces a hypertonic condition in the phloem. Water in the adjacent xylem moves into the phloem by osmosis.

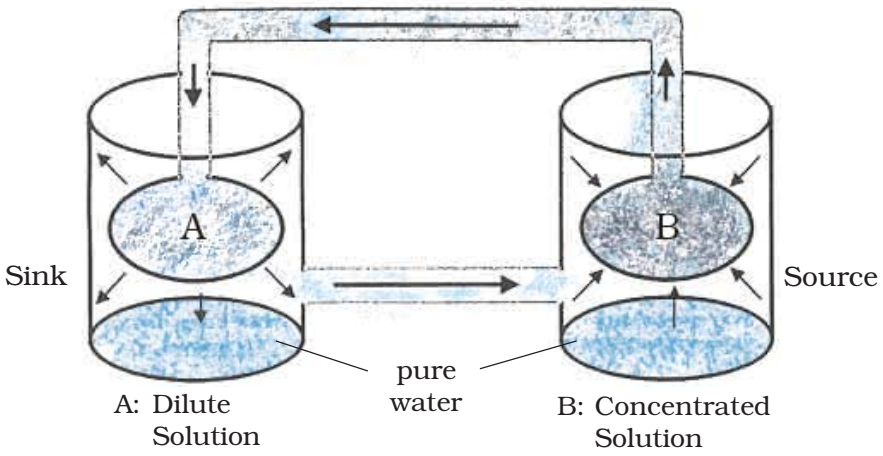


Figure 1.11 Illustration of Mass flow by Munch

As osmotic pressure builds up, the phloem sap moves to areas of lower pressure. At the sink osmotic pressure must be reduced. Again active transport is necessary to move the sucrose out of the phloem sap and into the cells which will use the sugar – converting it into energy, starch, or cellulose. As sugars are removed, the osmotic pressure decreases and water moves out of the phloem.

To summarise, the movement of sugars in the phloem begins at the source, where sugars are loaded (actively transported) into a sieve tube. Loading of the phloem sets up a water potential gradient that facilitates mass movement in the phloem.

Phloem tissue is composed of sieve tube cells which form long columns with holes in their end walls called sieve plates. Cytoplasmic strands pass through the holes in the sieve plates, forming continuous filaments. As hydrostatic pressure in the phloem sieve tube increases, pressure flow begins, and the sap moves through the phloem. Meanwhile, at the sink, incoming sugars are actively transported out of the phloem and removed as complex carbohydrates. The loss of solutes produces a high water potential in the phloem and water passes out, returning eventually to xylem.

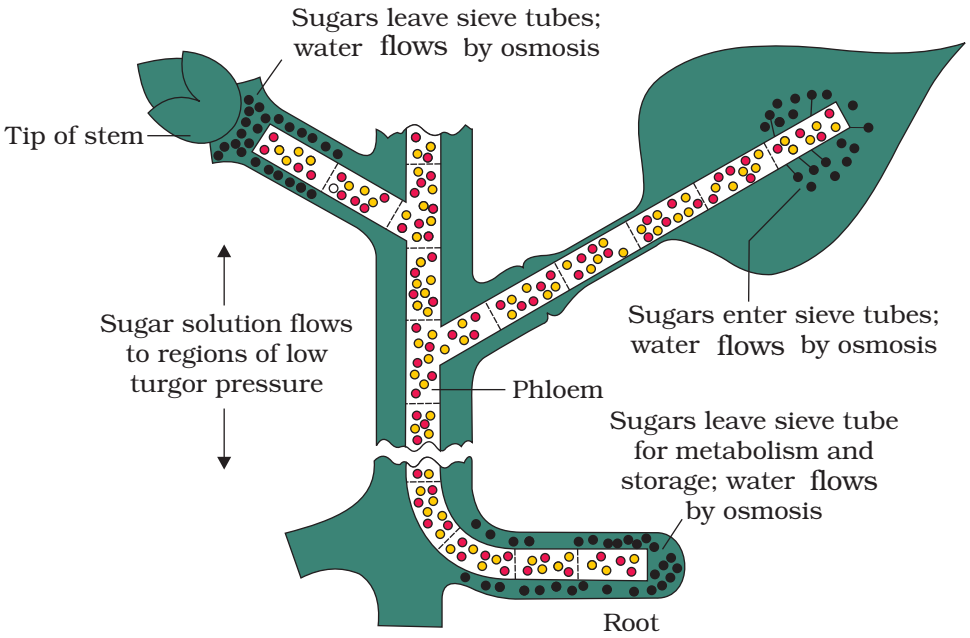


Figure 1.12 Diagrammatic presentation of mechanism of translocation

A simple experiment, called girdling, is conducted to identify the tissues through which food is transported. On the trunk of a tree a ring of bark up to a depth of the phloem layer is carefully removed. In the absence of downward movement of food the portion of the bark above the ring on the stem becomes swollen after a few weeks. This simple experiment shows that phloem is the tissue responsible for translocation of food and that transport takes place in one direction, i.e., towards the roots. This experiment can be performed by you easily.

SUMMARY

SUMMARY

Plants obtain a variety of inorganic elements (ions) and salts from their surroundings, especially from water and soil. The movement of these nutrients from the environment into the plant as well as from one plant cell to another plant cell essentially involves movement across a cell membrane. Transport across cell membrane can be through diffusion, facilitated transport or active transport. Water and minerals absorbed by roots are transported by xylem and the organic material synthesised in the leaves is transported to other parts of plant through phloem.

Passive transport (diffusion, osmosis) and active transport are the two modes of nutrient transport across cell membranes in living organisms. In passive transport, nutrients move across the membrane by diffusion, without any use of energy as it is always down the concentration gradient and hence entropy driven. Diffusion of substances depends on their size, solubility in water or organic solvents. Osmosis is a special type of diffusion of water across a semi-permeable membrane which depends on pressure gradient and concentration gradient. In active transport, energy in the form of ATP is utilised to pump molecules against a concentration gradient across membranes. Water potential is the potential energy of water which helps in the movement of water. It is determined by solute potential and pressure potential. The behaviour of the cells depends on the surrounding solution. If the surrounding solution of the cell is hypertonic, it gets plasmolysed. The absorption of water by seeds and dry wood takes place by a special type of diffusion called imbibition.

In higher plants, there is a vascular system, xylem and phloem, responsible for translocation. Water, minerals and food cannot be moved within the body of a plant by diffusion alone. They are, therefore, transported by a mass flow system – movement of substance in bulk from one point to another as a result of pressure differences between the two points.

Water absorbed by root hairs moves deeper into the root by two distinct pathways, apoplast and symplast. Various ions and water from soil can be transported upto a small height in stems by root pressure. Transpiration pull is the most acceptable factor to explain the transport of water. Transpiration is the loss of water in the form of vapours from the plant parts through stomata. Temperature, light, humidity, wind speed and number as well as distribution of stomata affect the rate of transpiration. Excess water is also removed through the tips of leaves of plants by guttation.

Phloem is responsible for transport of food (primarily sucrose) from the source to the sink. The translocation in phloem is bi-directional; the source-sink relationship is variable. The translocation in phloem is explained by the pressure-flow hypothesis.

GLOSSARY

Apoplast: The path of water within the plant system that moves without crossing membranes, i.e., through intercellular spaces or through the space between cell wall and plasma membrane or through the non-living xylem tissue. Hence apoplastic path of water is considered to be non-living path.

Diffusion: The movement of solute particles from a region of their higher concentration to the region of their lower concentration without having any membrane. This is a passive downhill movement which is driven by concentration gradient and not by any metabolic energy.

Flaccid cell: A cell when kept in hypertonic solution tends to loose water through osmosis and such a cell is said to be flaccid cell.

Guttation: The loss of water from the leaves of plants in liquid form.

Hypertonic solution: When two solutions are separated by a membrane, the solution whose concentration is higher is called hypertonic solution.

Hypotonic solution: When two solutions are separated by a membrane, the solution whose concentration is lower is called hypotonic solution.

Imbibition: The phenomenon of adsorption of liquids to the surface of solids such as water adsorption by dry seeds.

Incipient plasmolysis: The stage of the cell at the beginning of plasmolysis wherein the membrane separation can be noticed at the corners.

Isotonic condition: If both the solutions across the membrane have equal concentrations, then they are said to be in isotonic condition.

Osmosis: The physical phenomenon of diffusion of solvent from a region of lower concentrated solution to a region of higher concentrated solution through semi-permeable membrane.

Plasmolysis: The phenomenon of shrinkage of protoplast due to osmotic diffusion of water from the cells into surrounding environment.

Root pressure: The positive pressure built up in the xylem of roots due to absorption of water and ions from the soil.

Symplast: The path of water movement in the plant systems that crosses the membrane.

Turgid Cell: The state of a normal living cell in which the plasma membrane remains appressed against the cell wall wherein the turgor pressure and wall pressure are at their maximum values but in opposite direction.

QUESTIONS

Very Short Answer Type Questions

1. What are porins? What role do they play in diffusion?
2. Define water potential. What is the value of water potential of pure water?
3. Differentiate osmosis from diffusion.
4. Compare transpiration and evaporation.
5. What are apoplast and symplast?
6. How does guttation differ from transpiration?
7. What happens when a pressure greater than the atmospheric pressure is applied to pure water or a solution?
8. Explain what will happen to a plant cell if it is kept in a solution having higher water potential?
9. What are the physical factors responsible for the ascent of sap through xylem in plants?
10. Explain why xylem transport is unidirectional while that in phloem is bidirectional?
11. With reference to transportation within plant cells, what are source and sink?
12. Does transpiration occur at night? Give an example.
13. Compare the pH of guard cells during the opening and closing of stomata.
14. In the wake of transpirational loss, why do the C_4 plants are more efficient than C_3 plants?
15. What is meant by transport saturation? How does it influence facilitated diffusion?
16. Pressure potential in plant systems can be negative. Elaborate.
17. How does ABA bring about the closure of stomata under water stress conditions?

Short Answer Type Questions

1. Define and explain water potential.
2. Write short notes on facilitated diffusion.
3. What is meant by plasmolysis? How is it practically useful to us?
4. How does ascent of sap occur in tall tress?
5. Stomata are turgor operated valves. Explain.
6. Explain pressure flow hypothesis of translocation of sugars in plants.
7. "Transpiration is a necessary evil". Explain.
8. A gardener forgot to water a potted plant for a day in summer. What will happen to the plant? Do you think it is reversible? Explain.
9. Explain the type of molecular movement which is highly selective and requires special membrane proteins, but does not require any metabolic energy.
10. How does most of the water move within a healthy plant body and by which path?
11. Transpiration and Photosynthesis – a compromise. Explain.
12. Do different species of plants growing in the same area show the

same rate of transpiration at a particular time? Justify your answer.

Long Answer Type Questions

1. Explain how plants absorb water.
2. Define transpiration. Explain the structure and mechanism of opening and closing of stomata.

Exercises

1. Differentiate uphill and downhill transport.
2. Compare facilitated diffusion and simple diffusion.
3. What happens when two solutions of different concentrations are separated by egg membrane? State the reason.
4. Compare imbibing capacities of pea and wheat seeds.
5. In general in a plant which path of water movement is more and why?
6. Why *Pinus* seeds fail to germinate in the absence of mycorrhizae?
7. Which structures do you think the *Pinus* plant does not posses due to which its seeds fail to germinate?
8. What do you think is the driving force for ascent of sap?
9. Why do stomata close under water stress conditions?
10. How are stomata distributed in a typical monocot plant?
11. In what form the sugars are transported through phloem?
12. The inward movement of water into a plant begins either as symplast or apoplast. How does it conclude before entering into xylem?
13. Why does the root endodermis transports ions in one direction only?
14. If a ring of bark is removed from an actively growing plant, what will happen and why?
15. A flowering plant is planted in an earthen pot and watered. Urea is added to make the plant grow faster, but after sometime the plant dies. Why?

Chapter 2
Mineral Nutrition

The basic needs of all living organisms are essentially the same. All living organisms require macromolecules, such as carbohydrates, proteins and fats, and water and minerals for their growth and development.

This chapter focusses mainly on inorganic plant nutrition, wherein you will study the methods to identify elements essential for the growth and development of plants and the criteria for establishing the essentiality. You will also study the role of the essential elements and major deficiency symptoms, as well as the mechanism of absorption of these essential elements. The chapter also introduces you briefly to the significance and the mechanism of biological nitrogen fixation.

2.1 Methods to Study the Mineral Requirements of Plants

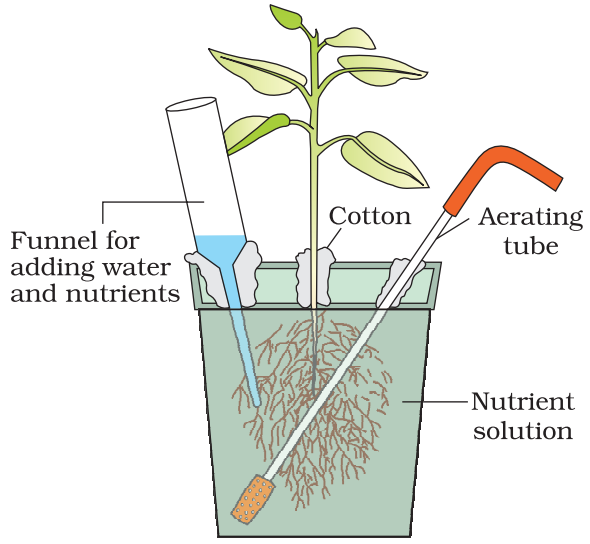


Figure 2.1 Diagram of a typical set-up for nutrient solution culture

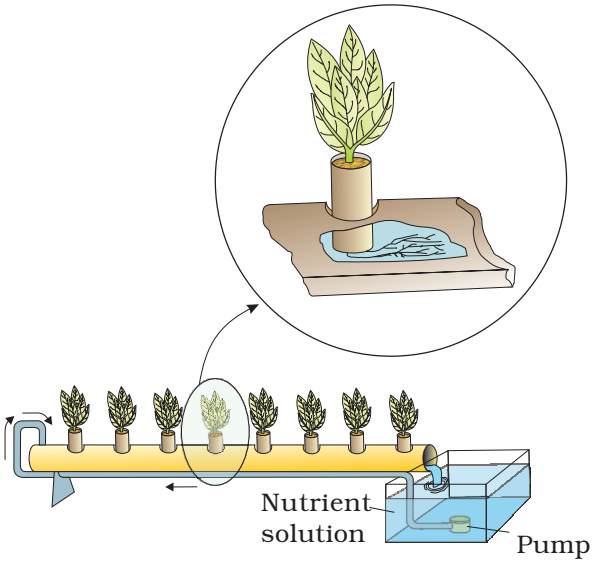


Figure 2.2 Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots are continuously bathed in aerated nutrient solution. The arrows indicate the direction of the flow.

In 1860 Julius von Sachs, a prominent German botanist, demonstrated for the first time that plants could be grown to maturity in a defined nutrient solution in complete absence of soil.

> **"The technique of growing plants in a specified nutrient solution is known as hydroponics".**

Since then, a number of improvised methods have been employed to try and determine the mineral nutrients essential for plants. The essence of all these methods involves the culture of plants in a soil-free, defined mineral solution. These methods require purified water and mineral nutrient salts. *Can you explain why this is so essential?*

After a series of experiments in which the roots of the plants were immersed in nutrient solutions and wherein an element was added / removed or given in varied concentration, a mineral solution suitable for the plant growth was obtained. By this method, essential elements were identified and their deficiency symptoms discovered. Hydroponics has been successfully employed as a technique for the commercial production of vegetables such as tomato, seedless cucumber and lettuce. It must be emphasised that the nutrient solutions must be adequately aerated to obtain optimum

growth. What would happen if solutions were poorly aerated? Diagrammatic views of the hydroponic technique is given in Figures 2.1 and 2.2.

2.2 Essential Mineral Elements

Most of the minerals present in soil can enter plants through roots. In fact, more than sixty elements are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium. There are techniques that are able to detect the minerals even at a very low concentration (10^{-8} g/mL). The question is, whether all the diverse mineral elements present in a plant, for example, gold and selenium as mentioned above, are really necessary for plants? How do we decide what is essential for plants and what is not?

2.2.1 Criteria for Essentiality

The criteria for essentiality of an element are given below:

(a) The element must be absolutely necessary for supporting normal growth and reproduction. In the absence of the element the plants do not complete their life cycle or set the seeds.

(b) The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element cannot be met by supplying some other element.

(c) The element must be directly involved in the metabolism of the plant.

Based upon the above criteria only a few elements have been found to be absolutely essential for plant growth and metabolism. These elements are further divided into two broad categories based on their quantitative requirements.

(i) Macronutrients

(ii) Micronutrients

Macronutrients are generally present in plant tissues in large amounts (in excess of 10 mmole Kg^{-1} of dry matter). They include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium and magnesium. Of these, the framework elements-carbon, hydrogen and oxygen are mainly obtained from CO_2 and H_2O, while the others are absorbed from the soil as mineral nutrients.

Micronutrients or trace elements are needed in very small amounts (less than 10 mmole Kg^{-1} of dry matter). These include iron, manganese, copper, molybdenum, zinc, boron, chlorine and nickel.

In addition to the 17 essential elements named above, there are some beneficial elements such as sodium, silicon, cobalt and selenium. They are required by higher plants.

Essential elements can also be grouped into four broad categories on the basis of their diverse functions. These categories are:

(i) Essential elements that are components of biomolecules and hence are structural elements of cells (e.g., carbon, hydrogen, oxygen and nitrogen etc.).

(ii) Essential elements that are components of energy-related chemical compounds in plants (e.g., magnesium in chlorophyll and phosphorous in ATP).

(iii) Essential elements that activate or inhibit enzymes. For example, Mg^{2+} is an activator for both ribulose bisphosphate carboxylase-oxygenase and phosphoenol pyruvate carboxylase, both of which are critical enzymes in photosynthetic carbon fixation; Zn^{2+} is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism. *Can you name a few more elements that fall in this category?* For this, you will need to refer to some of the biochemical pathways which you will study in the later chapters.

(iv) Some essential elements can alter the osmotic potential of a cell. Potassium plays an important role in the opening and closing of stomata. You may recall the role of minerals as solutes in determining the water potential of a cell.

2.2.2 Role of Macro- and Micro-nutrients

Essential elements perform several functions. They participate in various metabolic processes in the plant cells such as permeability of cell membrane, maintenance of osmotic concentration of cell sap, electron-transport systems, buffering action and enzymatic activity. They also act as major constituents of macromolecules and co-enzymes.

The various forms and functions of essential nutrient elements are given below:

Nitrogen: This is the essential mineral nutrient element required by plants in the greatest amount. It is absorbed mainly as NO_3^- though sometimes it is also taken up as NO_2^- or NH_4^+. Nitrogen is required by all parts of a plant, particularly the meristematic tissues and the metabolically active cells. Nitrogen is one of the major constituents of proteins, nucleic acids, enzymes, vitamins and hormones.

Phosphorus: Phosphorus is absorbed by plants from the soil in the form of phosphate ions (either as $H_2PO_4^-$ or HPO_4^{2-}). Phosphorus is a constituent of cell membranes, certain proteins, all nucleic acids and nucleotides, and is required for all phosphorylation reactions.

Potassium: It is absorbed as potassium ion (K^+). It is required in more abundant quantities in the meristematic tissues, buds, leaves and root tips. Potassium helps to maintain an anion-cation balance in cells and is involved in protein synthesis, opening and closing of stomata, activation of enzymes and in the maintenance of the turgidity of cells.

Calcium: Plants absorb calcium from the soil in the form of calcium ions (Ca^{2+}). Calcium is required by meristematic and differentiating tissues. During cell division, it is used in the synthesis of cell wall, particularly as calcium pectate in the middle lamella. It is also needed during the formation of mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes. It activates certain enzymes and plays an important role in regulating metabolic activities. Calcium is also an important element that helps in photolysis of water during photosynthesis.

Magnesium: It is absorbed by plants in the form of divalent Mg^{2+} ions. It activates the enzymes of respiration and photosynthesis and is involved in the synthesis of DNA and RNA. Magnesium is a constituent of the ring structure of chlorophyll and helps to maintain the ribosome structure.

Sulphur: Plants obtain sulphur in the form of sulphate SO_4^{2-} ions. Sulphur is present in two amino acids – cysteine and methionine, and is the main constituent of several coenzymes, vitamins (thiamine, biotin, coenzyme A) and ferredoxin. Sulphur forms disulphide bridges which help in stabilizing the protein structure.

Iron: Plants obtain iron in the form of ferric ions (Fe^{3+}). Iron is required in larger amounts compared to other micronutrients. It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochromes. It is reversibly oxidised from Fe^{2+} to Fe^{3+} during electron transfer. It activates catalase enzyme, and is essential for the formation of chlorophyll.

Manganese: It is absorbed in the form of manganous ions (Mn^{2+}). It activates many enzymes involved in photosynthesis, respiration and nitrogen metabolism. The best defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis. It is also an activator for IAA oxidase enzyme.

Zinc: Plants obtain zinc as Zn^{2+} ions. Zinc activates various enzymes, especially carboxylases. It is also needed in the synthesis of auxin.

Copper: It is absorbed as cupric ions (Cu^{2+}). It is essential for the overall metabolism in plants. Like iron, it is associated with certain enzymes involved in redox reactions and is reversibly oxidised from Cu^{+} to Cu^{2+}. It is also a structural component of electron carriers like cytochrome C oxidase in mitochondria and plastocyanin in chloroplast.

Boron: It is absorbed as BO_3^{3-} or $B_4O_7^{2-}$ ions. Boron is required for the uptake and utilisation of Ca^{2+}, membrane functioning, pollen germination, cell elongation, cell differentiation and carbohydrate translocation.

Molybdenum: It is obtained in the form of molybdate ions (MoO_2^{2+}). It is a component of several enzymes, including nitrogenase and nitrate reductase, both of which participate in nitrogen metabolism.

Chlorine: It is absorbed in the form of chloride anion (Cl^{-}). Along with Na^{+} and K^{+}, it helps in determining the solute concentration and the anion-cation balance in cells. It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.

Nickel: It is recently recognized as the 17th essential nutrient as it acts as an activator for urease, an important enzyme of nitrogen metabolism. It is also found to induce disease resistance in some plants.

2.2.3 Deficiency Symptoms of Essential Elements

Whenever the supply of an essential element becomes limited, plant growth is retarded. The concentration of the essential element below which plant growth is retarded is termed as **critical concentration.** The element is said to be deficient when present below the critical concentration.

Since each element has one or more specific structural or functional role in plants, in the absence of any particular element, plants show certain morphological changes. These morphological changes are indicative of deficiencies of certain elements and are called deficiency symptoms. The deficiency symptoms vary from element to element and they disappear when the deficient mineral nutrient is provided to the plant. However, if deprivation continues, it may eventually lead to the death of the plant. The parts of the plants that show the deficiency symptoms also depend on the mobility of the element within the plant. For elements that are actively mobilised within the plants and exported to young developing tissues, the deficiency symptoms

tend to appear first in the older tissues. For example, the deficiency symptoms of nitrogen, potassium and magnesium are visible first in the senescent leaves. In the older leaves, biomolecules containing these elements are broken down, making these elements available for mobilising to younger leaves.

Deficiency symptoms tend to appear first in the young tissues whenever the elements are relatively immobile and are not transported out of the mature organs. For example, elements like sulphur and calcium are a part of the structural component of the cell and hence are not easily released. This aspect of mineral nutrition of plants is of a great significance and importance in agriculture and horticulture.

The kind of deficiency symptoms shown in plants include chlorosis, necrosis, stunted plant growth, premature fall of leaves and buds, and inhibition of cell division. Chlorosis is the loss of chlorophyll leading to yellowing in leaves. This symptom is caused by the deficiency of elements N, K, Mg, S, Fe, Mn, Zn and Mo. Likewise, necrosis, or death of tissue, particularly leaf tissue, is due to the deficiency of Ca, Mg, Cu, K. Lack or low level of N, K, S, Mo causes inhibition of cell division. Some elements like N, S, Mo delay flowering if their concentration in plants is low.

You can see from the above that the deficiency of any element can cause multiple symptoms and that the same symptoms may be caused by the deficiency of one of several different elements. Hence, to identify the deficient element, one has to study all the symptoms developed in all the various parts of the plant and compare them with the available standard tables. We must also be aware that different plants respond differently to the deficiency of the same element.

Deficiency of certain micronutrients like Zn, Cu, B, Mo, Cl and Ni cause physiological diseases such as **mottled leaf**, **die-back** in citrus, **heart-rot** in beets, **whip tail** in cauliflower, **bronzing** in legumes and **mouse ear** in pecan respectively.

2.2.4 Toxicity of Micronutrients

The requirement of micronutrients is always in low amounts. While a moderate decrease causes deficiency symptoms, a moderate increase causes toxicity. In other words, there is a narrow range of concentration at which the elements are at an optimum level. Any mineral ion concentration in tissues that reduces the dry weight of tissues by about 10 per cent is considered toxic. Such critical concentrations vary widely among different micronutrients. Toxicity symptoms

are difficult to identify. Toxicity levels of any element also vary for different plants. Many a time, excess of an element may inhibit the uptake of another element. For example, the prominent symptom of manganese toxicity is the appearance of brown spots surrounded by chlorotic veins. It is important to know that manganese competes with iron and magnesium for uptake and with magnesium for binding with enzymes. Manganese also inhibits calcium translocation in the shoot apex. Therefore, excess of manganese may, in fact, induce deficiencies of iron, magnesium and calcium. Thus, what appears as symptoms of manganese toxicity may actually be the deficiency symptoms of iron, magnesium and calcium. Can this knowledge be of some importance to a farmer? A gardener? Or even for you in your kitchen-garden?

2.3 Mechanism of Absorption of Elements

Most studies on the mechanism of absorption of elements by plants have been carried out on isolated cells, tissues or organs. These studies revealed that the process of absorption can be demarcated into two main phases. In the first phase, there is an initial *rapid* uptake of ions into the 'free space' or 'outer space' of cells – the apoplast. It is a passive process. In the second phase of uptake, the ions are taken in *slowly* into the 'inner space' – the symplast of the cells.

The **passive** movement of ions into the apoplast from the cell along the concentration gradient usually occurs through ion-channels. The trans-membrane proteins function as selective pores. The entry or exit of ions to and from the symplast against the concentration gradient requires the expenditure of metabolic energy which is an **active** process.

The movement of ions is called **flux;** the inward movement into the cells is **influx** and the outward movement, **efflux**.

2.4 Translocation of Solutes

Mineral salts are translocated through the xylem along with the ascending stream of water, which is pulled up through the plant by transpirational pull. Analysis of xylem sap shows the presence of mineral salts in it. The use of radioisotopes of mineral elements also substantiate the view that they are transported through the xylem. We have already discussed the movement of water in xylem in Chapter 1.

2.5 Soil as Reservoir of Essential Elements

The majority of the nutrients that are essential for the growth and development of plants become available to the roots due to weathering and breakdown of rocks. These processes enrich the soil with dissolved ions and inorganic salts. Since they are derived from the rock minerals, their role in plant nutrition is referred to as mineral nutrition. Soil consists of a wide variety of substances. Soil not only supplies minerals but also harbours nitrogen-fixing bacteria, other microbes, holds water, supplies air to the roots and acts as a matrix that stabilises the plant. Since deficiency of essential minerals affect the crop-yield, there is often a need for supplying minerals through fertilisers. Both macro-nutrients (N, P, K, S, etc.) and micro-nutrients (Cu, Zn, Fe, Mn, etc.) form components of fertilisers and are applied as per need.

2.6 Metabolism of Nitrogen

As Nitrogen is the essential mineral element required in the largest quantity by plants in general, its metabolism is discussed here.

2.6.1 Nitrogen Cycle

Apart from Carbon, Hydrogen and Oxygen, Nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls and many vitamins. Plants compete with microbes for the limited nitrogen that is available in soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural eco-systems. Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond (N ≡ N). The process of conversion of molecular nitrogen (N_2) to ammonia or nitrogen oxides, nitrites and nitrates is termed as **nitrogen-fixation.** In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO, NO_2, NO_3). Industrial combustions, forest fires, automobile exhausts and power-generating stations are also sources of atmospheric nitrogen oxides.

The nitrates and ammonia thus formed are absorbed by plants and converted into amino acids, proteins, enzymes, nucleic acids, pigments and hormones etc., in the plant body. These constitute the organic form of nitrogen. When plants are eaten by animals this organic nitrogen is passed on into animal body. The process of abosorbing nitrates, ammonia and chemically binding the nitrogen with other elements to produce organic nitrogen in plants and thereby into animals constitutes nitrogen **assimilation**.

Decomposition of organic nitrogen of dead plants and animals into ammonia is called **ammonification**. Some of this ammonia volatilises and re-enters the atmosphere but most of it is converted into nitrate by soil bacteria in the following steps:

$$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H^+ + 2H_2O \quad \text{.... (i)}$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \quad \text{..... (ii)}$$

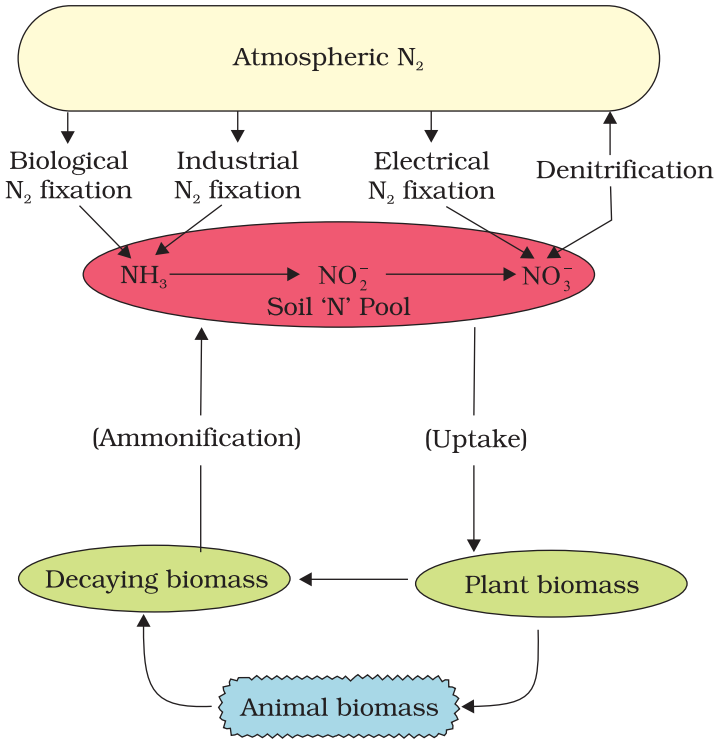


Figure 2.3 The nitrogen cycle showing the relationship between the three main nitrogen pools – atmosphere, soil, and biomass

Ammonia is first oxidised to nitrite by the bacteria *Nitrosomonas* and/or *Nitrococcus*. The nitrite is further oxidised to nitrate with the help of the bacterium *Nitrobacter*. These steps are called **nitrification** (Figure 2.3). These nitrifying bacteria are **chemoautotrophs**.

The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of **denitrification**. Denitrification is carried out by the bacteria *Pseudomonas* and *Thiobacillus*.

2.6.2 Biological Nitrogen Fixation

Very few living organisms can utilise the nitrogen in the form of N_2, available abundantly in the air. Only certain prokaryotic species are capable of fixing nitrogen. Reduction of nitrogen to ammonia by living organisms is called **biological nitrogen fixation.** The enzyme, nitrogenase, which is capable of nitrogen reduction is present exclusively in prokaryotes. Such microbes are called N_2- fixers.

$$N \equiv N \xrightarrow{\text{Nitrogenase}} NH_3$$

The nitrogen-fixing microbes can be free-living or symbiotic. Examples of free-living nitrogen-fixing aerobic microbes are *Azotobacter* and *Beijernickia* while *Rhodospirillum* is anaerobic and *Bacillus* free-living. In addition, a number of cyanobacteria such as *Anabaena* and *Nostoc* are also free-living nitrogen-fixers.

Symbiotic nitrogen fixation

Several types of symbiotic nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* has such a relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, *Frankia,* also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., *Alnus*). Both *Rhizobium* and *Frankia* are free-living in soil but as symbionts can fix atmospheric nitrogen.

Uproot any one plant of a common pulse just before it flowers. You will see near-spherical outgrowths on the roots. These are nodules. If you cut through them you will notice that the central portion is red or pink. What makes the nodules pink? This is due to the presence of leguminous haemoglobin or leg-haemoglobin.

Nodule Formation

Nodule formation involves a sequence of multiple interactions between *Rhizobium* and roots of the host plant. The Principal stages in the nodule formation are summarised as follows:

Rhizobia attracted by the sugars, amino acids etc., released by the host legume, multiply and colonise the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair. An infection thread is produced, carrying the bacteria into the cortex of the root. Bacteria initiate nodule formation in the cortex of the root. Then the bacteria released from the thread into the cortical cells of the host stimulate the host cells to divide. Thus leads to the differentiation of specialised nitrogen fixing cells. The nodule thus formed establishes a direct vascular connection with the host for exchange of nutrients. These events are depicted in Figure 2.4.

The nodule contains all the necessary biochemical components, such as the enzyme nitrogenase and leghaemoglobin. The enzyme nitrogenase is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia,

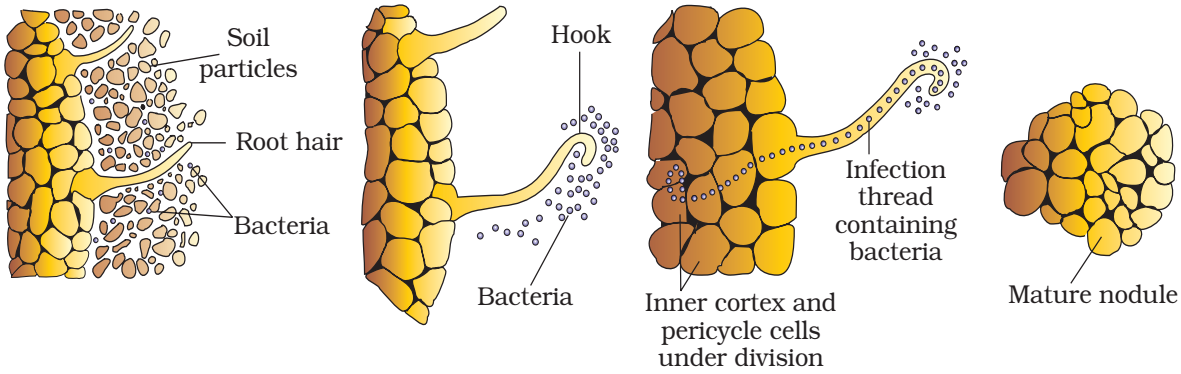


Figure 2.4 Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it. (b) Successful infection of the root hair causes it to curl. (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation. (d) A mature nodule is complete with vascular tissues continuous with those of the root.

(Figure 2.5) the first stable product of nitrogen fixation. The reaction is as follows:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \longrightarrow 2H_3 + H_2 + 16ADP + 16Pi$$

The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contains an **oxygen scavenger** called *leg-haemoglobin*. It is interesting to note that

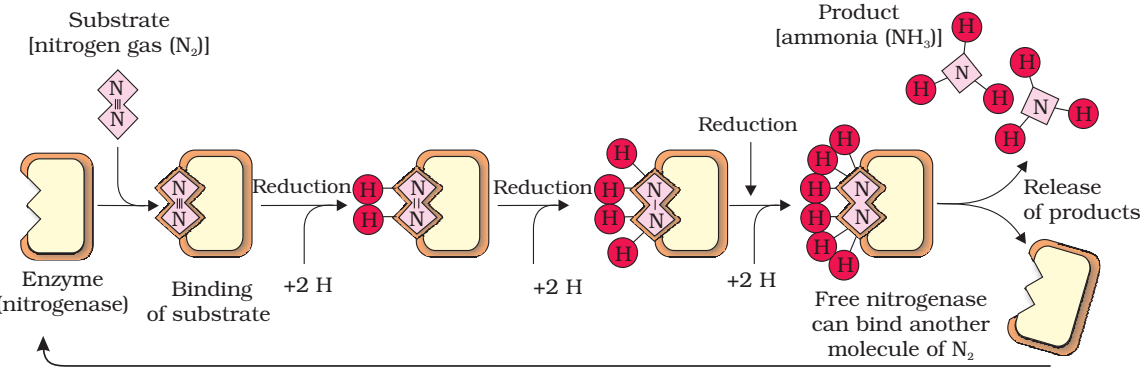


Figure 2.5 Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

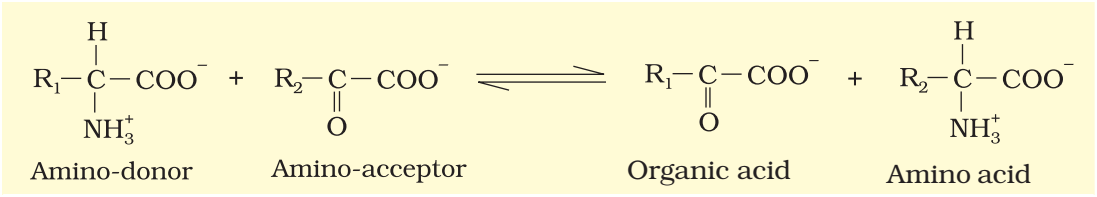
these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they adapt to anaerobic conditions, thus protecting the nitrogenase enzyme. You must have noticed in the above reaction that the ammonia synthesis by nitrogenease requires a very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.

Fate of ammonia: At physiological pH, the ammonia is protonated to form NH_4^+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the latter is quite toxic to plants and hence cannot accumulate in them. Let us now see how the NH_4^+ is used to synthesise amino acids in plants. There are two main ways in which this can take place:

(i) Reductive amination: In these processes, ammonia reacts with α-ketoglutaric acid and forms glutamic acid as indicated in the equation given below :

$$\alpha\text{-ketoglutaric acid} + NH^+_4 + NADPH \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{glutamate} + H_2O + NADP$$

(ii) Transamination : It involves the transfer of an amino group from an amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH_2, the amino group, takes place and other amino acids are formed through transamination. The enzyme **transaminase** catalyses all such reactions. For example,



The two most important amides – asparagine and glutamine – found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid, respectively, by addition of another amino group to each. The hydroxyl part of the acid is replaced by another NH_2^- radicle. Since amides contain more nitrogen than amino acids, they are transported to other parts of the plant via xylem vessels. In addition, along with the transpiration stream, the nodules of some plants (e.g., soyabean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

SUMMARY

SUMMARY

Plants obtain their inorganic nutrients from air, water and soil. Plants absorb a wide variety of mineral elements. Not all the mineral elements that are absorbed are required by plants. Out of the more than 65 elements found in plants, less than 21 are defined as essential and are beneficial for normal plant growth and development. The elements required in large quantities are called macronutrients while those required in less quantities or in traces are termed as micronutrients. These elements are either essential constituents of proteins, carbohydrates, fats, nucleic acids etc., and/or take part in various metabolic processes. Deficiency of any of these essential elements may lead to symptoms called deficiency symptoms. Chlorosis, necrosis, stunted growth, impaired cell division, etc., are some prominent deficiency symptoms. Plants absorb minerals through roots by either passive or active processes. These minerals are carried to all parts of the organism through xylem along with water transport.

Nitrogen is very essential for the sustenance of life. Plants cannot use atmospheric nitrogen directly. But some of the plants in association with N_2-fixing bacteria, especially roots of legumes, can fix this atmospheric nitrogen into biologically usable forms. Nitrogen fixation requires a strong reducing agent and energy in the form of ATP. N_2– fixation is accomplished with the help of nitrogen-fixing microbes, mainly *Rhizobium*. The enzyme nitrogenase which plays an important role in biological N_2– fixation is very sensitive to oxygen. Most of the processes take place in anaerobic environment. The energy, ATP, required is provided by the respiration of the host cells. Ammonia produced following N_2 fixation is incorporated into amino acids as the amino group.

GLOSSARY

Active process: A biological process that occurs due to utilization of energy.

Ammonification: Accumulation of nitrogen in the form of ammonia in soil mainly due to decomposition of organic nitrogen by microorganisms.

Chemoautotroph: An organism that derives its carbon from inorganic carbon source while the energy is obtained from the oxidation of chemical substances.

Critical concentration: The minimum quantum of a particular mineral element required by plants for growth.

Denitrification: The process of conversion of usable form of nitrogen (nitrate) into unusable form (molecular nitrogen).

Flux: The movement of ions either into or out of cells.

Framework Elements: The essential elements that are constituents of organic substances which make about every chemical substance of life forms.

Leg-haemoglobin: The red pigment produced in legume root nodules that removes oxygen from the vicinity of Nitrogenase enzyme. It acts as *oxygen scavenger*.

Photoheterotroph: An organism which derives its carbon from organic sources and energy from sunlight.

QUESTIONS

Very Short Answer Type Questions

1. Define hydroponics.
2. How do you categorize a particular essential element as a macro or micronutrient?
3. Give two examples of essential elements that act as activators for enzymes.
4. Name the essential mineral elements that play an important role in photolysis of water.
5. Out of the 17 essential elements which elements are called non-mineral essential elements?
6. Name two amino acids in which sulphur is present.
7. When is an essential element said to be deficient?
8. Name two elements whose symptoms of deficiency first appear in younger leaves.
9. Explain the role of the pink colour pigment in the root nodule of legume plants. What is it called?
10. Excess Mn in soils leads to deficiency of Ca, Mg and Fe. Justify.
11. What acts as a reservoir of essential elements for plants? By what process is it formed?
12. Which element is regarded as the 17th essential element? Name a disease caused by its deficiency.
13. Nitrogen fixation is shown by prokaryotes only. Why not by eukaryotes?
14. Give an example for each of the aerobic and anaerobic nitrogen fixing prokaryotes.
15. Non-legume plants also form root nodules. Justify.
16. Name the essential elements present in nitrogenase enzyme. What type of essential elements are they?
17. Write the balanced equation of nitrogen fixation.
18. How many ATPs of energy is required to fix one molecule of atmospheric nitrogen by biological mode? What is the source of that energy?
19. Why are amides transported through xylem?
20. Name any two essential elements and the deficiency diseases caused by them.

Short Answer Type Questions

1. 'All elements that are present in a plant need not be essential for its survival'. Justify.
2. Name at least five different deficiency symptoms in plants. Describe them and correlate them with the concerned mineral deficiency.
3. Explain the steps involved in the formation of root nodule.

4. Some angiospermic plants have adapted to absorb molecular nitrogen from atmosphere. Explain, citing two examples.
5. Write in brief how plants synthesize amino acids.
6. What will happen if a healthy plant is supplied with excess essential elements? Explain.
7. Explain in brief how plants absorb essential elements.
8. Nitrogen is fixed into the soil not only by biological processes. Elaborate.

Long Answer Type Questions

1. Explain the nitrogen cycle, giving relevant examples.
2. Trace the events starting from the coming in contact of *Rhizobium* with a leguminous root till nodule formation. Add a note on the importance of leg haemoglobin.

Exercises

1. Who should be credited for initiation of hydroponics?
2. Are all the essential elements required by plants mineral elements? Explain.
3. Which essential element is needed to activate the enzymes required for CO_2 fixation?
4. Name a cation and an anion that maintain osmotic balance in cells.
5. Which element is required for the formation of mitotic spindle?
6. What is the role of sulphur in plant life?
7. Which microelement is required in more quantity than the other micronutrients?
8. Which element is necessary for the synthesis of the chief photosynthetic pigment without being its structural component?
9. Which micronutrient necessary for photolysis of water is absorbed by plants in anionic form?
10. Which enzyme is activated by the 17^{th} essential element?
11. When is an element considered to be toxic?
12. Which element when supplied in excess leads to appearance of brown spots surrounded by chlorotic veins?
13. Name an anaerobic, free living, photo-heterotrophic nitrogen fixing bacterium.
14. Which microorganism produces nitrogen-fixing nodules in *Alnus*?
15. When the cross section of root nodules of ground nut plants are observed under microscope, they appear pinkish. Why?
16. Apart from the cortical cells, which other cells are stimulated to divide by the bacteroids inside the root nodules?
17. What is the ratio of electrons and protons required for the fixation of atmospheric molecular nitrogen through biological mode?
18. What acts as *oxygen scavenger* in the legume-root nodule combination?

19. In what way does aspargine differ from aspartic acid?
20. Through which tissue the amino acids are transported inside the plant body?
21. Plants like the Pitcher and Venus-fly trap have special nutritional adaptations. Name the essential element and its source for which they show such adaptations.

Chapter 3

Enzymes

Almost all enzymes are proteins. There are some nucleic acids that behave like enzymes. These are called ribozymes (eg. 23s rRNA). One can depict an enzyme by a line diagram. An enzyme like any protein has a primary structure, i.e., amino acid sequence of the protein. An enzyme, like any protein, has a secondary and the tertiary structure too. When you look at a tertiary structure (Figure 10.4 1st year) you will notice that the backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence, many crevices or pockets are made. One such pocket is the 'active site'. An active site of an enzyme is a crevice or pocket into which the substrate fits. Thus enzymes, through their active site, catalyse reactions at a high rate. Enzyme catalysts differ from inorganic catalysts in many ways, but one major difference needs mention. Inorganic catalysts work efficiently at high temperatures and high pressures, while enzymes get damaged at high temperatures (say above 40°C). However, enzymes isolated from organisms which normally live under extremely high temperatures (e.g., hot vents and sulphur springs), are stable and retain their catalytic power even at high temperatures (upto 80°-90°C). Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms.

3.1 Chemical Reactions

How do we understand these enzymes? Let us first understand a chemical reaction. Chemical compounds undergo two types of changes. A physical change simply refers to a change in shape without breaking of bonds. This is a physical process. Another physical process is a change in the state of matter: e.g., when ice melts into water, or when water becomes a vapour. These are also physical processes. However, when bonds are broken and new bonds are formed during transformation, it is called a chemical reaction. For example:

$$Ba(OH)_2 + H_2SO_4 \longrightarrow BaSO_4 + 2H_2O$$

is an inorganic chemical reaction. Similarly, hydrolysis of starch into glucose is an organic chemical reaction. The rate of a physical or chemical process refers to the amount of product formed per unit time. It can be expressed as:

$$\text{rate} = \frac{\delta p}{\delta t}$$

Rate can also be called velocity if the direction is specified. Rates of physical and chemical processes are influenced by temperature among other factors. A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones. When enzyme catalysed reactions are observed, the rate is vastly higher than the same but uncatalysed reaction. For example

$$\underset{\text{carbon dioxide}}{CO_2} + \underset{\text{water}}{H_2O} \xrightleftharpoons{\text{Carbonicanhydrase}} \underset{\text{carbonic acid}}{H_2CO_3}$$

In the absence of any enzyme this reaction is very slow, with about 200 molecules of H_2CO_3 being formed in an hour. However, by using the enzyme present within the cytoplasm, called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme accelerates the reaction rate by about 10 million times. The power of enzymes is incredible indeed!

There are thousands of types of enzymes, each catalysing a unique chemical or metabolic reaction. A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway.

For example,

$$\text{Glucose} \longrightarrow 2 \text{ Pyruvic acid}$$

$$C_6H_{12}O_6 + O_2 \longrightarrow 2C_3H_4O_3 + 2H_2O$$

is actually a metabolic pathway in which glucose becomes pyruvic acid through ten different enzyme catalysed metabolic reactions. When you study respiration in Chapter 5 you will study these reactions. At this stage you should know that this very metabolic pathway with one or two additional reactions gives rise to a variety of metabolic end products. In our skeletal muscle, under anaerobic conditions, lactic acid is formed. Under normal aerobic conditions, pyruvic acid is formed. In yeast, during fermentation, the same pathway leads to the production of ethanol (alcohol). Hence, in different conditions different products are possible.

3.2 How do Enzymes bring about such High Rates of Chemical Conversions?

To understand this we should study enzymes a little more. We have already understood the idea of an 'active site'. The chemical or metabolic conversion refers to a reaction. The chemical which is converted into a product is called a 'substrate'. Hence enzymes, i.e. proteins with three dimensional structures including an 'active site', convert a substrate (S) into a product (P). Symbolically, this can be depicted as:

$$S \longrightarrow P$$

It is now understood that the substrate 'S' has to bind the enzyme at its 'active site' within a given cleft or pocket. The substrate has to diffuse towards the 'active site'. There is thus, an obligatory formation of an 'ES' complex. E stands for enzyme. This complex formation is a transient phenomenon. During the state where substrate is bound to the enzyme active site, a new structure of the substrate called transition state structure is formed. Very soon, after the expected bond breaking/making is completed, the product is released from the active site. In other words, the structure of substrate gets transformed into the structure of product(s). The pathway of this transformation must go through the so-called transition state structure. There could be many more 'altered structural states' between the stable substrate and the product. Implicit in this statement is the fact that all other intermediate structural states are

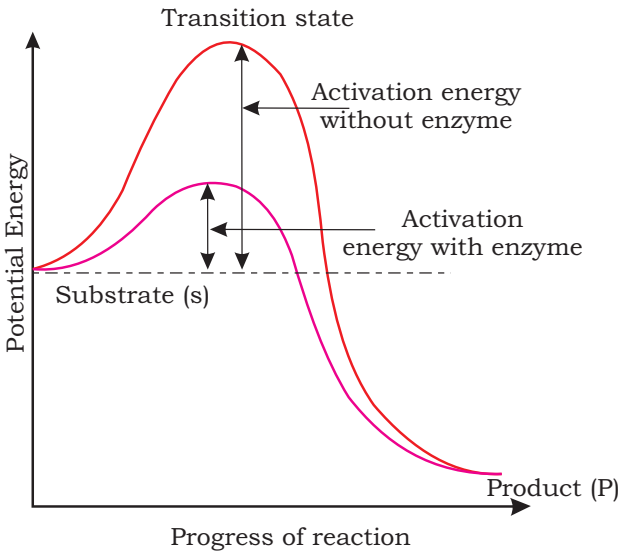


Figure 3.1 Concept of activation energy

unstable. Stability is something related to energy status of the molecule or the structure. When we represent this pictorially through a graph it looks like what is given in Figure 3.1.

The y-axis represents the potential energy content. The x-axis represents the progression of the structural transformation or states through the 'transition state'. You would notice the energy level difference between S and P. If 'P' is at a lower level than 'S', it is an exothermic reaction. One need not supply energy (by heating) in order to form the product. However, whether it is an exothermic or spontaneous reaction or an endothermic or energy requiring reaction, the 'S' has to go through a much higher energy state or transition state. The difference between average energy content of 'S' and that of this transition state is called 'activation energy'.

Enzymes eventually bring down this energy barrier, making the transition of 'S' to 'P' more easy.

3.3 Nature of Enzyme Action

Each enzyme (E) has a substrate (S) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme, with an intermediate formation of the enzyme-product complex (EP).

The formation of the ES complex is essential for catalysis.

$$E + S \longrightarrow [ES] \longrightarrow [EP] \longrightarrow E + P$$

Formation of [ES] complex has been explained with 'Lock and Key' hypothesis by Emil Fisher (1884) and much later with 'Induced-Fit' hypothesis by Daniel E.Koshland (1973).

The catalytic cycle of an enzyme action can be described in the following steps:

1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity to the substrate, breaks the chemical bonds of the substrate and the new enzyme- product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and runs through the catalytic cycle once again.

3.4 Factors Affecting Enzyme Activity

The activity of an enzyme can be affected by a change in the conditions which alter the tertiary structure of the protein. These include temperature, pH, change in substrate concentration or binding of specific chemicals that regulate its activity.

Temperature and pH

Enzymes generally function in a narrow range of temperature and pH (Figure 3.2). Each enzyme shows its highest activity at a particular temperature and pH, called the optimum temperature and optimum pH. Activity declines both

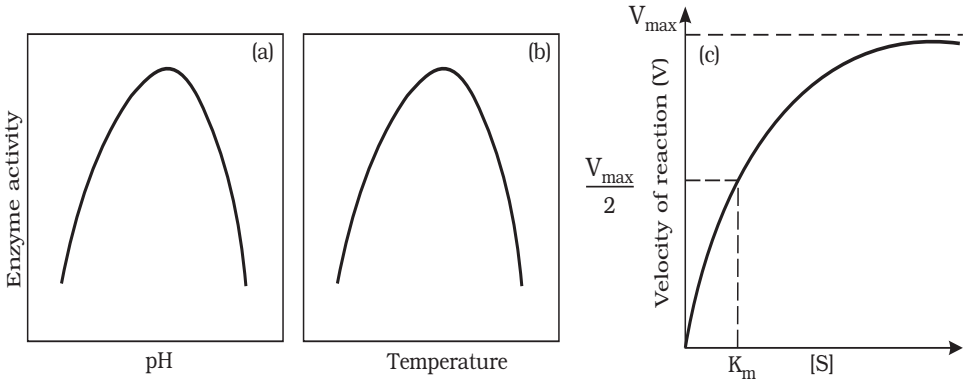


Figure 3.2 Effect of change in: (a) pH (b) Temperature and (c) Concentration of substrate on enzyme activity

below and above the optimum value. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature destroys enzymatic activity because proteins are denatured by heat.

Concentration of Substrate

With the increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches a maximum velocity (V_{max}) which is not exceeded by any further rise in concentration of the substrate. This is because the enzyme molecules are fewer than the substrate molecules and after saturation of these molecules, there are no free enzyme molecules to bind with the additional substrate molecules (Figure 3.2). Substrate concentration required to cause half the maximal reaction rate is termed as Michaelis-Menten constant (Km). Km values represent approximate inverse measures of the affinity of the enzyme for a given substrate.

The activity of an enzyme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off enzyme activity, the process is called **inhibition** and the chemical is called an **inhibitor**.

When the inhibitor closely resembles the substrate in its molecular structure and inhibits the activity of the enzyme, it is known as **competitive inhibitor**. Due to its close structural similarity with the substrate, the inhibitor competes with the substrate for the substrate-binding site of the enzyme. Consequently, the substrate cannot bind and, as a result, the enzyme action declines, e.g., inhibition of succinic dehydrogenase by malonate which closely resembles the substrate succinate in structure. Such competitive inhibitors are often used in the control of bacterial pathogens.

In non-competitive enzyme inhibition, the inhibitor has no structural similarity with the substrate and forms an enzyme inhibitor complex at a point other than its active site, so that the globular structure of the enzyme is changed. As a result catalysis cannot take place eg: Metal ions of copper, mercury, silver etc. In 'Feedback inhibition', the end product of a chain of enzyme catalysed reactions inhibits the enzyme of the first reaction as part of homeostatic control of metabolism.

3.5 Classification and Nomenclature of Enzymes

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. Enzymes are divided into 6 classes each with 4-13

subclasses and designated accordingly by a four-digit number.

Oxidoreductases/dehydrogenases: Enzymes which catalyse oxidoreduction between two substrates S and S' e.g.,

$$S \text{ reduced} + S' \text{ oxidised} \longrightarrow S \text{ oxidised} + S' \text{ reduced.}$$

E.g.: Malate + NAD^+ $\xrightarrow{\text{Malate dehydrogenase}}$ Oxaloacetate + NADH + H^+

Transferases: Enzymes catalysing a transfer of a group, G (other than hydrogen) between a pair of substrate S and S' e.g.,

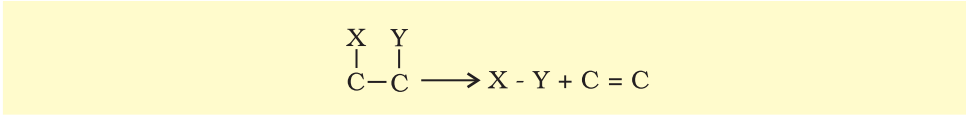
$$S - G + S' \longrightarrow S + S' - G$$

E.g.: Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-phosphate + ADP

Hydrolases: Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds.

E.g.: Fructose -1,6- bisphosphate + H_2O $\xrightarrow{\text{Fructose-1, 6-bisphosphotase}}$ Fructose - 6 - Phosphate + Inorganic phosphate

Lyases: Enzymes that catalyse removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.



E.g.: Arginosuccinic acid $\xrightarrow{\text{Arginosuccinase}}$ Arginine + Fumaric acid

Isomerases: Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers.

E.g.: Ribose - 5 - phosphate $\xrightarrow{\text{Ribose-5-phospho isomerase}}$ Ribulose - 5 - phosphate

Ligases: Enzymes catalysing the linking together of 2 compounds, e.g., enzymes which catalyse joining of C-O, C-S, C-N, P-O etc. bonds.

E.g.:Glutamic acid + NH_3 + ATP $\xrightarrow{\text{Glutamine synthetase}}$ Glutamine + ADP + ip

The above classification provides for a four digit code to identify individual enzymes. For instance, Glucose-6-phosphotransferase has the enzyme code (E.C.) 2.7.1.2. The first digit of the code indicates the major class of the

enzyme, while the second and the third digits indicate sub-class and sub-subclass respectively. The last digit of the code is the serial number of the enzyme in a particular sub-subclass.

3.6 Co-factors

Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called co-factors are bound to the enzyme to make the enzyme catalytically active. In these instances, the protein portion of the enzyme is called the apoenzyme. Three kinds of cofactors may be identified: prosthetic groups, co-enzymes and metal ions.

Prosthetic groups are organic compounds and are distinguished from other cofactors in that they are tightly bound to the apoenzyme. For example, in peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme.

Co-enzymes are also organic compounds but their association with the apoenzyme is only transient, usually occurring during the course of catalysis. Furthermore, co-enzymes serve as co-factors in a number of different enzyme catalyzed reactions. The essential chemical components of many coenzymes are vitamins, e.g., both coenzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin.

A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more cordination bonds with the substrate, e.g., zinc is a cofactor for the proteolytic enzyme carboxypeptidase.

Catalytic activity is lost when the co-factor is removed from the enzyme which testifies that co-factors play a crucial role in the catalytic activity of the enzyme.

SUMMARY

Enzymes are proteins which catalyse biochemical reactions in the cells. Ribozymes are nucleic acids with catalytic power. Proteinaceous enzymes exhibit substrate specificity, require optimum temperature and pH for maximal activity. They are denatured at high temperatures. Enzymes lower activation energy of reactions and enhance greatly the rate of the reactions.

GLOSSARY

Activation energy: Energy that is required for the substrate to get converted to product.

Active site: a cleft or pocket in the 3-D structure of a catalytic protein into which the substrate fits in

Apo enzyme: the protein part of a holoenzyme.

Co-enzyme: An organic co-factor that is loosly bound to the apoenzyme.

Co-factor: non-protein part of a holenzyme. It could be a metal ion or an organic compound.

Competitive inhibitor: An inhibitor of enzyme activity that is structurally similar to the substrate.

Enzyme: A protein with catalytic property

Enzyme code: A systematic code number, indicating scientific and specific enzyme activity as per IUB system. It is a four digit code.

Prosthetic group: An organic co-factor that is tightly bound to the apoenzyme.

Substrate: The substance which is converted to product in a enzyme catalysed reaction

Thermophiles: organisms that survive in hot water springs i.e. at high temperature.

QUESTIONS

Very Short Answer Type Questions

1. How are prosthetic groups different from co-factors?
2. What is meant by 'feed-back inhibition'?
3. Why are 'oxido reductases', so named?'
4. Distinguish between apoenzyme and cofactor.
5. What are competitive enzyme inhibitors? Mention one example.
6. What are non-competitive enzyme inhibitors? Mention one example.
7. What do the four digits of an enzyme code indicate?
8. Who proposed 'Lock and Key hypothesis' and Induced fit hypothesis?

Short Answer Type Questions

1. Explain how pH affects enzyme activity with the help of a graphical representation.
2. Explain the importance of [ES] complex formation.
3. Write briefly about enzyme inhibitors.
4. Explain different types of cofactors.

Long Answer Type Questions

1. Write an account of the classification of enzyme.
2. Explain the mechanism of enzyme action.

Excersises

1. Enumerate the properties of enzymes.
2. What is Michaelis constant?
3. Distinguish between feedback inhibition and allosteric inhibition.
4. What are isoenzymes?
5. What is turnover number? What is the fastest acting enzyme?

Chapter 4

Photosynthesis in Higher Plants

All animals including human beings depend on plants for their food. Have you ever wondered from where plants get their food? Green plants, in fact, have to make or rather synthesise the food they need and all other organisms depend on them for their needs. Green plants carry out 'photosynthesis', a physico-chemical process by which they use light energy to drive the synthesis of carbohydrates. Ultimately, all living forms on earth depend on sunlight for energy. The use of energy from sunlight by plants doing photosynthesis is the basis of life on earth. Photosynthesis is important due to two reasons: it is the primary source of all food on earth. It is also responsible for the release of oxygen into the atmosphere. *Have you ever thought what would happen if there were no oxygen to breath?* This chapter focusses on the structure of the photosynthetic machinery and the various reactions that transform light energy into chemical energy.

4.1 What do we Know?

Let us try to find out what we already know about photosynthesis. Some simple experiments you may have done in the earlier classes have shown that chlorophyll (green pigment of the leaf), light and CO_2 are required for photosynthesis to occur.

You may have carried out the experiment to look for starch formation in two leaves – a variegated leaf or a leaf that was partially covered with black paper, and one that was exposed to light. On testing these leaves for starch it was clear that photosynthesis occurred only in the green parts of the leaves in the presence of light.

Another experiment you may have carried out is the half-leaf experiment, in which a part of a leaf is enclosed in a test tube containing some KOH soaked cotton (which absorbs CO_2), while the other half is exposed to air. The setup is then placed in light for some time. On testing for starch later in the two halves of the leaf, you must have found that the exposed part of the leaf tested positive for starch while the portion that was in the tube, tested negative. This showed that CO_2 is required for photosynthesis. *Can you explain how this conclusion could be drawn?*

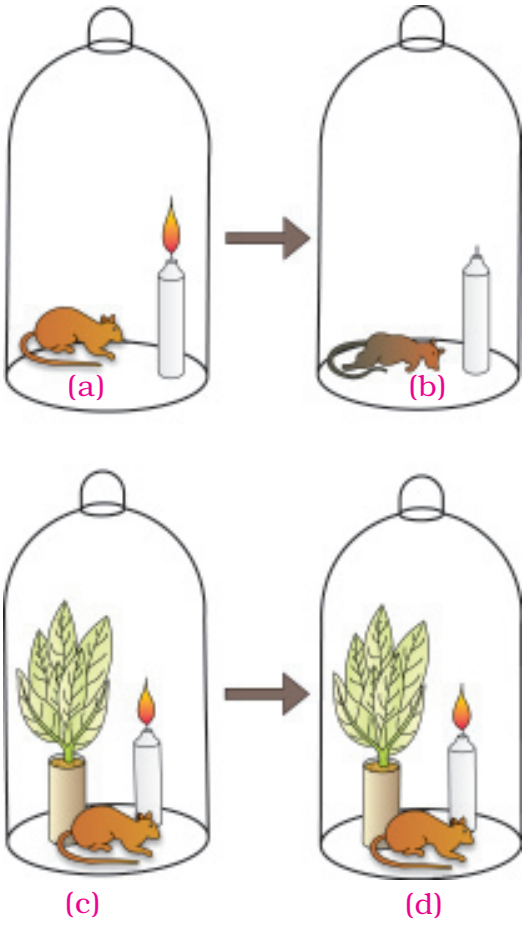


Figure 4.1 Priestley's experiment

4.2 Early Experiments

It is interesting to learn more about those simple experiments that led to a gradual development in our understanding of photosynthesis.

Joseph Priestley (1733-1804) in 1770 performed a series of experiments that revealed the essential role of air in the growth of green plants. Priestley, you may recall, discovered oxygen in 1774. Priestley observed that candle burning in a closed space – a bell jar soon gets extinguished (Figure 4.1 a, b, c, d). Similarly, a mouse would soon

suffocate in a closed space. He concluded that both the burning candle or the animal that breathe air, somehow, damage the air. But when he placed a mint plant in the same bell jar, he found that the mouse stayed alive and the candle continued to burn. Priestley hypothesised as follows: Plants restore to the air whatever breathing animals and burning candles remove.

Can you imagine how Priestley would have conducted the experiment using a candle and a plant? Remember, he would need to rekindle the candle to test whether it burns after a few days. *How many different ways can you think of lighting the candle without disturbing the set-up?*

Using a similar setup as the one used by Priestley, but by placing it once in the dark and once in the sunlight, Jan Ingenhousz (1730-1799) showed that sunlight is essential to the plant process that somehow purifies the air fouled by burning candles or breathing animals. Ingenhousz in an elegant experiment with an aquatic plant showed that in bright sunlight, small bubbles were formed around the green parts while in the dark they did not. Later he identified these bubbles to be those of oxygen. Hence he showed that it is only the green part of the plants that could release oxygen.

It was not until about 1854 that Julius von Sachs provided evidence for the production of glucose during the growth of plants. Glucose is usually stored as starch. Sach's later studies showed that the green substance in plants (chlorophyll as we know it now) is located in special bodies (later called chloroplasts) within plant cells. He found that the green parts in plants is where glucose is made, and that the glucose is usually stored as starch.

Now consider the interesting experiments done by T.W Engelmann (1843 – 1909). Using a prism Engelmann split light into its spectral components and then illuminated a green alga, *Cladophora*, placed in a suspension of aerobic bacteria. The bacteria were used to detect the sites of O_2 evolution. He observed that the bacteria accumulated mainly in the region of blue and red light of the split spectrum. A first action spectrum of photosynthesis was thus described. It resembles roughly the absorption spectra of chlorophyll *a* and *b* (discussed in section 4.4).

By the middle of the nineteenth century the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO_2 and water. The empirical equation representing the total process of photosynthesis for oxygen evolving organisms was then understood as:

$$CO_2 + H_2O \xrightarrow{\text{Light}} [CH_2O] + O_2$$

where [CH_2O] represented a carbohydrate (e.g., glucose, a six-carbon sugar).

A milestone contribution to the understanding of photosynthesis was that made by a microbiologist, Cornelius van Niel (1897-1985), who, based on his studies of purple and green bacteria, demonstrated that photosynthesis is essentially a light-dependent reaction in which hydrogen from a suitable oxidisable compound reduces carbon dioxide to carbohydrates. This can be expressed by:

$$2H_2A + CO_2 \xrightarrow{\text{Light}} 2A + CH_2O + H_2O$$

In green plants H_2O is the hydrogen donor and is oxidised to O_2. Some organisms do not release O_2 during photosynthesis. When H_2S is the hydrogen donor for purple and green sulphur bacteria, the 'oxidation' product is sulphur or sulphate depending on the organism, and not O_2. Hence, Niel inferred that the O_2 evolved by the green plant comes from H_2O, not from carbon dioxide. This was later proved by using isotopic techniques. The correct equation that would represent the overall process of photosynthesis, is therefore:

$$6CO_2 + 12H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

where $C_6\ H_{12}\ O_6$ represents glucose. The O_2 released is from water; this was proved using radio isotopic techniques (^{18}O, a heavy isotope). Note that this is not a single reaction but a description of a multistep process called photosynthesis. *Can you explain why twelve molecules of water as substrate are used in the equation given above?*

4.3 What is the site of Photosynthesis?

You would ofcourse answer: in 'the green leaf' or you may add, 'in the chloroplasts' based on what you earlier read in Chapter 9 of the First Year book. You are definitely right. Photosynthesis does take place in the green leaves of plants but it does so also in other green parts of the plants. *Can you name some other parts where you think photosynthesis may occur?*

You would recollect that the mesophyll cells in the leaves have a large number of chloroplasts. Usually the chloroplasts align themselves along the walls of the mesophyll cells such that they get the maximum quantity of the incident light. *When do you think the chloroplasts will be aligned with their flat surfaces parallel to the walls? When would they be perpendicular to the incident light?*

You have studied the structure of chloroplast earlier. Within the chloroplast there is the membranous system consisting of grana, the stroma lamellae,

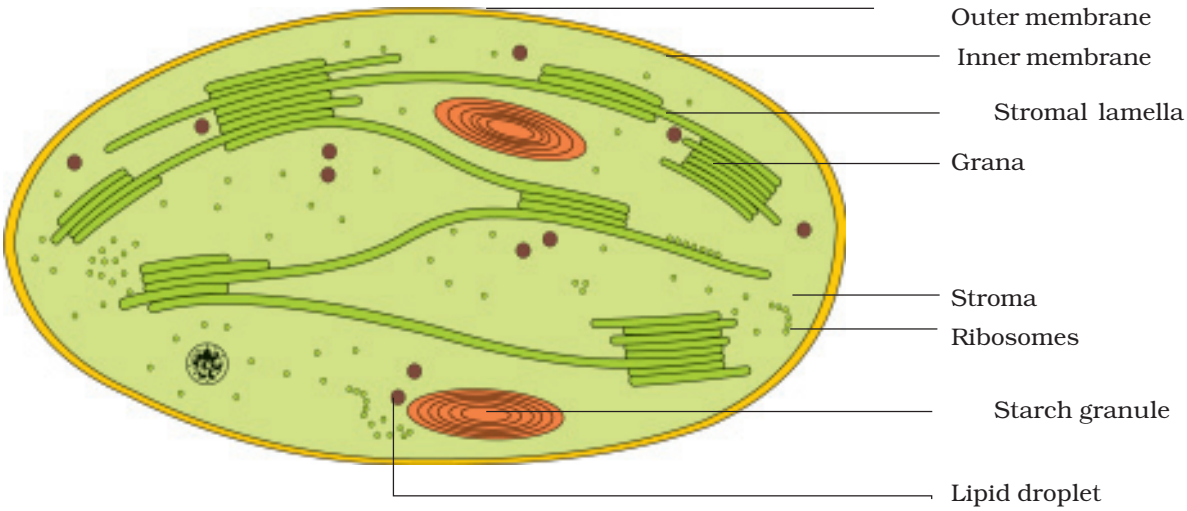


Figure 4.2 Diagrammatic representation of an electron micrograph of a section of chloroplast

and the fluid stroma (Figure 4.2). There is a clear division of labour within the chloroplast. The membrane system is responsible for trapping the light energy and also for the synthesis of ATP and NADPH. In stroma, enzymatic reactions incorporate CO_2 into the plant, leading to the synthesis of sugar, which in turn forms starch. The former set of reactions, since they are directly light driven, are called **light reactions.** The latter are not directly light driven but are dependent on the products of light reactions (ATP and NADPH) and are called, by convention, **dark reactions**. However, this should not be taken to mean that they occur in darkness or that they are not light-dependent.

4.4 How many Pigments are Involved in Photosynthesis?

Have you ever wondered why and how there are so many shades of green in leaves – even in the same plant? We can look for an answer to this question by trying to separate the leaf pigments of any green plant through paper chromatography. A chromatographic separation of the leaf pigments shows that the colour that we see in leaves is not due to a single pigment but due to four pigments: **Chlorophyll *a*** (bright or blue green in the chromatogram), **chlorophyll *b*** (yellow green), **xanthophylls** (yellow) and **carotenoids** (yellow to yellow-orange). Let us now see what roles the various pigments play in photosynthesis.

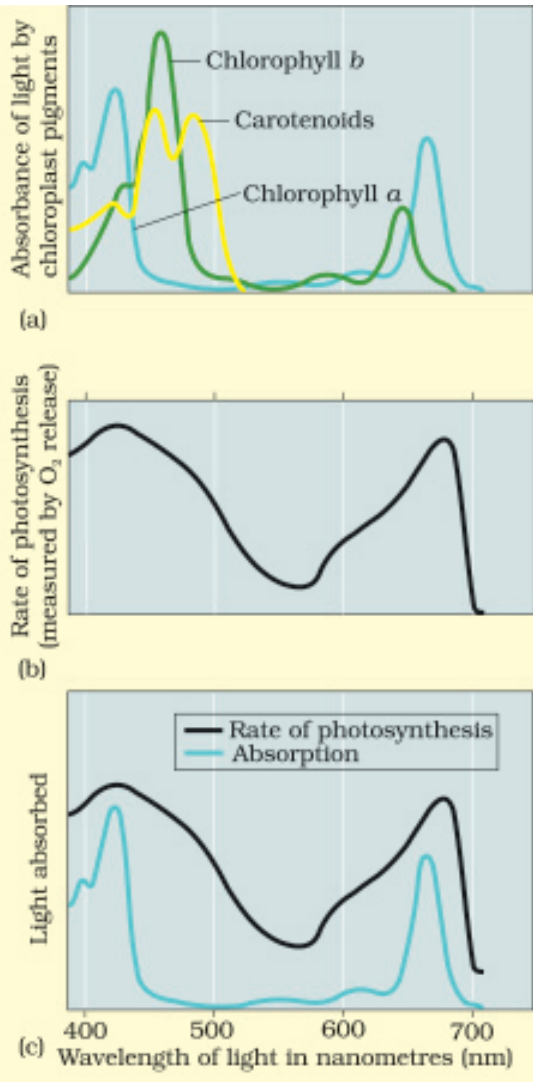


Figure 4.3a Graph showing the absorption spectrum of chlorophyll *a*, *b* and the carotenoids

Figure 4.3b Graph showing action spectrum of photosynthesis

Figure 4.3c Graph showing action spectrum of photosynthesis superimposed on absorption spectrum of chlorophyll *a*

Pigments are substances that have an ability to absorb light at specific wavelengths. *Can you guess which is the most abundant plant pigment in the world?* Let us study the graph showing the ability of chlorophyll *a* pigment to absorb lights of different wavelengths (Figure 4.3 a). Of course, you are familiar with the wavelength of the visible spectrum of light as well as the VIBGYOR.

From Figure 4.3a can you determine the wavelength (colour of light) at which chlorophyll a shows the maximum absorption? Does it show another absorption peak at any other wavelengths too? If yes, which one?

Now look at Figure 4.3b showing the wavelengths at which maximum photosynthesis occurs in a plant. You can see that the wavelengths at which there is maximum absorption by chlorophyll *a*, i.e., in the blue and the red regions, also shows higher rate of photosynthesis. Hence, we can conclude that chlorophyll *a* is the chief pigment associated with photosynthesis. *But by looking at Figure 4.3c can you say that there is a complete one-to-one overlap between the absorption spectrum of chlorophyll a and the action spectrum of photosynthesis?*

These graphs, together, show that most of the photosynthesis takes place in the blue and red regions of the spectrum; some photosynthesis does take place at the other wavelengths of the visible spectrum. Let us see how this happens. Though chlorophyll is the major pigment

responsible for trapping light, other thylakoid pigments like chlorophyll *b*, xanthophylls and carotenoids which are called **accessory pigments** also absorb light and transfer the energy to chlorophyll *a*. Indeed, they not only enable a wider range of wavelength of incoming light to be utilised for photosyntesis but also protect chlorophyll *a* from photo-oxidation.

4.5 Light Dependent Reactions

Light reactions or the 'Photochemical' phase include light absorption, water splitting, oxygen release, and the formation of high-energy chemical intermediates, ATP and NADPH. Several complexes are involved in the process. The pigments are organised into two discrete photochemical **light harvesting complexes (LHC)** within the **Photosystem I (PS I)** and **Photosystem II (PS II)**. These are named in the sequence of their discovery, The LHC are made up of hundreds of pigment molecules bound to proteins. Each photosystem has all the pigments forming a light harvesting system also called **antennae** (Figure 4.4). These pigments help to make photosynthesis more efficient by absorbing different wavelengths of light. A special chlorophyll *a* forms the **reaction centre.** The reaction centre is different in both the photosystems. In PS I, the reaction centre, chlorophyll *a*, has an absorption peak at 700 nm, hence is called **P700**, while in PS II, it has absorption maxima at 680 nm and is called **P680**.

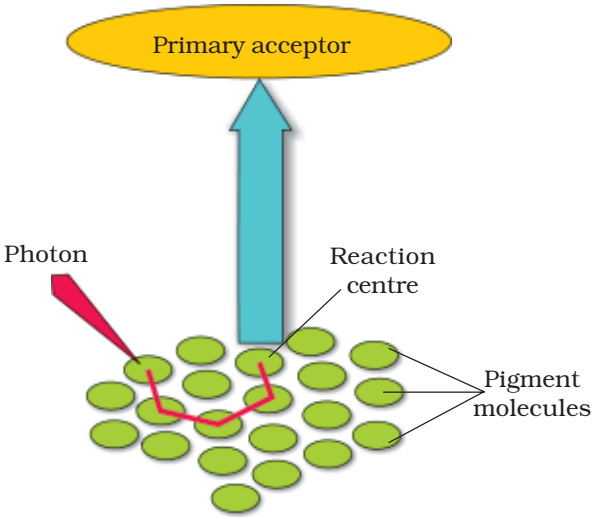


Figure 4.4 The light harvesting complex

4.6 The Electron Transport

In photosystem II the reaction centre chlorophyll *a* absorbs 680 nm wavelength of red light causing electrons to become excited and jump into an orbit farther from the atomic nucleus. These electrons are picked up by an electron acceptor which passes them to an **electron transport system consisting of cytochromes** (Figure 4.5). This movement of electrons from pheophytin to $P_{700}$is downhill, in terms of an oxidation-reduction or redox potential scale. The electrons are not used up as they pass through the electron transport chain, but are passed on to the pigments of photosystem PSI. Simultaneously, electrons in the reaction centre of PS I are also excited when they receive red

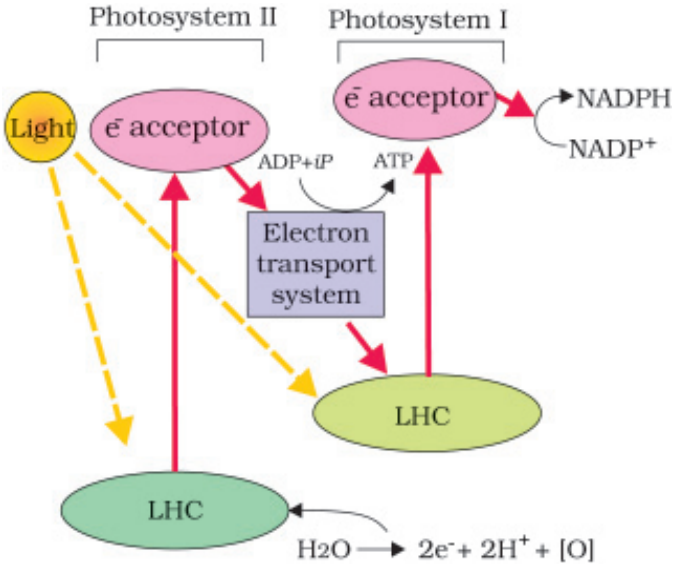


Figure 4.5 Z scheme of light reaction

light of wavelength 700 nm and are transferred to another accepter molecule that has a greater redox potential. These electrons then are moved downhill again, this time to a molecule of energy-rich $NADP^+$. The addition of these electrons reduces $NADP^+$ to NADPH + H^+. This whole scheme of transfer of electrons, starting from the PS II, uphill to the acceptor, down the electron transport chain to PS I, excitation of electrons, transfer to another acceptor, and finally downhill to $NADP^+$ causing it to be reduced to NADPH + H^+ is called the **Z scheme**, due to its characteristic shape (Figure 4.5). This shape is formed when all the carriers are placed in a sequence on a redox potential scale.

4.6.1 Splitting of Water

You would then ask, *How does PS II supply electrons continuously?* The electrons that were moved from photosystem II must be replaced. This is achieved by electrons available due to splitting of water. The splitting of water is associated with the PS II; water is split into protons, oxygen and electrons. This creates oxygen, one of the net products of photosynthesis. The electrons needed to replace those removed from photosystem I are provided by photosystem II.

$$2H_2O \longrightarrow 4H^+ + O_2 + 4e^-$$

We need to emphasise here that the water splitting complex (Oxygen Evolving Complex OEC) is associated with the PS II, which itself is physically located on the inner side of the membrane of the thylakoid. *Then, where are the protons and O_2 formed likely to be released – in the lumen? or on the outer side of the membrane?*

4.6.2 Cyclic and Non-cyclic Photo-phosphorylation

Living organisms have the capability of extracting energy from oxidisable substances and storing this in the form of bond energy. Special substances like ATP carry this energy in their chemical bonds. The process through which ATP is synthesised by cells (in mitochondria and chloroplasts) is named phosphorylation. Photo-phosphorylation is the synthesis of ATP from ADP and

inorganic phosphate in the presence of light. When the two photosystems work in a series, first PS II and then PS I, a process called non-cyclic photo-phosphorylation occurs. The two photosystems are connected through an electron transport chain, as seen in the Z scheme. Both ATP and NADPH+H$^+$ are synthesised by this kind of electron flow (Figure 4.5).

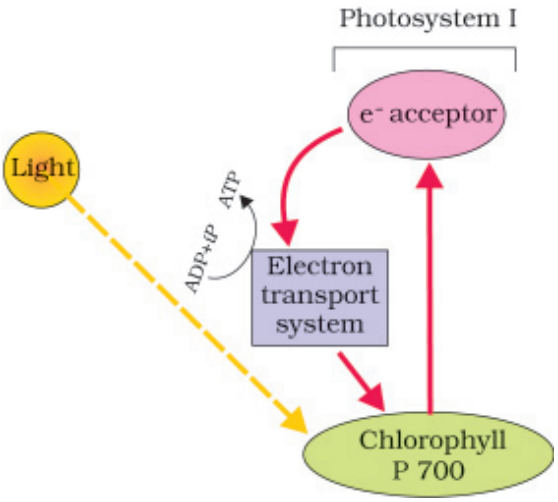


Figure 4.6 Cyclic photophosphorylation

When only PS I is functional, the electron is circulated within the photosystem and the phosphorylation occurs due to the cyclic flow of electrons (Figure 4.6). A possible location where this could be happening is in the stroma lamellae. While the membrane or lamellae of the grana have both PS I and PS II, the stroma lamellae membranes lack PS II as well as NADP reductase enzyme. The excited electron does not pass on to NADP$^+$ but is cycled back to the PS I complex through the

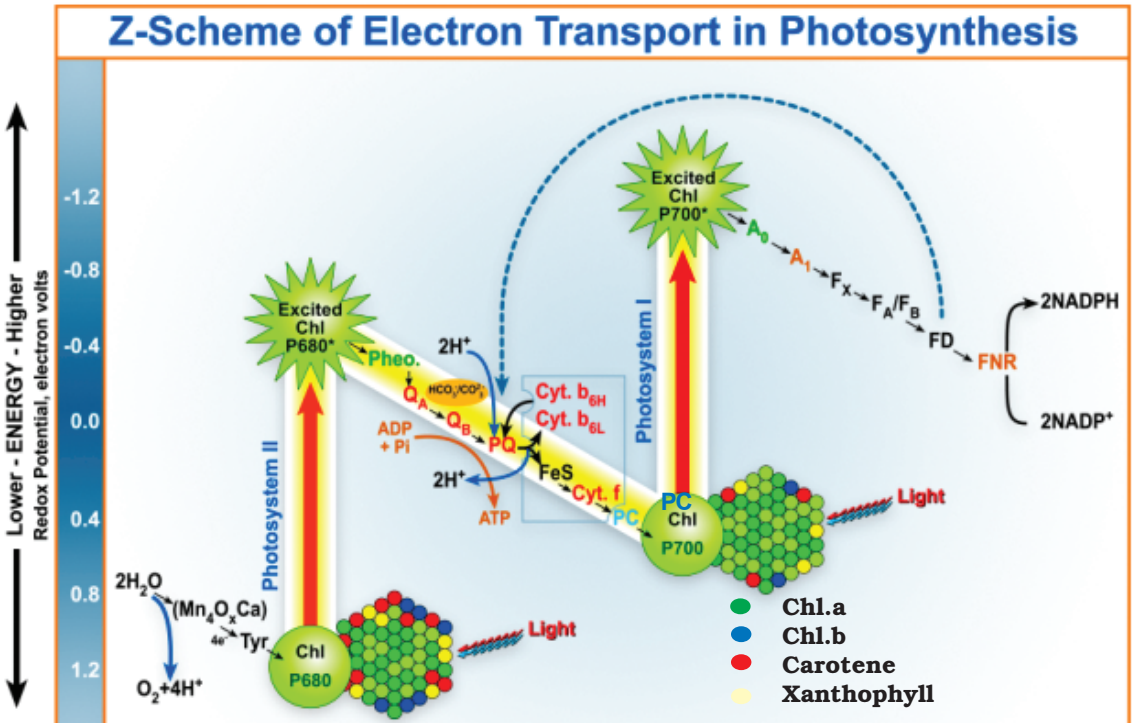


Figure 4.6a Modern scheme of photosynthetic electron transport

electron transport chain (Figure 4.6). The cyclic flow hence, results only in the synthesis of ATP, but not of NADPH+H^+. Cyclic photophosphorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

In green plants, cyclic photo-phosphorylation is an additional source of ATP required for chloroplast activities over and above that is required in the Calvin cycle.

A great deal of detail about photosynthetic electron transport has been worked out in the recent past. A modern scheme of photosynthentic electron transport is provided in Fig.4.6a

4.6.3 Chemiosmotic Hypothesis

Let us now try and understand how actually ATP is synthesised in the chloroplast. The chemiosmotic hypothesis has been put forward to explain the mechanism. Like in respiration, in photosynthesis too, ATP synthesis is linked to development of a proton gradient across a membrane. This time these are membranes of the thylakoid. There is one difference though. Here the proton accumulation is towards the inside of the membrane, i.e., in the lumen while in respiration, protons accumulate in the intermembrane space of the mitochondria when electrons move through the ETS (Chapter 5).

Let us understand what causes the proton gradient across the membrane. We need to consider again the processes that take place during the activation of electrons and their transport to determine the steps that cause a proton gradient to develop (Figure 4.7).

(a) Since splitting of the water molecule takes place on the inner side of the membrane, the protons or hydrogen ions that are produced by the splitting of water accumulate within the lumen of the thylakoids.

(b) As electrons move through the transport chain, protons are transported across the membrane. This happens because the primary accepter of electron which is located towards the outer side of the membrane transfers its electron, not to an electron carrier, but to a H carrier (PQ). Hence, it removes a proton from the stroma while transporting an electron. When this molecule passes on its electron to the electron carrier on the inner side of the membrane, the proton is released into the inner side or the lumen side of the membrane. Proton gradient across the membrane increases due to quinone cycle (cyclic flow of electrons between PQ and cytochrome b).

(c) The NADP reductase enzyme is located on the stroma side of the membrane. Along with electrons that come from the acceptor of electrons of PS I, protons are necessary for the reduction of $NADP^+$ to NADPH+ H^+. These protons are also removed from the stroma.

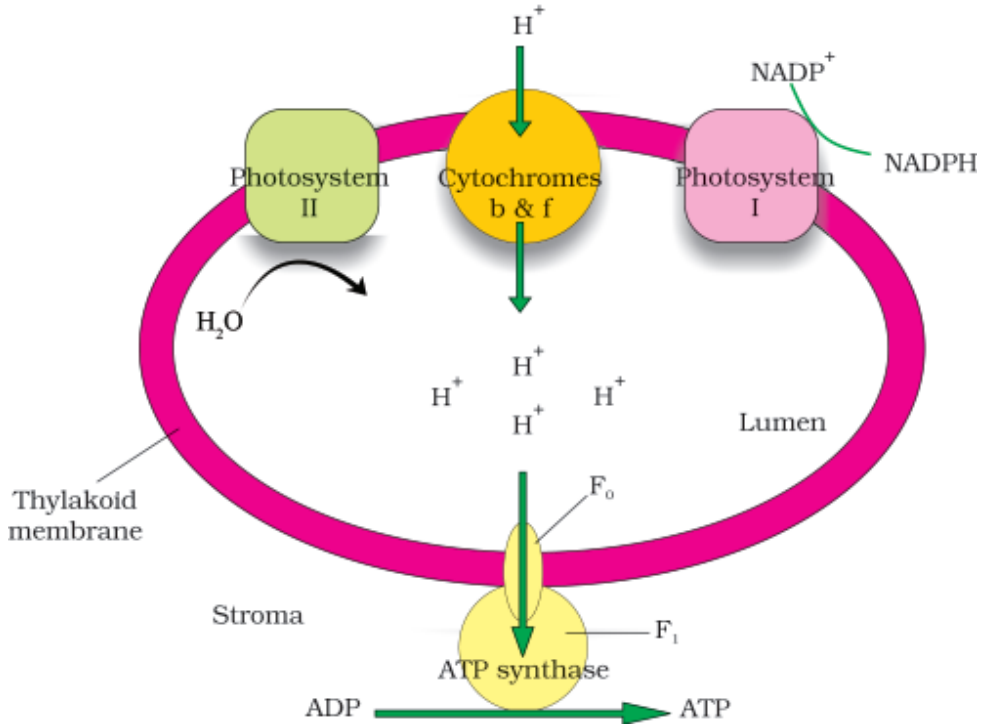


Figure 4.7 ATP synthesis through chemiosmosis

Hence, within the chloroplast, protons in the stroma decrease in number, while in the lumen, there is accumulation of protons. This creates a proton gradient across the thylakoid membrane as well as a measurable decrease in pH in the lumen.

Why are we so interested in the proton gradient? This gradient is important because it is the breakdown of this gradient that leads to release of energy. The gradient is broken down due to the movement of protons across the membrane to the stroma through the transmembrane channel of the F_0 of the ATPase. The ATPase enzyme consists of two parts: one called the F_0 is embedded in the membrane and forms a transmembrane channel that carries out facilitated diffusion of protons across the membrane. The other portion is called F_1 and protrudes on the outer surface of the thylakoid membrane on the side that faces the stroma. The break down of the gradient provides enough energy to cause a conformational change in the F_1 particle of the ATPase, which makes the enzyme synthesise several molecules of energy-packed ATP.

Chemiosmosis requires a membrane, a proton pump, a proton gradient and ATPase. Energy is used to pump protons across a membrane, to create a gradient or a high concentration of protons within the thylakoid lumen. ATPase

has a channel that allows diffusion of protons back across the membrane; this releases enough energy to activate ATPase enzyme that catalyses the formation of ATP.

Along with the NADPH produced by the movement of electrons, the ATP is used immediately in the biosynthetic reaction taking place in the stroma for fixing CO_2 and for the synthesis of sugars.

4.7 Where are the ATP and NADPH used?

We learnt that the products of light reaction are ATP, NADPH and O_2. Of these O_2 diffuses out of the chloroplast while ATP and NADPH are used to drive the processes leading to the synthesis of food, more accurately, sugars. This is the **biosynthetic phase** of photosynthesis. This process does not directly depend on the presence of light but is dependent on the products of the light reaction, i.e., ATP and NADPH, besides CO_2 and H_2O. Hence they are called Light Independent Reactions. You may wonder how this could be verified; it is simple: immediately after light becomes unavailable, the biosynthetic process continues for some time, and then stops. If then, light is made available, the synthesis starts again.

*Can we, hence, say that calling the biosynthetic phase the **dark reaction** is a misnomer? Discuss this amongst yourselves.*

Let us now see how the ATP and NADPH are used in the biosynthetic phase. We saw earlier that CO_2 is combined with H_2O to produce $(CH_2O)_n$ or sugars. It was of interest to scientists to find out how this reaction proceeded and to find out what is the first product formed when CO_2 is taken into a reaction or fixed. Just after World War II, among the several efforts to put radioisotopes to beneficial use, the work of Melvin Calvin is exemplary. The use of radioactive ^{14}C by him in algal photosynthesis studies led to the discovery that the first CO_2 fixation product was a 3-carbon organic acid. Calvin also contributed to working out the complete biosynthetic pathway; hence it was called **Calvin cycle**. The first product identified was **3-phosphoglyceric** acid or, in short, **PGA**. *How many carbon atoms does it have?*

Scientists also tried to know whether all plants have PGA as the first product of CO_2 fixation, or whether any other product was formed in other plants. Experiments conducted over a wide range of plants led to the discovery of another group of plants, where the first stable product of CO_2 fixation was again an organic acid, but one which had 4 carbon atoms in it. This acid was identified to be **oxaloacetic acid** or OAA. Since then CO_2 assimilation during photosynthesis is said to be of two main types: those plants in which the first

product of CO_2 fixation is a C_3 acid (PGA), i.e., the **C_3 pathway,** and those in which the first product is a C_4 acid (OAA), i.e., the **C_4 pathway**. These two groups of plants showed other associated characteristics that we will discuss later.

4.7.1 Light Independent Reactions

Let us now ask ourselves a question that was asked by the scientists who were struggling to understand the 'light independent reaction'. *How many carbon atoms would a molecule have which, after accepting (fixing) CO_2, would have 3 carbons (of PGA)?*

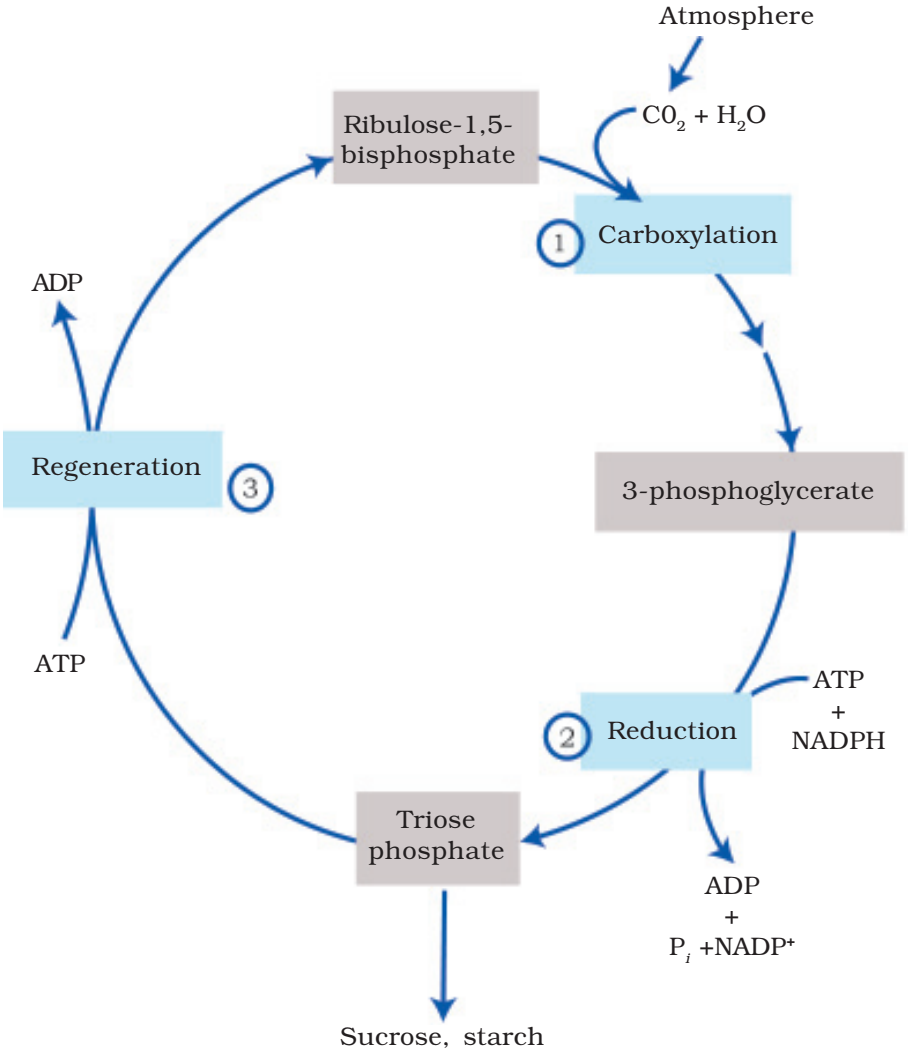


Figure 4.8 The Calvin cycle proceeds in three stages : (1) carboxylation, during which CO_2 combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the CO_2 acceptor ribulose-1,5-bisphosphate is formed again so that the cycle continues.

The studies very unexpectedly showed that the acceptor molecule was a 5-carbon ketose sugar – ribulose bisphosphate (RuBP). *Did any of you think of this possibility?* Do not worry; the scientists also took a long time and conducted many experiments to reach this conclusion. They also believed that since the first product was a C_3 acid, the primary acceptor would be a 2-carbon compound. They spent many years trying to identify a 2-carbon compound before they discovered the 5-carbon RuBP.

4.7.2 The Calvin Cycle

Calvin and his co-workers then worked out the whole pathway and showed that the pathway operated in a cyclic manner; the RuBP was regenerated. Let us now see how the Calvin pathway operates and where the sugar is synthesised. Let us at the outset understand very clearly that the Calvin pathway occurs in **all photosynthetic plants**; it does not matter whether they have C_3 or C_4 (or any other) pathways (Figure 4.8).

For ease of understanding, the Calvin cycle can be described in three stages: carboxylation, reduction and regeneration.

1. **Carboxylation**: Carboxylation is the fixation of CO_2 into a stable organic intermediate. Carboxylation is the most crucial step of the Calvin cycle where CO_2 is utilised for the carboxylation of RuBP. This reaction is catalysed by the enzyme RuBP carboxylase which results in the formation of two molecules of 3-PGA. Since this enzyme also has an oxygenation activity it would be more correct to call it RuBP carboxylase-oxygenase or **RuBisCO**.

   $$6RUBP + 6CO_2 + 6H_2O \xrightarrow{\text{RuBisCo}} 12 \text{ Phosphoglyceric acid}$$

2. **Reduction**: This is a two step reaction that leads to the formation of trioses (G-3-P). The steps involve utilisation of 2 molecules of ATP for phosphorylation and two of NADPH for reduction per CO_2 molecule fixed. The fixation of six molecules of CO_2 and 6 turns of the cycle are required for the removal (net gain) of two molecules of triose (= one glucose molecule) from the pathway.

a. $$12 \text{ Phosphoglyceric acid} + 12 \text{ ATP} \xrightarrow{\text{Phosphoglycerokinase}} 12 \text{ Bisphosphoglyceric acid} + 12 \text{ ADP}$$

b. $$12 \text{ Bisphosphoglyceric acid} + 12 \text{ NADPH} \xrightarrow{\text{G-3-P dehydrogenase}} 12 \text{ Glyceraldehyde - 3 - phosphate (G-3-P)} + 12 \text{ NADP}^+ + 12 \text{ } H_2PO_4^-$$

(of the 12 G-3-P, net gain is 2; 10 G-3-P are used to regenerate 6 RuBP)

3. Regeneration: Regeneration of the CO_2 acceptor molecule RuBP is crucial if the cycle is to continue uninterrupted. The regeneration steps require one ATP for phosphorylation to form RuBP.

G-3-P and DHAP (Dihydroxy acetone phosphate) are isomers and the interconversion is catalysed by triose phosphate isomerase.

a. 2G-3-P + 2DHAP $\xrightarrow{\text{Aldoase}}$ 2 Fructose -1,6- bisphosphate

b. 2 Fructose -1,6- bisphosphate $\xrightarrow{\text{Fructose-1, 6-bisphosphatase}}$ 2 Fructose–6 phosphate + 2Pi

c. 2 Fructose -6 phosphate + 2G-3-P $\xrightarrow{\text{Transketolase}}$ 2 Xylulose-5-phosphate(X-5-P) + 2 Erythrose - 4 - Phosphate (E-4-P)

d. 2 E-4-P + 2DHAP $\xrightarrow{\text{Aldolase}}$ 2 Sedoheptulose -1,7 - bisphosphate

e. 2 Sedoheptulose -1,7-bisphosphate $\xrightarrow{\text{Sedoheptulose-1, 7-bisphosphatase}}$ 2 Sedoheptulose -7-phosphate +2Pi

f. 2 Sedoheptulose-7-phosphate + 2 G-3-P $\xrightarrow{\text{Transketolase}}$ 2 Xylulose-5-P + 2 Ribose - 5- phosphate

g. 4 Xylulose-5-P $\xrightarrow{\text{Ribulose-5-phosphate epimerase}}$ 4 Ribulose-5-phosphate

h. 2 Ribose-5-P $\xrightarrow{\text{Ribose -5-phosphate isomerase}}$ 2 Ribulose-5-phospate

i. 6 Ribulose-5-phosphate + 6ATP $\xrightarrow{\text{Ribulose–5–phospho kinase}}$ 6 Ribulose-1, 5-bisphosphate + 6 ADP

Hence for every CO_2 molecule entering the Calvin cycle, 3 molecules of ATP and 2 of NADPH are required.

To make one molecule of glucose 6 turns of the cycle are required. *Work out how many ATP and NADPH molecules will be required to make one molecule of glucose through the Calvin pathway.*

It might help you to understand all of this if we look at what goes in and what comes out of the Calvin cycle.

In	Out
Six CO_2	Two trioses (One glucose)
18 ATP	18 ADP
12 NADPH	12 NADP

4.8 The C_4 Pathway

Plants that are adapted to dry tropical regions have the C_4 pathway mentioned earlier. Though these plants have the C_4 oxaloacetic acid as the first CO_2 fixation product they use the C_3 pathway or the Calvin cycle as the main biosynthetic pathway. Then, in what way are they different from C_3 plants? This is a question that you may reasonably ask.

C_4 plants are special: They have a special type of leaf anatomy, they tolerate higher temperatures, they show a response to high light intensities, they lack a process called photorespiration and have greater productivity of biomass. Let us understand these one by one.

Study vertical sections of leaves, one of a C_3 plant and the other of a C_4 plant. *Do you notice the differences? Do both have the same types of mesophylls? Do they have similar cells around the vascular bundle sheath?*

The particularly large cells around the vascular bundles of the C_4 pathway plants are called **bundle sheath cells**, and the leaves which have such anatomy are said to have **'Kranz' anatomy**. 'Kranz' means 'wreath' and is a reflection of the arrangement of cells. Bundle sheath cells are characterised by a large number of chloroplasts, thick walls impervious to gaseous exchange and no intercellular spaces. You may like to cut a section of the leaves of C_4 plants – maize or sorghum – to observe the Kranz anatomy and the distribution of mesophyll cells.

It would be interesting for you to collect leaves of diverse species of plants around you and take cross sections of the leaves. Observe under the microscope – look for the bundle sheath around the vascular bundles. The presence of the bundle sheath would help you identify the C_4 plants.

Now study the pathway shown in Figure 4.9. This pathway that has been named the Hatch and Slack Pathway, is again a cyclic process. Let us study the pathway by listing the steps.

The primary CO_2 acceptor is a 3-carbon molecule **phosphoenol pyruvate (PEP)** and is present in the mesophyll cells. The enzyme responsible for this fixation is **PEP carboxylase** (PEPcase). It is important to register that the mesophyll cells lack RuBisCO enzyme. C_4 acid OAA is formed in the mesophyll cells.

PEPcase, then forms other 4-carbon compounds like malic acid or aspartic acid in the mesophyll cells itself, which are transported to the bundle sheath cells. In the bundle sheath cells these C_4 acids are broken down to release CO_2 and a 3-carbon molecule.

The 3-carbon molecule is transported back to the mesophyll where it is converted to PEP again, thus, completing the cycle.

The CO_2 released in the bundle sheath cells enters the C_3 or the Calvin pathway, a pathway common to all plants. The bundle sheath cells are rich in an enzyme, Ribulose bisphosphate carboxylase-oxygenase **(RuBisCO)**, but lack PEPcase. Thus, the basic pathway that results in the formation of the sugars, the Calvin pathway, is common to C_3 and C_4 plants.

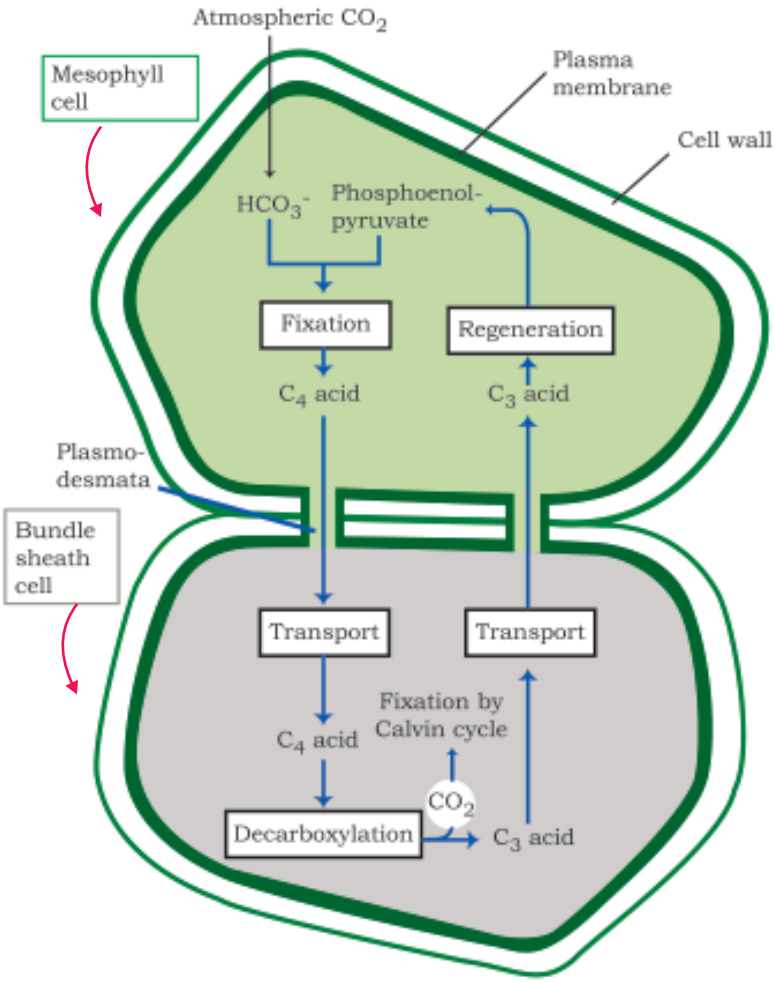


Figure 4.9 Diagrammatic representation of the Hatch and Slack Pathway

Did you note that the Calvin pathway occurs in all the mesophyll cells of the C_3 plants? In the C_4 plants it does not take place in the mesophyll cells but does so only in the bundle sheath cells.

Crassulacean Acid Metabolism (CAM) is an alternative to the C_3 and C_4 pathways of CO_2 fixation found in plants in dry and hot climates. eg. Cacti. Crassulaceae is one family of such plants.

CAM pathway is similar to C_4 pathway in that CO_2 is trapped by highly efficient PEP carboxylase. However, instead of having two kinds of photosynthetic cells, CAM plant has only one kind of photosynthetic cell in which CO_2 is fixed during night and used to make glucose during day, In C_4 plants, CO_2 fixation and Calvin cycle are separated in space, while in CAM plants they are sepearated in time.

4.9 Photorespiration

Let us try and understand one more process that creates an important difference between C_3 and C_4 plants – **Photorespiration**. To understand photorespiration we must know a little more about the first step of the Calvin pathway – the first CO_2 fixation step. This is the reaction where RuBP combines with CO_2 to form 2 molecules of 3PGA that are catalysed by RuBisCO.

$$RuBP + CO_2 \xrightarrow{RuBisCo} 2 \times 3PGA$$

RuBisCO is the most abundant enzyme in the world (Do you wonder why?). It is characterised by the fact that its active site can bind to both CO_2 and O_2 – hence the name. *Can you think how this could be possible?* RuBisCO has a much greater affinity for CO_2 than for O_2. Imagine what would happen if this were not so! This binding is competitive. It is the relative concentration of O_2 and CO_2 that determines which of the two will bind to the enzyme.

In C_3 plants some O_2 does bind to RuBisCO, and hence CO_2 fixation is decreased. Here the RuBP, instead of being converted to 2 molecules of PGA, binds with O_2 to form one molecule of phosphoglycerate and phosphoglycolate in a pathway called photorespiration. In the photorespiratory pathway, there is synthesis neither of sugars, nor of ATP. Rather there is a release of CO_2 with the utilisation of ATP. In the photorespiratory pathway there is no synthesis of ATP or NADPH. Therefore, photorespiration is a wasteful process.

In C_4 plants photorespiration does not occur. This is because they have a mechanism that increases the concentration of CO_2 at the enzyme site. This takes place when the C_4 acid from the mesophyll is broken down in the bundle sheath cells to release CO_2 – this results in increasing the intracellular concentration of CO_2. In turn, this ensures that the RuBisCO functions as a carboxylase, minimising the oxygenase activity.

Now that you know that the C_4 plants lack photorespiration, you probably can understand why productivity and yields are better in these plants. In addition these plants show tolerance to higher temperatures.

Based on the above discussion can you compare plants showing the C_3 and the C_4 pathway? Use the table format given and fill in the information.

TABLE 4.1 Fill in the Columns 2 and 3 in this table to highlight the differences between C_3 and C_4 Plants

Characteristics	C_3 Plants	C_4 Plants	Choose from
Cell type in which the Calvin cycle takes place			Mesophyll/Bundle sheath/both
Cell type in which the initial carboxylation reaction occurs			Mesophyll/Bundle sheath /both
How many photosynthetic cell types does the leaf have that fix CO_2.			Two: Bundle sheath and mesophyll One: Mesophyll Three: Bundle sheath, palisade, spongy mesophyll
Which is the primary CO_2 acceptor			RuBP/PEP/PGA
Number of carbons in the primary CO_2 acceptor			5 / 4 / 3
Which is the primary CO_2 fixation product			PGA/OAA/RuBP/PEP
No. of carbons in the primary CO_2 fixation product			3 / 4 / 5
Does the plant have RuBisCO?			Yes/No/Not always
Does the plant have PEP Case?			Yes/No/Not always
Which cells in the plant have Rubisco?			Mesophyll/Bundle sheath/none
CO_2 fixation rate under high light conditions			Low/ high/ medium
Whether photorespiration is present at low light intensities			High/negligible/sometimes
Whether photorespiration is present at high light intensities			High/negligible/sometimes
Whether photorespiration would be present at low CO_2 concentrations			High/negligible/sometimes
Whether photorespiration would be present at high CO_2 concentrations			High/negligible/sometimes
Temperature optimum			30-40 C/20-25C/above 40 C
Examples			Take cross sections of leaves of different plants and observe under the microscope for Kranz anatomy and list them in the appropriate columns.

4.10 Factors affecting Photosynthesis

An understanding of the factors that affect photosynthesis is necessary. The rate of photosynthesis is very important in determining the yield of plants including crop plants. Photosynthesis is influenced by several factors, both internal (plant) and external. The plant factors include the number, size, age and orientation of leaves, mesophyll cells and chloroplasts, internal CO_2 concentration and the amount of chlorophyll. The plant or internal factors are dependent on the genetic predisposition and the growth of the plant.

The external factors include the availability of sunlight, temperature, CO_2 concentration and water. All these factors simultaneously affect the rate of photosynthesis. Though several factors interact and simultaneously affect photosynthesis or CO_2 fixation, usually one factor is predominant or is the one that limits the rate. Hence, at any point the rate of photosynthesis is determined by the factor available at sub-optimal levels.

When several factors affect any [bio] chemical process, Blackman's (1905) **Law of Limiting Factors** comes into effect. This states the following:

"If a process (like photosynthesis) is conditioned as to its rapidity by a number of seperate factors, the rate of the process is limited by the factor that is present in a relative minimal value."

For example, despite the presence of a green leaf and optimal light and CO_2 conditions, a plant may not photosynthesise if the temperature is very low. But if given the optimal temperature, the leaf will start photosynthesising.

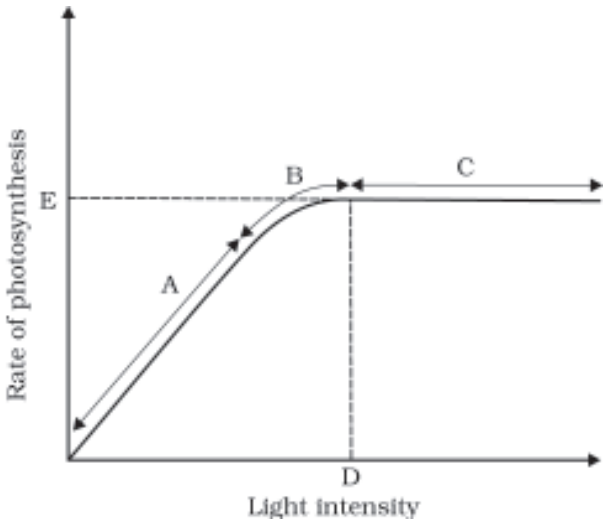


Figure 4.10 Graph showing effect of light intensity on the rate of photosynthesis

4.10.1 Light

We need to distinguish between light quality, light intensity and the duration of exposure to light while discussing light as a factor that affects photosynthesis. There is a linear relationship between incident light and CO_2 fixation rates at low light intensities. At higher light intensities, gradually the rate does not show further increase as other factors become limiting (Figure 4.10). What is interesting to note is that light saturation occurs at 10 per

cent of the full sunlight. Hence, except for plants in shade or in dense forests, light is rarely a limiting factor in nature. Increase in incident light beyond a point causes the breakdown of chlorophyll and a decrease in photosynthesis.

4.10.2 Carbon dioxide Concentration

Carbon dioxide is the major limiting factor for photosynthesis. The concentration of CO_2 is very low in the atmosphere (between 0.03 and 0.04 per cent). Increase in concentration upto 0.05 per cent can cause an increase in CO_2 fixation rates; beyond this the levels can become damaging over longer periods.

C_3 and C_4 plants respond differently to CO_2 concentrations. At low light conditions neither group responds to high CO_2 conditions. At high light intensities, both C_3 and C_4 plants show increase in the rates of photosynthesis. What is important to note is that C_4 plants show saturation at about 360 $\mu l L^{-1}$ while C_3 plants respond to increased CO_2 concentration and saturation is seen only beyond 450 $\mu l L^{-1}$. Thus, current availability of CO_2 levels is limiting photosynthesis in the C_3 plants.

The fact that C_3 plants respond to higher CO_2 concentration by showing increased rates of photosynthesis leading to higher productivity has been used for some greenhouse crops such as tomatoes and bell pepper. These crops are allowed to grow in carbon dioxide enriched atmosphere that leads to higher yields.

4.10.3 Temperature

The dark reactions, being enzymatic, are temperature controlled. Though the light reactions are also temperature sensitive they are affected to a much lesser extent. C_4 plants respond to higher temperatures and show higher rate of photosynthesis while C_3 plants have a much lower temperature optimum. The temperature optimum for photosynthesis of different plants also depends on the habitat that they are adapted to. Tropical plants have a higher temperature optimum than the plants adapted to temperate climates.

4.10.4 Water

Even though water is one of the reactants in the light reaction, the effect of water as a factor is more through its effect on the plant, rather than directly on photosynthesis. Water stress causes the stomata to close, thereby reducing the CO_2 availability. Besides, water stress also makes leaves wilt, thus, reducing the surface area of the leaves and their metabolic activity as well.

SUMMARY

SUMMARY

Green plants make their own food by photosynthesis. During this process carbon dioxide from the atmosphere is taken in by leaves through stomata and used for making carbohydrates, principally sucrose and starch. Photosynthesis takes place only in the green parts of the plants, mainly the leaves. Within the leaves, the mesophyll cells have a large number of chloroplasts that are responsible for CO_2 fixation. Within the chloroplasts, the membranes are sites for the light reaction, while the chemosynthetic pathway occurs in the stroma. Photosynthesis has two stages: the light reaction and the carbon fixing reactions. In the light reaction the light energy is absorbed by the pigments present in the antenna and funnelled to special chlorophyll *a* molecules called reaction centre chlorophylls. There are two photosystems, PS I and PS II. PS I has a 700 nm absorbing chlorophyll *a* P700 molecule at its reaction centre, while PS II has a P680 reaction centre that absorbs red light at 680 nm. After absorbing light, chlorophylls are excited and electrons are transferred through PS II and PS I and finally to NAD forming NADH. During this process a proton gradient is created across the membrane of the thylakoid. The breakdown of the proton gradient due to movement through the F_0 part of the ATPase enzyme releases enough energy for synthesis of ATP. Splitting of water molecules is associated with PS II resulting in the release of O_2, protons and transfer of electrons to PS II.

In the carbon fixation cycle, CO_2 is added by the enzyme, RuBisCO, to a 5-carbon compound RuBP that is converted to 2 molecules of 3-carbon PGA. This is then converted to sugar by the Calvin cycle, and the RuBP is regenerated. During this process ATP and NADPH synthesised in the light reaction are utilised. RuBisCO also catalyses a wasteful oxygenation reaction in C_3 plants: photorespiration.

Some tropical plants show a special type of photosynthesis called C_4 pathway. In these plants the first product of CO_2 fixation that takes place in the mesophyll is a 4-carbon compound. In the bundle sheath cells the Calvin pathway is carried out for the synthesis of carbohydrates.

GLOSSARY

Absorption spectrum: Graph showing light absorption by photosynthetic pigments as a function of wavelength of light.

Action spectrum: Graph showing rate of photosynthesis as a function of wavelength of light.

Assimillatory power: Chemical energy generated as a result of light reaction. It is in the form of ATP, NADPH. Also known as reducing power.

CAM: Crassulacean Acid Metabolism, an alternative pathway of CO_2 fixation found in plants living in dry and hot climates eg: Cacti.

Chemiosmosis: Proposed by Mitchell, explains ATP generation from ADP and iP driven by proton gradient across membrane in mitochondria and chloroplasts.

Dark reaction: Phase of photosynthesis not directly light driven, but dependent on the products of light reaction. Now more appropriately known as biosynthetic phase.

Kranz anatomy: Typical of C_4 leaf with particularly large cells around vascular bundles i.e. wreath like bundle sheath.

Law of limiting factors: In a process participated by a number of seperate factors, the rate of the process is limitted by the factor which is present in minimal value.

Light harvesting complex (LHC): A group of molecules, associated with reaction centre of a photosystem, collecting light energy to be transferred to the reaction centre eventually.

Light reaction: Phase of photosynthesis driven directly by light.

Photolysis of H_2O: Splitting of water by photosystem II in the presence of light.

Photorespiration: Light dependent release of CO_2 and uptake of O_2 by the green tissue of particularly C_3 plants.

Reaction centre: Special chlorophyll which routinely undergoes photooxidation, designated as P_{700} in photosystem I and P_{680} in photosystem II.

Redox potential: The tendency of a molecule or atom to give or take up electrons.

RUBISCO: Enzyme that catalyses addition of CO_2 to RUBP. The most abundent protein on earth.

Z-Scheme: Schematic representation of photosynthetic electron transport from H_2O to $NADP^+$, in line with redoxpotential gradient. First proposed by Hill and Bendall.

QUESTIONS

Very Short Answer Type Questions

1. Name the processes which take place in the grana and stroma regions of chloroplasts.
2. Can chloroplasts be passed on to progeny? How?
3. Where does the photolysis of H_2O occur? What is its significance?
4. Where is the enzyme NADP reductase located? What is released if the proton gradient breaksdown?
5. Which tissue transports photosynthates? What experiments prove this?
6. How many molecules of ATP and NADPH are needed to fix a molecule of CO_2 in C_3 plants? Where does this process occur?
7. Explain the terms:
   (a) Hatch-Slack pathway
   (b) Calvin cycle
   (c) PEP carboxylase
   (d) Bundle sheath cells
8. What is the role of NADP reductase in the development of proton gradient?
9. Mention the components of ATPase enzyme. What is their location? Which part of the enzyme shows conformational change?
10. What products drive Calvin Cycle? What process regenerates them?
11. What is the basis for designating C_3 and C_4 pathway of photosynthesis?
12. Distinghish between action spectrum and absorption spectrum.
13. Of the basic raw materials of photosynthesis, what is reduced? What is oxidised?
14. Define the law of limitting factors proposed by Blackman.
15. What is Joseph Priestley's contribution to the study of photosynthesis?
16. Comment on the contribution of Van Niel to the understanding of photosynthesis.
17. With reference to photosystem, bring out the meaning of the terms (a) antennae (b) reaction centre.
18. Why is photosynthetic electron transport from H_2O to $NADP^+$, named as Z-scheme?
19. What is the primary acceptor of CO_2 in C_3 plants? What is first stable compound formed in a Calvin cycle?
20. What is the primary acceptor of CO_2 in C_4 plants? What is the first compound formed as a result of primary carboxylation in the C_4 pathway?

Short Answer Type Questions

1. Succulents are known to keep their stomata closed during the day to check transpiration. How do they meet their photosynthetic CO_2 requirements?
2. Cholorophyll 'a' is the primary pigment for light reaction. What are accessory pigments? What is their role in photosynthesis?
3. Does 'dark reaction' of photosynthesis require light? Explain.
4. How are photosynthesis and respiration related to each other?
5. What conditons enable 'RUBISCO' to function as oxygenase? Explain the ensuing process.
6. Why does the rate of photosynthesis decrease at higher temperatures?
7. Explain how, during light reaction of photosynthesis, ATP synthesis is a chemiosmotic phenomenon.
8. Explain how Calvin worked out the complete biosynthetic pathway for the synthesis of sugar.
9. Six turns of calvin cycle are required to generate one mole of glucose. Explain,
10. With the help of diagram, explain briefly the process of cyclic photosphorylation.
11. In what type of plants do you come across 'kranz' anatomy? To which conditons are those plants better adapted? How are these plants better adapted than the plants, which lack this anatomy?
12. Explain the structure of the chloroplast? Draw a neat labelled diagram.
13. Explain why `12 molecules of water are used as substrate, instead of 6 molecules of water, in the folliwng equation.

$$6CO_2 + 12H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

14. Compare and contrast the absorption spectrum of chlorophylls and carotenoids.
15. Which group of plants exhibits two types of photosynthetic cells? What is the first product of carboxylation? What carboxylating enzyme is present in bundle sheath cells and mesophyll cells?
16. A cyclic process is occuring in a C_3 plant, which is light dependent and needs O_2. This process does not produce energy rather it consumes energy.
    (a) Can you name the given process?
    (b) Is it essential for survival?
    (c) What are the end products of this process?
    (d) Where does it occur?

17. Suppose Euphorbia and Maize are grown in the tropical area.
    (a) Which one of them do you think will be able to survive under such conditions?
    (b) Which one of them is more efficient interms of photosynthetic activity?
    (c) What differences do you think are there in the leaf?

Long Answer Type Questions

1. The entire process of photosynthesis consists of a number of reactions. Where in the cell do each of these take place?
    (a) Synthesis of ATP and NADPH.
    (b) Photolysis of water.
    (c) Fixation of CO_2
    (d) Synthesis of sugar molecule
    (e) Synthesis of starch.
2. Which property of pigments is responsible for its ability to initiate the process of photosynthesis? Why is the rate of photosynthesis higher in red and blue regions of the spectrum of light?
3. Under what conditions are C_4 plants superior to C_3?
4. (a) How can we plot an action spectrum? What does the action spectrum indicate? Explain with an example.
    (b) How can we derive an absorption spectrum for any substance?
    (c) If chloropyll 'a' is responsible for light reaction of photosynthesis, why do the action spectrum and absorption spectrum not overlap?
5. What are the important events and end products of light reactions?
6. Explain various aspects of Mitchell chemiosmotic hypothesis, with the help of diagrams.
7. Comment on the dual role of RUBISCO. What is the basis for its oxygenation activity? Why is this activity absent or negligible in C_4 plants?

Exercises

1. By looking at a plant externally, can you tell whether a plant is C_3 or C_4? Why and how?
2. By looking at which internal structure of a plant can you tell whether a plant is C_3 or C_4? Explain.
3. Even though a very few cells in a C_4 plant carry out the biosynthetic – Calvin pathway, yet they are highly productive. Can you discuss why?

4. RuBisCO is an enzyme that acts both as a carboxylase and oxygenase. Why do you think RuBisCO carries out more carboxylation in C_4 plants?
5. Suppose there were plants that had a high concentration of Chlorophyll *b*, but lacked chlorophyll *a*, would it carry out photosynthesis? Then why do plants have chlorophyll *b* and other accessory pigments?
6. Why is the colour of a leaf kept in the dark frequently yellow, or pale green? Which pigment do you think is more stable?
7. Look at leaves of the same plant on the shady side and compare it with the leaves on the sunny side. Or, compare the potted plants kept in the sunlight with those in the shade. Which of them has leaves that are darker green ? Why?
8. Figure 4.10 shows the effect of light on the rate of photosynthesis. Based on the graph, answer the following questions:
   (a) At which point/s (A, B or C) in the curve is light a limiting factor?
   (b) What could be the limiting factor/s in region A?
   (c) What do C and D represent on the curve?
9. Give comparison between the following:
   (a) C_3 and C_4 pathways
   (b) Cyclic and non-cyclic photophosphorylation
   (c) Anatomy of leaf in C_3 and C_4 plants
10. Cyanobacteria and some other photosynthetic bacteria do not have chloroplasts. How do they conduct photosynthesis?
11. Does moonlight support photosynthesis?
12. Why photorespiration does not occur in C_4 plants?
13. Tamatoes, chillis and carrots are red in colour due to the presence of one pigment. Name the pigment. Is it a photosynthetic pigment?
14. If a green plant is kept in dark with proper ventilation, can this plant carry out photosynthesis? Can anything be given as supplement to maintain its growth or survival?
15. Photosynthetic organisms occur at different depths in the ocean. Do they receive qualitatively and quantitatively the same light? How do they adapt to carry out photosynthesis under these conditions?
16. In tropical rain forests, the canopy is thick and shorter plants growing below it, receive filtered light. How are they able to carry out photosynthesis?

17. Why do you believe chloroplast and mitochondria to be semi-autonomous organelle.
18. Is it correct to say that photosynthesis occurs only in the leaves of a plant? Besides leaves, what are the other parts that may be capable of carrying out photosynthesis? Justify.
19. What can we conclude from the statement that the action and absorption spectrum of photosynthesis overlap? At which wavelength do they show peaks?

Chapter 5

Respiration in Plants

All of us breath to live, but why is breathing so essential to life? What happens when we breathe? Also, do all living organisms, including plants and microbes, breathe? If so, how?

All living organisms need energy for carrying out daily life activities, be it absorption, transport, movement, reproduction or even breathing. Where does all this energy come from? We know we eat food for energy – but how is energy taken from food? How is this energy utilised? Do all foods give the same amount of energy? Do plants 'eat'? Where do plants get their energy from? And do micro-organisms too eat 'food'?

You may wonder at the several questions raised above – they may seem to be disconnected. But in reality, the process of breathing is very much connected to the process of release of energy from food. Let us try and understand how this happens.

All the energy required for 'life' processes is obtained by oxidation of some macromolecules that we call 'food'. Only green plants and cyanobacteria can prepare their own food; by the process of photosynthesis they trap light energy and convert it into chemical energy that is stored in the bonds of carbohydrates

like glucose, sucrose and starch. We must remember that in green plants too, not all cells, tissues and organs photosynthesise; only cells containing chloroplasts, that are most often located in the superficial layers, carry out photosynthesis. Hence, even in green plants all other organs, tissues and cells that are non-green, need food for oxidation. Hence, food has to be translocated to all non-green parts. Animals are heterotrophic, i.e., they obtain food from plants directly (herbivores) or indirectly (carnivores). Saprophytes like fungi are dependent on dead and decaying matter. What is important is that ultimately all the food that is respired for life processes comes from photosynthesis. This chapter deals with **cellular respiration** or the mechanism of breakdown of food materials within the cell to release energy, and the trapping of this energy for synthesis of ATP.

Photosynthesis, of course, takes place within the chloroplasts (in the eukaryotes), whereas the breakdown of complex molecules to yield energy takes place in the cytoplasm and in the mitochondria (also only in eukaryotes). The breaking of the C-C bonds of complex compounds through oxidation within the cells, leading to release of considerable amount of energy, is called **respiration**. The compounds that are oxidised during this process are known as **respiratory substrates.** Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substances in some plants, under certain conditions. During oxidation within a cell, all the energy contained in respiratory substrates is not released free into the cell, or in a single step. It is released in a series of slow step-wise reactions controlled by enzymes, and it is trapped as chemical energy in the form of ATP. Hence, it is important to understand that the energy released by oxidation in respiration is not (or rather cannot be) used directly but is used to synthesise ATP, which is broken down whenever (and wherever) energy needs to be utilised. Hence, ATP acts as the energy currency of the cell. This energy trapped in ATP is utilised in various energy-requiring processes of the organisms, and the carbon skeleton produced during respiration is used as a precursor for biosynthesis of other molecules in the cell.

5.1 Do Plants Breathe?

Well, the answer to this question is not quite so direct. Yes, plants require O_2 for respiration to occur and they also give out CO_2. Hence, plants have systems in place that ensure the availability of O_2. Plants, unlike animals, have no specialised organs for gaseous exchange but they have stomata and lenticels for this purpose. There are several reasons why plants can get along without respiratory organs. First, each plant part takes care of its own gas-exchange

needs. There is very little transport of gases from one plant part to another. Second, plants do not present great demands for gas exchange. Roots, stems and leaves respire at rates far lower than animals do. Only during photosynthesis are large volumes of gases exchanged and, each leaf is well adapted to take care of its own needs during these periods. When cells photosynthesise, availability of O_2 is not a problem in these cells since O_2 is released within the cell. Third, the distance that gases must diffuse even in large, bulky plants is not great. Each living cell in a plant is located quite close to the surface of the plant. 'This is true for leaves', you may ask, 'but what about thick, woody stems and roots?' In stems, the 'living' cells are organised in thin layers inside and beneath the bark. They also have openings called lenticels. The cells in the interior are dead and provide only mechanical support. Thus, most cells of a plant have at least a part of their surface in contact with air. This is also facilitated by the loose packing of parenchyma cells in leaves, stems and roots, which provide an interconnected network of air spaces.

The complete combustion of glucose, which produces CO_2 and H_2O as end products, yields energy most of which is given out as heat.

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

If this energy is to be useful to the cell, it should be able to utilise it to synthesise other molecules that the cell requires. The strategy that the plant cell uses is to catabolise the glucose molecule in such a way that not all the liberated energy goes out as heat. The key is to oxidise glucose not in one step but in several small steps enabling some steps to be just large enough such that the energy released can be coupled to ATP synthesis. How this is done is, essentially, the story of respiration.

During the process of respiration, oxygen is utilised, and carbon dioxide, water and energy are released as products. The combustion reaction requires oxygen. But some cells live where oxygen may or may not be available. *Can you think of such situations (and organisms) where O_2 is not available?* There are sufficient reasons to believe that the first cells on this planet lived in an atmosphere that lacked oxygen. Even among present-day living organisms, we know of several that are adapted to anaerobic conditions. Some of these organisms are facultative anaerobes, while in others the requirement for anaerobic condition is obligate. In any case, all living organisms retain the enzymatic machinery to partially oxidise glucose without the help of oxygen. This breakdown of glucose to pyruvic acid is called **glycolysis**.

5.2 Glycolysis

The term glycolysis has originated from the Greek words, *glycos* for sugar, and *lysis* for splitting. The scheme of glycolysis was given by Gustav Embden, Otto Meyerhof, and J. Parnas, and is often referred to as the EMP pathway. In anaerobic organisms, it is the only process in respiration. Glycolysis occurs in the cytoplasm of the cell and takes place in all living organisms. In this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid. In plants, this glucose is derived from sucrose, which is the end product of photosynthesis, or from storage carbohydrates. Sucrose is converted into glucose and fructose by the enzyme, invertase, and these two monosaccharides readily enter the glycolytic pathway. Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate by the activity of the enzyme hexokinase. This phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Subsequent steps of metabolism of glucose and fructose are the same. The various steps of glycolysis are depicted in Figure 5.1. In glycolysis, a chain of ten reactions, under the control of different enzymes, takes place to produce pyruvate from glucose. While studying the steps of glycolysis, please note the steps at which utilisation or synthesis of ATP or (in this case) NADH + H^+ take place.

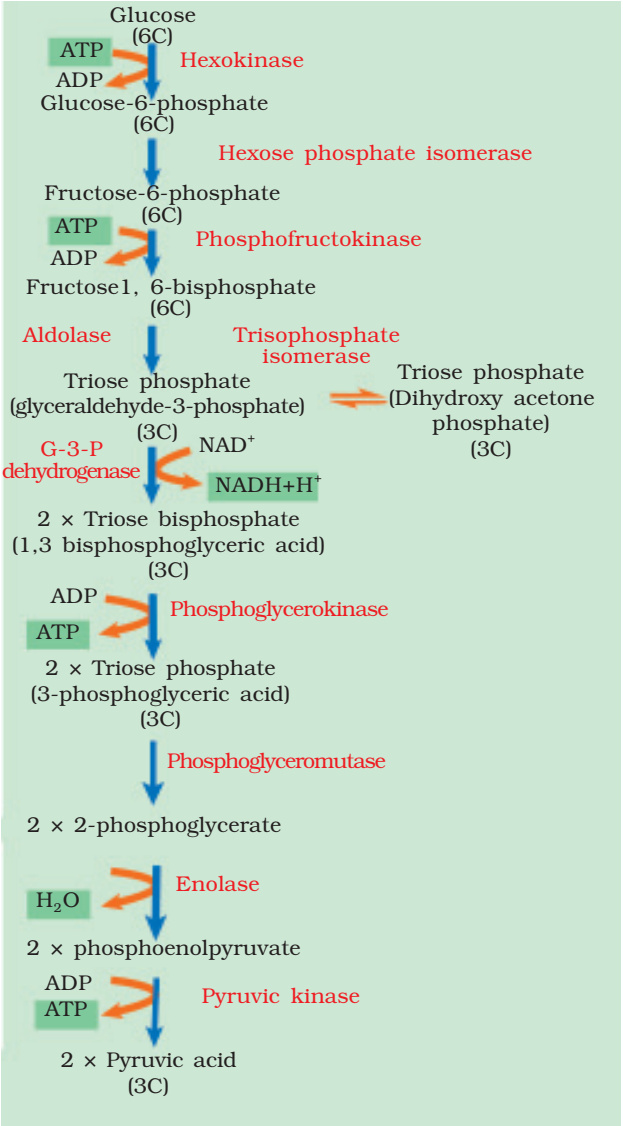


Figure 5.1 Steps of glycolysis

ATP is utilised at two steps: first in the conversion of glucose into glucose 6-phosphate and second in the conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate.

The fructose 1, 6-bisphosphate is split into dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (PGAL). We find that there is one step where NADH + H^+ is formed from NAD^+; this is when 3-

phosphoglyceraldehyde (PGAL) is converted to 1, 3-bisphosphoglycerate (BPGA). Two redox-equivalents are removed (in the form of two hydrogen atoms) from PGAL and transferred to a molecule of NAD^+. PGAL is oxidised and with inorganic phosphate to get converted into BPGA. The conversion of BPGA to -phosphoglyceric acid (PGA), is also an energy yielding process; this energy is trapped by the formation of ATP. Another ATP is synthesised during the conversion of PEP to pyruvic acid. *Can you then calculate how many ATP molecules are directly synthesised in this pathway from one glucose molecule?*

Pyruvic acid is then the key product of glycolysis. What is the metabolic fate of pyruvate? This depends on the cellular need. There are three major ways in which different cells handle pyruvic acid produced by glycolysis. These are lactic acid fermentation, alcoholic fermentation and aerobic respiration. Fermentation takes place under anaerobic conditions in many prokaryotes and unicellular eukaryotes. For the complete oxidation of glucose to CO_2 and H_2O, however, organisms adopt Krebs' cycle which is also called as aerobic respiration. This requires O_2 supply.

5.3 Fermentation

In fermentation, say by yeast, the incomplete oxidation of glucose is achieved under anaerobic conditions by sets of reactions where pyruvic acid is converted to CO_2 and ethanol. The enzymes, pyruvic acid decarboxylase and alcohol dehydrogenase catalyse these reactions. Other organisms like some bacteria produce lactic acid from pyruvic acid. The steps involved are shown in Figure 5.2. In animal cells also, like muscles during exercise, when oxygen is inadequate for cellular respiration, pyruvic acid is reduced to lactic acid by lactate dehydrogenase. The reducing agent is $NADH+H^+$ which is reoxidised to NAD^+ in both the processes.

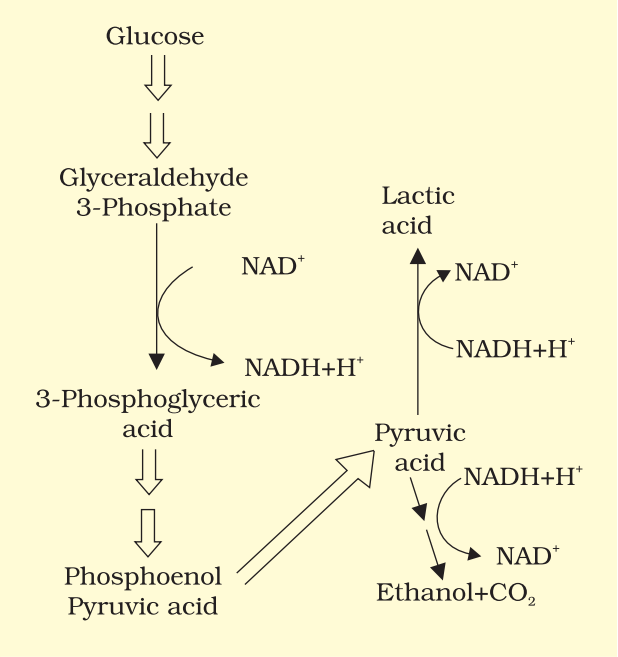


Figure 5.2 Major pathways of anaerobic respiration

In both lactic acid and alcohol fermentation not much energy is released; less than seven per cent of the energy in glucose is released and not all

of it is trapped as high energy bonds of ATP. Also, the processes are hazardous – either acid or alcohol is produced. What is the net ATPs that is synthesised (calculate how many ATP are synthesised and deduct the number of ATP utilised during glycolysis) when one molecule of glucose is fermented to alcohol or lactic acid? Yeasts poison themselves to death when the concentration of alcohol reaches about 13 per cent. *What then would be the maximum concentration of alcohol in beverages that are naturally fermented?* How do you think alcoholic beverages with alcohol content greater than this concentration are obtained?

What then is the process by which organisms can carry out complete oxidation of glucose and extract the energy stored to synthesise a larger number of ATP molecules needed for cellular metabolism? In eukaryotes these steps take place within the mitochondria and this requires O_2. **Aerobic respiration** is the process that leads to a complete oxidation of organic substances in the presence of oxygen, and releases CO_2, water and a large amount of energy present in the substrate. This type of respiration is most common in higher organisms. We will look at these processes in the following section.

5.4 Aerobic Respiration

For aerobic respiration to take place within the mitochondria, the final product of glycolysis, pyruvate, is transported from the cytoplasm into the mitochondria. The crucial events in aerobic respiration are:

- The complete oxidation of pyruvate by the stepwise removal of all the hydrogen atoms, leaving three molecules of CO_2.
- The passing one of the electrons removed as part of the hydrogen atoms to molecular O_2 with simultaneous synthesis of ATP.

What is interesting to note is that the first process takes place in the matrix of the mitochondria while the second process is located on the inner membrane of the mitochondria.

Pyruvate, which is formed by the glycolytic catabolism of carbohydrates in the cytosol, after entering mitochondrial matrix, undergoes oxidative decarboxylation by a complex set of reactions catalysed by pyruvic dehydrogenase. The reactions catalysed by pyruvic dehydrogenase require the participation of several coenzymes, including NAD^+ and Coenzyme A.

$$\text{Pyruvic acid} + \text{CoA} + NAD^+ \xrightarrow[\text{Pyruvate dehydrogenase}]{Mg^{2+}} \text{Acetyl CoA} + CO_2 + NADH + H^+$$

During this process, two molecules of NADH are produced from the metabolism of two molecules of pyruvic acid (produced from one glucose molecule during glycolysis).

The acetyl CoA then enters a cyclic pathway, tricarboxylic acid cycle, more commonly called Krebs cycle after the scientist Hans Krebs who first elucidated it.

5.4.1 Tricarboxylic Acid Cycle

The TCA cycle starts with the condensation of acetyl group with oxaloacetic acid (OAA) and water to yield citric acid (Figure 5.3). The reaction is catalysed by the enzyme citrate synthase and a molecule of CoA is released. Citrate is then isomerised to isocitrate. It is followed by two successive steps of decarboxylation, leading to the formation of α-ketoglutaric acid and then succinyl-CoA. In the remaining steps of the citric acid cycle, succinyl-CoA is oxidised to OAA, allowing the cycle to continue. During the conversion of succinyl-CoA to succinic acid a molecule of GTP is synthesised. This is a substrate level phosphorylation. In a coupled reaction GTP is converted to GDP with the simultaneous synthesis of ATP from ADP. Also there are three points in the cycle where NAD^+ is reduced to NADH + H^+ and one point where FAD^+ is reduced to $FADH_2$. The continued oxidation of acetyl CoA via the TCA cycle requires the continued replenishment of oxaloacetic acid, the first member of the cycle. In addition it also requires regeneration of NAD^+ and FAD^+ from NADH and $FADH_2$ respectively. The equations for this phase of respiration may be written as follows:

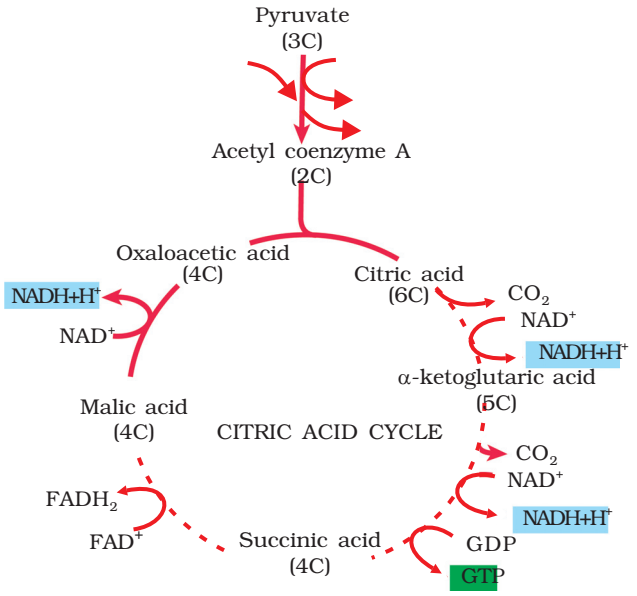


Figure 5.3 The Citric acid cycle

Condensation:

Oxaloacetic acid + Acetyl CoA $\xrightarrow{\text{citric synthetase}}$ Citric acid + Coenzyme A

Dehydration:

$$\text{Citric acid} \xrightarrow{\text{aconitase}} \text{Cis-aconitic acid} + H_2O$$

Hydration:

$$\text{Cis-aconitic acid} \xrightarrow{\text{Aconitase}} \text{Isocitric acid}$$

Oxidation I

$$\text{Isocitric acid} + NAD^+ \xrightarrow{\text{Isocitric dehydrogenase}} \text{Oxalosuccinic acid} + NADH$$

Decarboxylation:

$$\text{Oxalosuccinic acid} \xrightarrow{\text{Oxalosuccinic decarboxylase}} \alpha\text{-Ketoglutaric acid} + CO_2$$

Oxidative decarboxylation (oxidation II):

$$\alpha\text{-Ketoglutaric acid} + NAD^+ + CoA \xrightarrow{\alpha\text{-ketoglutaric dehydrogenase}} \text{Succinyl CoA} + NADH + CO_2$$

Cleavage:

$$\text{Succinyl CoA} + ADP + Pi \xrightarrow{\text{Succinic thiokinase}} \text{Succinic acid} + ATP + CoA$$

Oxidation III:

$$\text{Succinic acid} + FAD \xrightarrow{\text{Succinic dehydrogenase}} \text{Fumaric acid} + FADH_2$$

Hydration:

$$\text{Fumaric acid} + H_2O \xrightarrow{\text{Fumerase}} \text{Malic acid}$$

Oxidation IV:

$$\text{Malic acid} + NAD^+ \xrightarrow{\text{Malic dehydrogenase}} \text{Oxaloacetic acid} + NADH$$

Summary equation for the oxidation of pyruvic acid in mitochondrion is:

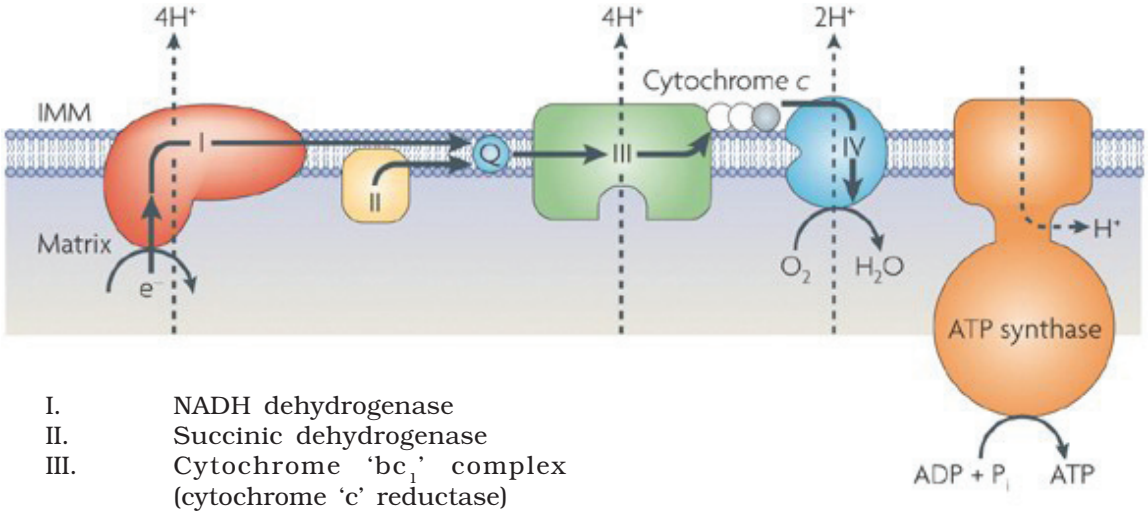
$$\text{Pyruvic acid} + 4NAD^+ + FAD^+ + 2H_2O + ADP + Pi \xrightarrow{\text{Mitochondrial Matrix}} 3CO_2 + 4NADH + 4H^+ + FADH_2 + ATP$$

We have seen till now that glucose has been broken down to release CO_2 and eight molecules of NADH + H^+; two of $FADH_2$ have been synthesised besides just two molecules of ATP. You may be wondering why we have been discussing respiration at all – neither O_2 has come into the picture nor the promised large number of ATP has yet been synthesised. Also what is the role of the NADH + H^+ and $FADH_2$ that is synthesised? Let us now understand the role of O_2 in respiration and how ATP is synthesised.

5.4.2 Electron Transport System (ETS) and Oxidative Phosphorylation

The following steps in the respiratory process take place in order to release and utilise the energy stored in $NADH+H^+$ and $FADH_2$. This is accomplished when, they are oxidised through the electron transport system and the electrons are passed on to O_2 resulting in the formation of H_2O. The metabolic pathway through which an electron passes from one carrier to another is called the **electron transport system** (ETS) (Figure 5.4) and it is present in the inner mitochondrial membrane. Electrons from NADH produced in the mitochondrial matrix during the citric acid cycle are oxidised by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via $FADH_2$ (complex II) that is generated during oxidation of succinate in the citric acid cycle. The reduced ubiquinone (ubiquinol) is then oxidised with the transfer of electrons to cytochrome *c* via cytochrome bc_1 complex (complex III). Cytochrome *c* is a small protein attached to the outer surface of the inner membrane and acts as a mobile carrier for the transfer of electrons between complex III and IV. Complex IV refers to cytochrome *c* oxidase complex containing cytochromes *a* and a_3, and two copper centres.

When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (complex V) for the production of ATP from ADP and inorganic phosphate. The number of ATP



I. NADH dehydrogenase
II. Succinic dehydrogenase
III. Cytochrome 'bc$_1$' complex (cytochrome 'c' reductase)
IV. Cytochrome 'c' oxidase

Figure 5.4 Respiratory Electron Transport and Oxidative Phosphorylation

molecules synthesised depends on the nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATP. Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is limited to the terminal stage of the process. Yet, the presence of oxygen is vital, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor. Unlike photophosphorylation where it is the light energy that is utilised for the production of proton gradient required for phosphorylation, in respiration it is the energy of oxidation-reduction utilised for the same process. It is for this reason that the process is called oxidative phosphorylation.

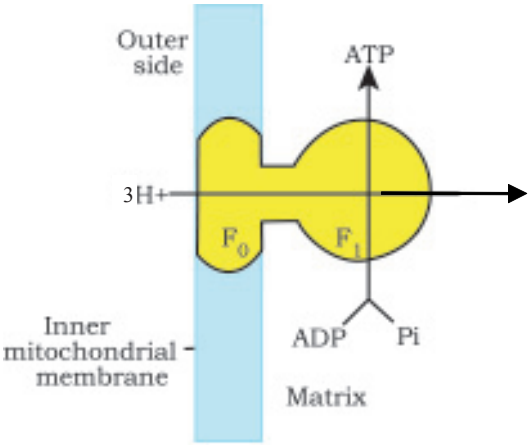


Figure 5.5 Diagramatic presentation of ATP synthesis in mitochondria

You have already studied about the mechanism of membrane-linked ATP synthesis as explained by chemiosmotic hypothesis in the earlier chapter. As mentioned earlier, the energy released during the electron transport system is utilised in synthesising ATP with the help of ATP synthase (complex V). This complex consists of two major components, F_1 and F_0 (Figure 5.5). The F_1 headpiece is a peripheral membrane protein complex and contains the site for synthesis of ATP from ADP and inorganic phosphate. F_0 is an integral membrane protein complex that forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP. For each ATP produced, $3H^+$ passes through F_0 from the intermembrane space to the matrix down the electrochemical proton gradient.

5.5 The Respiratory Balance Sheet

It is possible to make calculations of the net gain of ATP for every glucose molecule oxidised; but in reality this can remain only a theoretical exercise. These calculations can be made only on certain assumptions that:

- There is a sequential, orderly pathway functioning, with one substrate forming the next and with glycolysis, TCA cycle and ETS pathway following one after another.

- The NADH synthesised in glycolysis is transferred into the mitochondria and undergoes oxidative phosphorylation.
- None of the intermediates in the pathway are utilised to synthesise any other compound.
- Only glucose is being respired – no other alternative substrates are entering in the pathway at any of the intermediary stages.

But these kind of assumptions are not really valid in a living system; all pathways work simultaneously and do not take place one after another; substrates enter the pathways and are withdrawn from it as and when necessary; ATP is utilised as and when needed; enzymatic rates are controlled by multiple means. Yet, it is useful to do this exercise to appreciate the beauty and efficiency of the living system in extracting and storing energy. Hence, there can be a net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose.

Now let us compare fermentation and aerobic respiration:

- Fermentation accounts for only a partial breakdown of glucose whereas in aerobic respiration glucose completely degraded to CO_2 and H_2O.
- In fermentation there is a net gain of only two molecules of ATP for each molecule of glucose degraded to pyruvic acid whereas many more molecules of ATP are generated under aerobic conditions.
- NADH is oxidised to NAD^+ rather slowly in fermentation; however the reaction is very vigorous in the case of aerobic respiration.

Balance sheet of ATP production in aerobic oxidation of Glucose.

(1) Glycolysis:

1. ATP produced by substrate level phosphorylation

Bisphosphoglyceric acid to phosphoglyceric acid	:	2X1 = 2 ATP
Phosphoenol pyrvic acid to pyruvic acid	:	2X1 = 2 ATP
ATP consumed: for the phosphorylation of glucose and fructose-6-phosphate	:	-2 ATP
Net gain of ATP	:	+2 ATP

2. ATP from NADH generated in glycolysis:

G-3-P to BPGA (2NADH, each worth 2ATP)	:	2X2 = 4 ATP
ATP gain from glycolysis in the presence of O_2	:	[(a)]6 ATP

(2) Oxidative decarboxylation of pyruvic acid

Pyruvic acid to acetly COA

(2 NADH, each worth 3 ATP) : [b]2X3 = 6 ATP

(3) Krebs cycle

1. ATP produced in substrate level phosphorylation:

   Succinyl CoA to Succinic acid : 2X1 = 2 ATP

2. ATP from NADH: Isocitric acid to Oxalosuccinic acid : 2X3 = 6 ATP

   α-Ketoglutaric acid to Succinyl CoA : 2X3 = 6 ATP

   Malic acid to Oxaloacetic acid : 2X3 = 6 ATP

3. ATP from $FADH_2$: Succinic acid to Fumaric acid : 2X2 = 4 ATP

   ATP value of Krebs cycle : [c]24 ATP

   Net gain of ATP in aerobic respiration per mole glucose

   (a+b+c) : 36 ATP

5.6 Amphibolic Pathway

Glucose is the most favoured substrate for respiration. All carbohydrates are usually first converted into glucose before they are used for respiration. Other substrates can also be respired, as has been mentioned earlier, but then they do not enter the respiratory pathway at the first step. The points of entry of different substrates in the respiratory pathway are given in Figure 5.6. Fats would need to be broken down into glycerol and fatty acids first. If fatty acids were to be respired they would first be degraded to acetyl CoA and enter the pathway. Glycerol would enter the pathway after being converted to PGAL. The proteins would be degraded by proteases and the individual amino acids (after deamination), depending on their structure, would enter the pathway at some stage within the Krebs' cycle or even as pyruvate or acetyl CoA.

Since respiration involves the breakdown of substrates, the respiratory process has traditionally been considered a catabolic process and the respiratory pathway as a catabolic pathway. But is this understanding correct? We have discussed above, at which points in the respiratory pathway different substrates would enter if they were to be respired and used to derive energy. What is important to recognise is that it is these very compounds that would be withdrawn from the respiratory pathway for the synthesis of the said substrates.

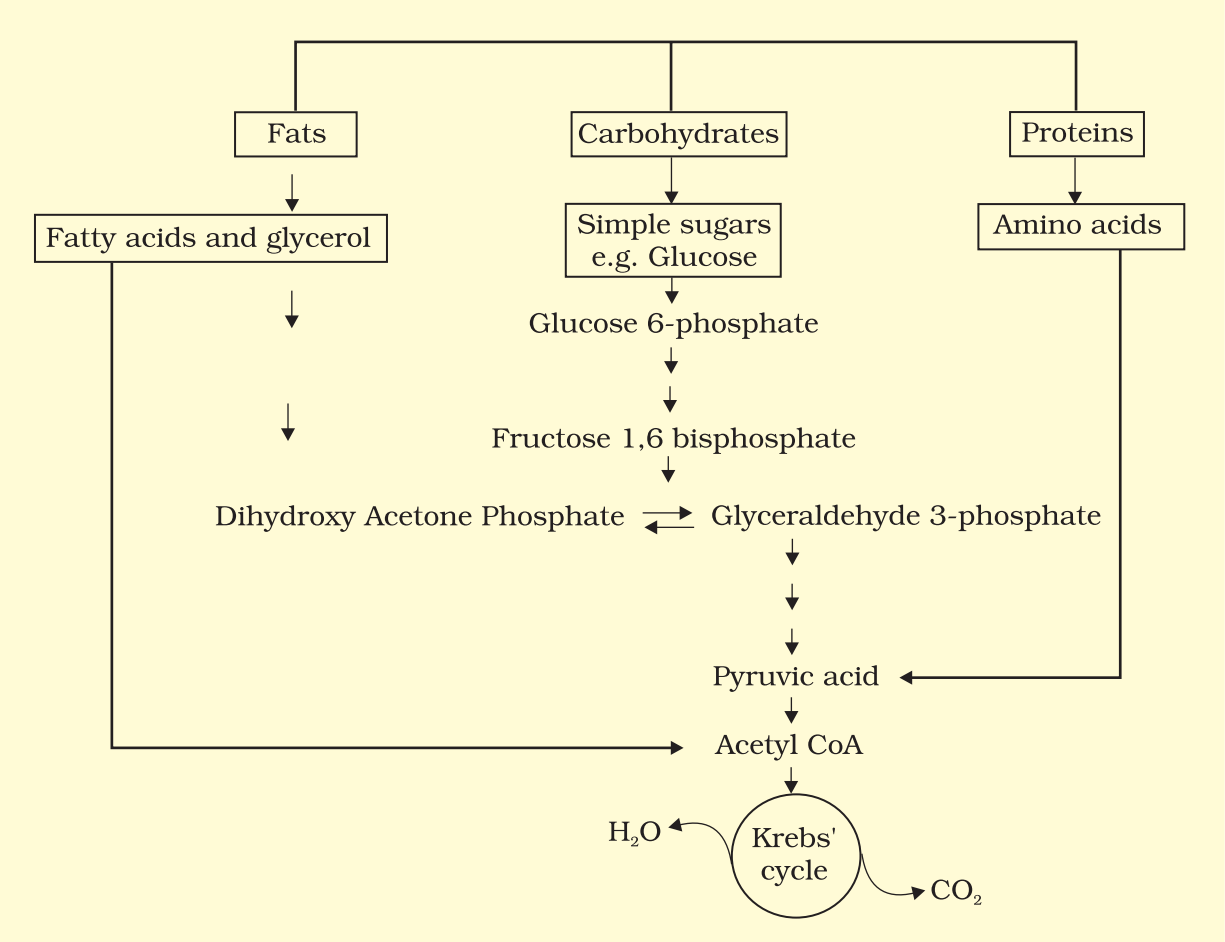


Figure 5.6 Interrelationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to CO_2 and H_20

Hence, fatty acids would be broken down to acetyl CoA before entering the respiratory pathway when it is used as a substrate. But when the organism needs to synthesise fatty acids, acetyl CoA would be withdrawn from the respiratory pathway for it. Hence, the respiratory pathway comes into the picture both during the breakdown and the synthesis of fatty acids. Similarly, during the breakdown and the synthesis of protein too, respiratory intermediates form the link. The breaking down process within the living organism constitute catabolism, while synthesis is anabolism. Because the respiratory pathway is involved in both anabolism and catabolism, it would be better to consider the respiratory pathway as an **amphibolic pathway** rather than as a catabolic one.

5.7 Respiratory Quotient

Let us now look at another aspect of respiration. As you know, during aerobic respiration, O_2 is consumed and CO_2 is released. The ratio of the volume of CO_2 evolved to the volume of O_2 consumed in respiration is called the **Respiratory Quotient** (RQ) or respiratory ratio.

$$RQ = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ consumed}}$$

The respiratory quotient depends upon the type of respiratory substrate used during respiration.

When carbohydrates are used as substrate and are completely oxidised, the RQ is 1, because equal amounts of CO_2 and O_2 are evolved and consumed, respectively, as shown in the equation below :

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

$$RQ = \frac{6CO_2}{6O_2} = 1.0$$

When fats are used in respiration, the RQ is less than 1. Calculations for a fatty acid, tripalmitin, if used as a substrate is shown:

$$2(C_{51}H_{98}O_6) + 145O_2 \longrightarrow 102CO_2 + 98H_2O + \text{energy}$$

Tripalmitin

$$RQ = \frac{102CO_2}{145O_2} = 0.7$$

When proteins are respiratory substrates the ratio would be about 0.9.

What is important to recognise is that in living organisms respiratory substrates are often more than one; pure proteins or fats are never used as respiratory substrates.

SUMMARY

Plants, unlike animals, have no special systems for breathing or gaseous exchange. Stomata and lenticels allow gaseous exchange by diffusion. Almost all living cells in a plant have their surfaces exposed to air.

The breaking of C-C bonds of complex organic molecules by oxidation in cells leading to the release of a lot of energy is called cellular respiration. Glucose is the favoured substrate for respiration. Fats and proteins can also be broken down to yield energy. The initial stage of cellular respiration takes place in the cytoplasm. Each glucose molecule is broken, through a series of enzyme catalysed reactions, into two molecules of pyruvic acid. This process is called glycolysis. The fate of pyruvate depends on the availability of oxygen and the type of organism. Under anaerobic conditions either lactic acid fermentation or alcohol fermentation occurs. Fermentation takes place under anaerobic conditions in many prokaryotes, unicellular eukaryotes and in germinating seeds. In eukaryotic organisms aerobic respiration occurs in the presence of oxygen. Pyruvic acid is transported into the mitochondria where it is converted into acetyl CoA with the release of CO_2. Acetyl CoA then enters the tricarboxylic acid pathway or Krebs' cycle operating in the matrix of the mitochondria. NADH + H^+ and $FADH_2$ are generated in Krebs' cycle. The energy in these molecules as well as that in the NADH + H^+ synthesised during glycolysis are used to synthesise ATP. This is accomplished through a system of electron carriers called electron transport system (ETS) located on the inner membrane of the mitochondria. The electrons, as they move through the system, release enough energy that is trapped, to synthesise ATP. This is called oxidative phosphorylation. In this process, O_2 is the ultimate acceptor of electrons and it gets reduced to water.

The respiratory pathway is an amphibolic pathway as it involves both anabolism and catabolism. The respiratory quotient depends upon the type of respiratory substance used during respiration.

GLOSSARY

ATP: Adenosine triphosphate, known as energy currency of the cell. On hydrolysis one mole of ATP yields 7.6k.cal of energy.

Cellular respiration: Breakdown of food materials within the cell to release energy as ATP.

Aerobic respiration: Respiration taking place in the presence of oxygen.

Anaerobic respiration: Respiration taking place in the absence of oxygen.

Fermentation: Enzymatic conversion of sugars to usually, ethylacohol by microbes/microbial enzymes under anaerobic condtions.

Glycolysis: A cytosolic process in which glucose is broken down to two molecules of pyruvic acid.

Krebs cycle: a cycle of reactions which oxidises acetyl CoA. Also called citric acid cycle or TCA cycle.

Oxidative phosporylation: Synthesis of ATP from ADP and iP coupled to electron transport from substrate to molecular oxygen.

Respiratory quotient: Ratio of the volume of CO_2 liberated to the volume of O_2 absorbed during respiration.

QUESTIONS

Very Short Answer Type Questions

1. Energy is released during the oxidation of compounds in respiration. How is this energy stored and released as and when it is needed?
2. Explain the term 'Energy currency'. Which substance acts as energy currency in plants and animals?
3. Different substrates get oxidised during respiration. How does respiratory quotient (RQ) indicate which type of substrate i.e. carbohydrate, fat or protein is getting oxidised?

   R.Q. = A/B

   What do A and B stand for?

   What type of substrates have RQ of 1, <1, >1?
4. What is the specific role of F_0-F_1 particles in respiration?
5. When does anaerobic repsiration occur in man and yeast?
6. Distinguish between obligate anaerobes and facultative anaerobes.
7. Explain the economic importance of fermenation.
8. What is the common pathway for aerobic and anaerobic respirations? Where does it take place?
9. Why are mitochondria termed as the power houses of the cell?
10. What is the reason for describing ATP synthesis in F_0-F_1 particles of mitochondria as oxidative phosphorylation?
11. Which substance is known as the connecting link between glycolysis and Krebs cycle? How many carbons does it have?
12. What cellular organic substances are never used as respiratory substrates?
13. Why is the RQ of fats less than that of carbohydrates?
14. What is meant by 'Amphibolic pathway'?
15. Name the mobile electron carriers of the respiratory electron transport chain in the inner mitochondrial membrane.
16. What is the final acceptor of electrons in aerobic respiration? From which complex does it receive electrons?

Short Answer Type Questions

1. What is meant by the statement 'Aerobic respiration is more efficient'?
2. Pyruvic acid is the end product of glycolysis. What are the three metabolic fates of pyruvic acid under aerobic and anaerobic conditions?

3. The energy yield in terms of ATP is higher in aerobic respiration than during anaerobic respiration. Why is there anaerobic respiration even in organisms that live in aerobic condition like human beings and angiosperms?
4. Oxygen is an essential requirement for aerobic respiration but it enters the respiratory process at the end. Discuss.
5. Respiration is an energy releasing and enzymatically controlled catablic process which involves a stepwise oxidative breakdown of organic substances? Inside living cells.

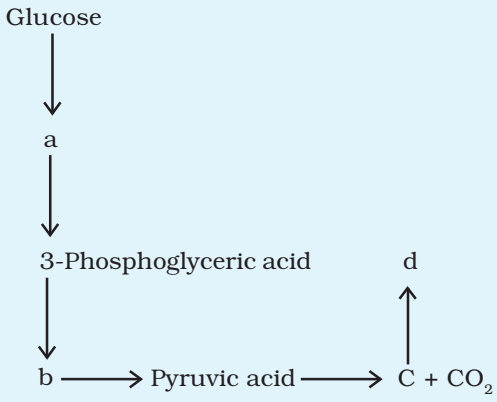
   In this statement about respiration, explain the meaning of (i) step-wise oxidative breakdown (ii) organic substance (used as substrates).
6. Comment on the statement - Respiration is an energy producing process but ATP is used in some steps of the process.
7. Explain briefly the process of glycolysis.
8. Why is the respiratory pathway refered to as an amphibolic pathway? Explain.
9. We commonly call ATP the energy currency of the cell. Can you think of some other energy carriers present in a cell? Name any two.
10. ATP produced during glycolysis is a result of substrate level phosphorylation. Explain.
11. Do you know of any step in Krebs cycle where there is a substrate level phosporylation? Explain.
12. When a substrate is being metabolised. Why doesn't all the energy that is produced get released in one step? Instead it is released in multiple steps. What is the advantage of step wise release of energy?
13. Respiration requires O_2. How did the first cells on earth manage to survive in an atmosphere that lacked oxygen?
14. The energy yield in terms of ATP is higher in aerobic respiration than during anaerobic respiration. Explain.
15. RUBP carboxylase, PEPcase, pyruvate dehydrogenase, ATPase, cytochrome oxidase, Hexokinase, Lactic dehydrogenase.

    Select the enzymes from the list above which are involved in
    (a) Photosynthesis
    (b) Respiration
    (c) Both photosynthesis and respiration
16. How does a tree trunk exchange gases with the environment although it lacks stomata?
17. Write about two energy yielding reactions of glycolysis.
18. Name the site(s) of pyruvate synthesis. Also write the chemical reaction wherein pyruvic acid dehydrogenase acts as a catalyst.

19. Mention the important series of events of aerobic respiration that occur in the matrix of the mitochondrion as well as the one that takes place in the inner membrane of the mitochondrion.
20. The respiratory pathway is believed to be a catabolic pathway. However, the nature of TCA cycle is amphibolic. Explain,
21. The net gain of ATP for the complete aerobic oxidation of glucose is 36. Explain.

Long Answer Type Questions

1. In the following flow chart, replace the symbols a,b,c and d with appropriate terms. Briefly explain the process and give any two applications of it.



2. Explain Mitchl's chemiosmosis in relation to oxidative phosphorylation.
3. Oxygen is critical for aerobic respiration. Explain its role with respect to ETS.
4. Enumerate the assumptions that we undertake in making the respiratory balance sheet. Are these assumptions valid for a living system?

   Compare fermentation and aerobic respiration in this context.
5. Give an account of glycolysis. Where does it occur? What are the end products? Trace the fate of these products in both aerobic and anaerobic respiration.
6. Explain the reactions of Krebs cycle.

Exercises

1. Differentiate between
   (a) Respiration and Combustion
   (b) Glycolysis and Krebs cycle
   (c) Aerobic respiration and Fermentation
2. What are respiratory substrates? Name the most common respiratory substrate.
3. Give the schematic representation of glycolysis?
4. What are the main steps in aerobic respiration? Where does it take place?
5. Give the schematic representation of an overall view of Krebs cycle.
6. Explain ETS.
7. Distinguish between the following:
   (a) Aerobic respiration and Anaerobic respiration

(b) Glycolysis and Fermentation

(c) Glycolysis and Citric acid Cycle

8. What are the assumptions made during the calculation of net gain of ATP?
9. Discuss "The respiratory pathway is an amphibolic pathway."
10. Define RQ. What is its value for fats?
11. What is oxidative phosphorylation?
12. What is the significance of step-wise release of energy in respiration?
13. Find the correct ascending sequence of the following, on the basis of energy released in respiratory oxidation.

(a) 1gm of fat (b) 1gm of protein

(c) 1gm of glucose

(d) 0.5gm of protein + 0.5gm of glucose

14. Name the products, respectively, in aerobic glycolysis in skeletal muscle and anaerobic fermentation in yeast.
15. If a person is feeling dizzy, glucose or fruit juice is given immediatley but not a cheese sandwich, which might have more energy. Why?
16. In a way green plants and cynobacteria have synthesised all the food on earth. comment.
17. It is known that red muscle fibres in animals can work for longer periods of time continuously. How is this possible?

Chapter 6

Plant Growth and Development

You have already studied the organisation of a flowering plant in the First Year Intermediate course. Have you ever thought about how structures like roots, stems, leaves, flowers, fruits and seeds arise and that too in an orderly sequence? You are, by now, aware of terms like seed, seedling, plantlet and mature plant. You have also seen that trees continue to increase in height and girth over a period of time. However, the leaves, flowers and fruits of the same tree not only have limited dimensions but also appear and fall periodically and sometime repeatedly. Why does the vegetative phase precede flowering in a plant? All plant organs are made up of a variety of tissues; is there any relationship between the structure of a cell, a tissue, an organ and the function they perform? Can the structure and the function of these be altered? All cells of a plant are descendents of the zygote. The question is, then, how do cells have different structural and functional attributes? Development is the sum of two processes: growth and differentiation. To begin with, it is essential to know that the development of a mature plant from a zygote (fertilised egg) follow a precise and highly ordered succession of events. During this process a complex body organisation (Figure 6.1) is formed whereby roots, leaves, branches, flowers, fruits, and seeds are produced and eventually die (Figure 6.1).

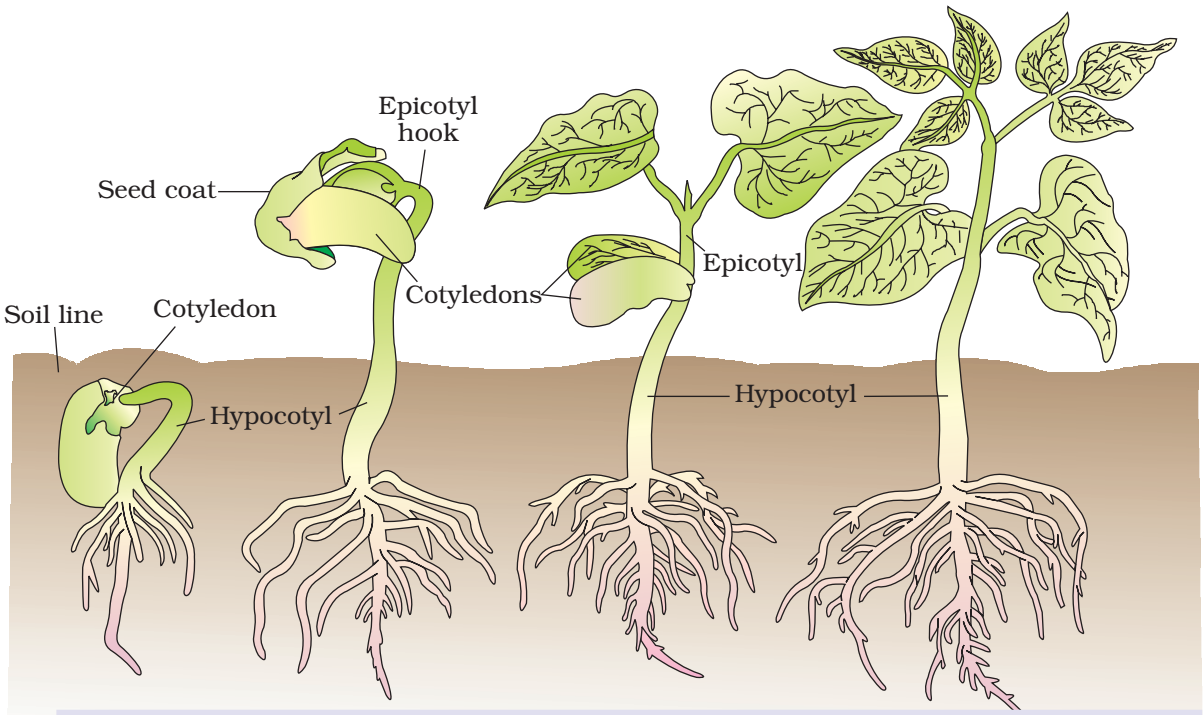


Figure 6.1 Germination and seedling development in bean

In this chapter, you will also study some of the factors which govern and control these developmental processes. These factors are both intrinsic (internal) and extrinsic (external) to the plant.

6.1 Growth

Growth is regarded as one of the most fundamental and conspicuous characteristics of a living being. What is growth? Growth can be defined as an irreversible permanent increase in size of an organism or its parts or even of an individual cell. Generally, growth is accompanied by metabolic processes (both anabolic and catabolic), that occur at the expense of energy. For example, expansion of a leaf is growth. How would you describe the swelling of piece of wood when placed in water?

6.1.1 Plant Growth Generally is Indeterminate

Plant growth is unique because plants retain the capacity for unlimited growth throughout their life. This ability of plants is due to the presence of meristems at certain locations in their body. The cells of such meristems have the capacity to divide and self-perpetuate. The product, however, soon loses the capacity

to divide and such cells make up the plant body. This form of growth wherein new cells are always being added to the plant body by the activity of the meristem is called the open form of growth. What would happen if the meristem ceases to divide? Does this ever happen?

In First Year, you studied about the root apical meristem and the shoot apical meristem. You know that they are responsible for the primary growth of the plants and principally contribute to the elongation of plants along their axis. You also know that in dicotyledonous plants and gymnosperms, the lateral meristems, vascular cambium and cork-cambium appear later in life. These are the meristems that cause the increase in the girth of the organs in which they are active. This is known as secondary growth of the plant (see Figure 6.2).

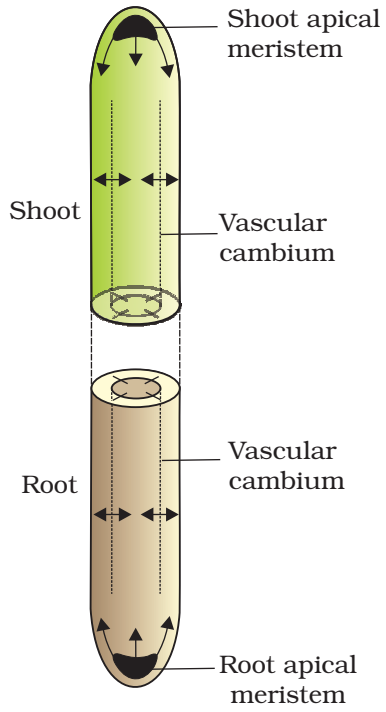


Figure 6.2 Diagrammatic representation of locations of root apical meristem, shoot apical meristem and vascular cambium. Arrows exhibit the direction of growth of cells and organ

6.1.2 Growth is Measurable

Growth, at a cellular level, is principally a consequence of increase in the amount of protoplasm. Since increase in protoplasm is difficult to measure directly, one generally measures some quantity which is more or less proportional to it. Growth is, therefore, measured by a variety of parameters some of which are: increase in fresh weight, dry weight, length, area, volume and cell number. You may find it amazing to know that a single maize root apical mersitem can give rise to more than 17,500 new cells per hour, while cells in a watermelon may increase in size by upto 3,50,000 times. In the former, growth is expressed as an increase in cell number; the latter expresses growth as an increase in the size of the cell. While the growth of a pollen tube is measured in terms of its length, an increase in surface area denotes growth in a dorsiventral leaf.

6.1.3 Phases of Growth

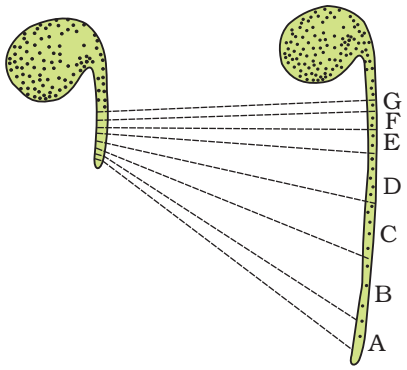


Figure 6.3 Detection of zones of elongation by the parallel line technique. Zones A, B, C, D immediately behind the apex have elongated most.

The period of growth is generally divided into three phases, namely, meristematic, elongation and maturation (Figure 6.3). Let us understand this by looking at the root tips. The constantly dividing cells, both at the root apex and the shoot apex, represent the meristematic phase of growth. The cells in this region are rich in protoplasm and possess large conspicuous nuclei. Their cell walls are primary in nature, thin and cellulosic with abundant plasmodesmatal connections. The cells proximal (just next, away from the tip) to the meristematic zone represent the phase of elongation. Increased vacuolation, cell enlargement and new cell wall deposition are the characteristics of the cells in this phase. Further away from the apex, i.e., more proximal to the phase of elongation, lies the portion of axis which is undergoing the phase of maturation. The cells of this zone attain their maximal size in terms of wall thickening and protoplasmic modifications. Most of the tissues and cell types you have studied in Chapter 12 of First Year Intermediate course represent this phase.

6.1.4 Growth Rates

The increased growth per unit time is termed as growth rate. Thus, rate of growth can be expressed mathematically. An organism, or a part of the organism, can produce more cells in a variety of ways.

The growth rate shows an increase that may be arithmetic or geometrical (Figure 6.4).

In arithmetic growth, following mitotic cell division, only one daughter cell continues to divide while the other differentiates and matures. The simplest expression of arithmetic growth is exemplified by a root elongating at a constant rate. Look at Figure 6.5. On plotting the length of the organ against time, a linear curve is obtained. Mathematically, it is expressed as

$L_t = L_0 + rt$

L_t = length at time 't'

L_0= length at time 'zero'

r = growth rate / elongation per unit time.

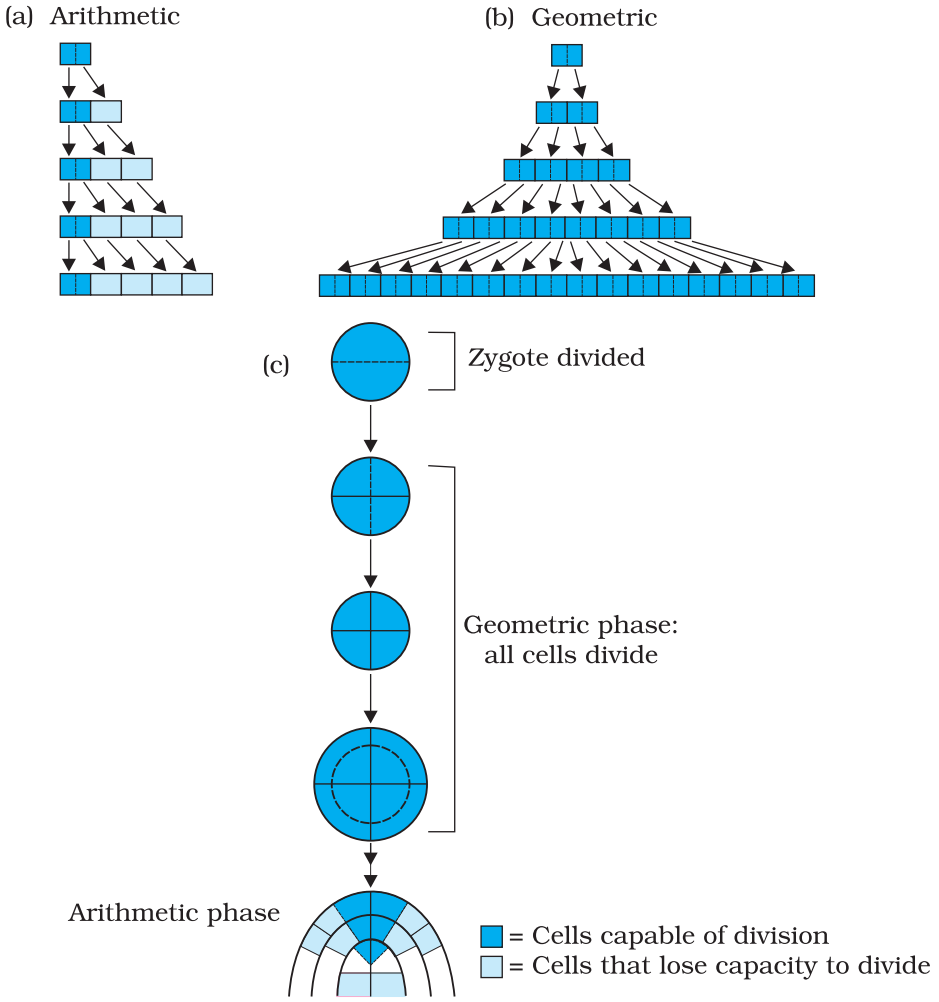


Figure 6.4 Diagrammatic representation of :
(a) Arithmetic
(b) Geometric growth and
(c) Stages during embryo development showing geometric and arithematic phases

Let us now see what happens in geometrical growth. In most systems, the initial growth is slow (lag phase). Growth increases rapidly thereafter at an exponential rate (log or exponential phase). Here, both the progeny cells following mitotic cell division retain the ability to divide and continue to do so. However, with limited nutrient supply, the growth slows down, leading to a stationary phase. If we plot the parameter of growth against time, we get a typical sigmoid or S-curve (Figure 6.6). A sigmoid curve is a characteristic of

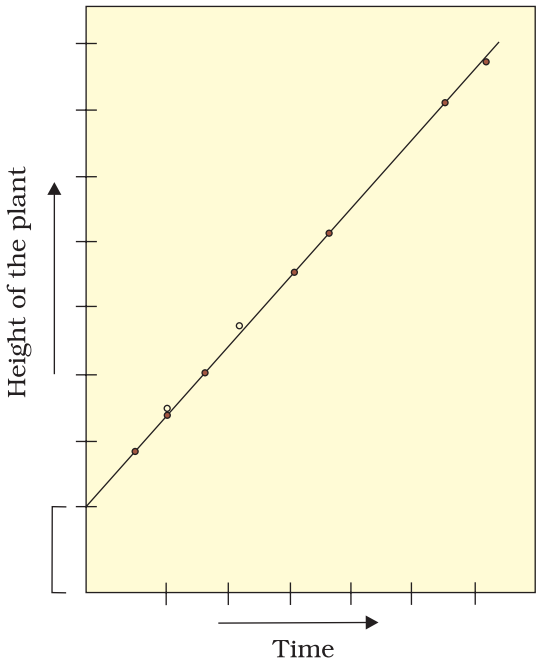


Figure 6.5 Constant linear growth, a plot of length L against time t

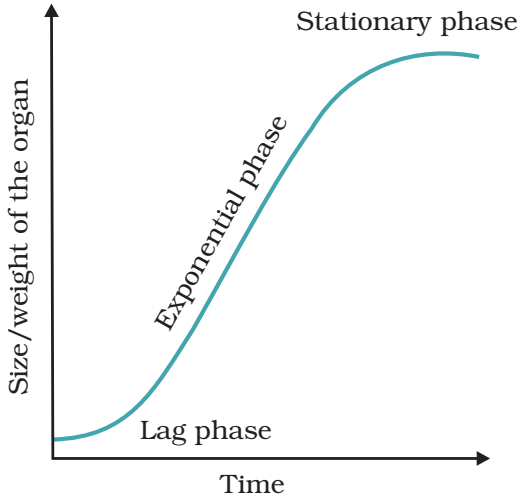


Figure 6.6 An idealised sigmoid growth curve typical of cells in culture, and many higher plants and plant organs

living organism growing in a natural environment. It is typical for all cells, tissues and organs of a plant. *Can you think of more such examples? What kind of curve can you expect in a tree showing seasonal activities?*

The exponential growth can be expressed as

$W_1 = W_0 e^{rt}$

W_1 = final size (weight, height, number etc.)

W_0 = initial size at the beginning of the period

r = growth rate

t = time of growth

e = base of natural logarithms

Here, r is the relative growth rate and is also the measure of the ability of the plant to produce new plant material, referred to as efficiency index. Hence, the final size of W_1 depends on the initial size, W_0.

Quantitative comparisons between the growth of living systems can be of two kinds (i) Measurement and comparison of the total growth per unit time is called the absolute growth rate. (ii) The growth of the given system per unit time expressed on a common basis, e.g., per unit initial parameter is called the relative growth rate. In Figure 6.7 two leaves, A and B, are of different sizes but show the same absolute increase in area in the given time to give leaves, A^1 and B^1. However, one of them shows much higher relative growth rate. Which one and why?

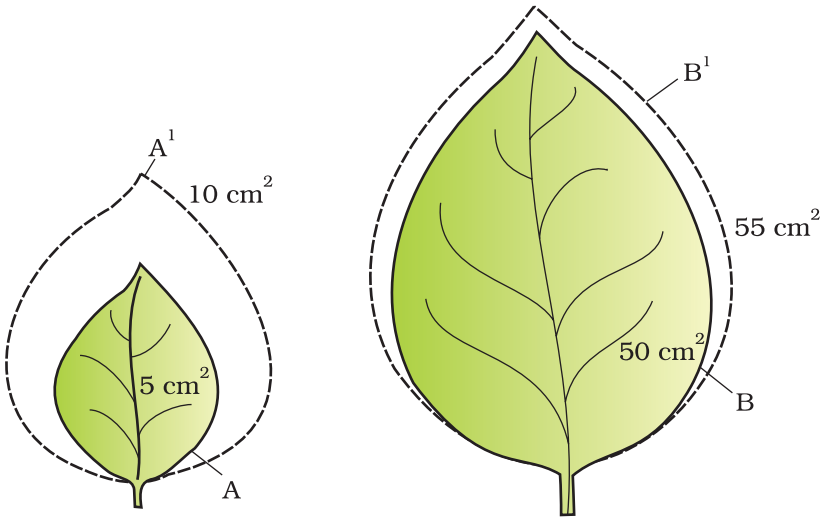


Figure 6.7 Diagrammatic comparison of absolute and relative growth rates. Both leaves A and B have increased their area by 5 cm^2 in a given time to produce A^1, B^1 leaves.

6.1.5 Conditions for Growth

Try to write down what you think are the necessary conditions for growth, your list may have water, oxygen and nutrients as they are all very essential for growth. Plant cells grow in size by cell enlargement which in turn requires water. Turgidity of cells helps in extension growth. Thus, plant growth and further development is intimately linked to the water status of the plant. Water also provides the medium for enzymatic activities needed for growth. Oxygen helps in releasing metabolic energy essential for growth activities. Nutrients (macro and micro essential elements) are required by plants for the synthesis of protoplasm and act as a source of energy.

In addition, every plant has an optimum temperature range best suited for its growth. Any deviation from this range could be detrimental to its survival. Environmental signals such as light and gravity also affect certain phases/ stages of growth.

6.2 Differentiation, Dedifferentiation and Redifferentiation

The cells derived from root apical and shoot-apical meristems and cambium differentiate and mature to perform specific functions. This act leading to maturation is termed as **differentiation**. During differentiation, cells undergo minor as well as major structural changes both in their cell walls and protoplasm. For example, to form a tracheary element, the cells would lose their protoplasm. They also develop very strong, elastic, lignocellulosic secondary cell walls to carry water to long distances even under extreme tension. Try to correlate the various anatomical features you encounter in plants to the functions they perform.

Plants show another interesting phenomenon. The living differentiated cells, that by now have lost the capacity to divide, can regain the capacity of division under certain conditions. This phenomenon is termed as **dedifferentiation**. For example, the formation of meristems – interfascicular cambium and cork cambium from fully differentiated parenchyma cells. In the process, such meristems/tissues are able to divide and produce cells that once again lose the capacity to divide but mature to perform specific functions, i.e., they get **redifferentiated**. List some of the tissues in a woody dicotyledenous plant that are the products of redifferentiation. How would you describe a tumour? What would you call the parenchyma cells that are made to divide under controlled laboratory conditions during plant tissue culture?

Recall, in Section 6.1.1, it has been mentioned that the growth in plants is open, i.e., it can be indeterminate or determinate. Now, we may say that even differentiation in plants is open, because cells/tissues arising out of the same meristem have different structures at maturity. The final structure at maturity of a cell/tissue is also determined by the location of the cell within. For example, cells positioned away from root apical meristems differentiate as root-cap cells, while those pushed to the periphery mature as epidermis. Can you add a few more examples of open differentiation correlating the position of a cell to its position in an organ?

6.3 Development

Development is a term that includes all changes that an organism goes through during its life cycle, from germination of the seed to senescence. Diagrammatic representation of the sequence of processes which constitute the development of a cell of a higher plant is given in Figure 6.8. It is also applicable to tissues/organs.

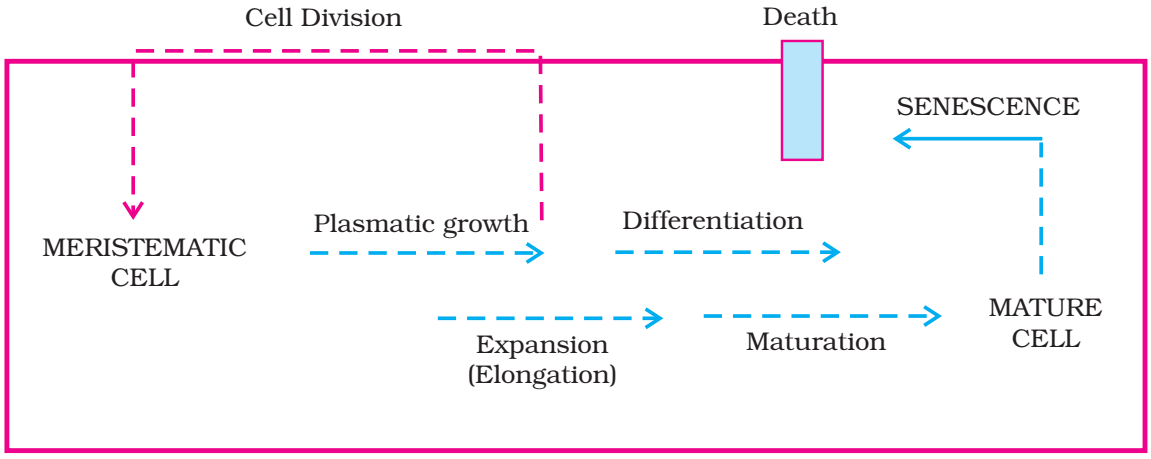


Figure 6.8 Sequence of the developmental process in a plant cell

Plants follow different pathways in response to the environment or phases of life to form different kinds of structures. This ability is called **plasticity**, e.g., heterophylly in cotton, coriander and larkspur. In such plants, the leaves of the juvenile plant are different in shape from those in mature plants. Difference in shapes of leaves produced in air and those produced in water in buttercup also

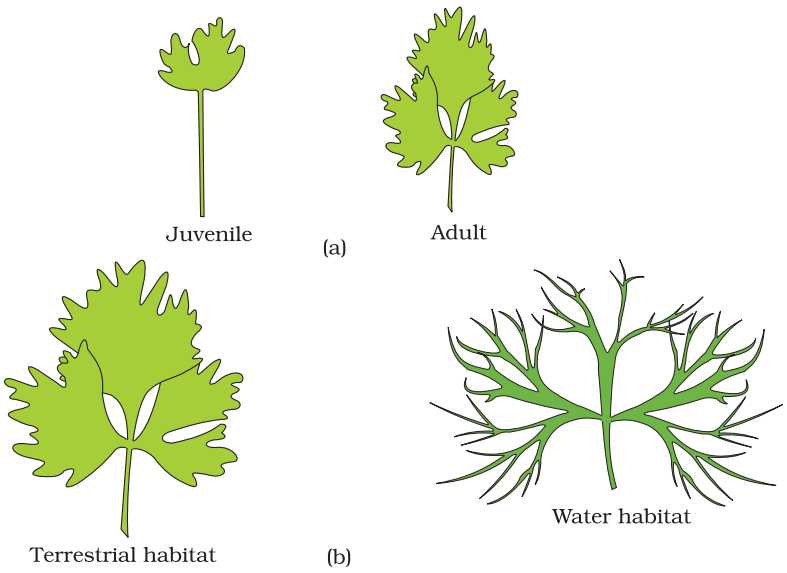


Figure 6.9 Heterophylly in (a) larkspur (*Delphinium*) and (b) buttercup (*Ranunculus*)

represent heterophyllous development due to environment (Figure 6.9). This phenomenon of heterophylly is an example of plasticity.

Thus, growth, differentiation and development are very closely related events in the life of a plant. Broadly, development is regarded as the sum of growth and differentiation. Development in plants (i.e., both growth and differentiation) is dependant on both intrinsic and extrinsic factors. Intrinsic factors may be intracellular (genetic) or intercellular (chemicals such as plant growth regulators) while extrinsic factors include light, temperature, water, oxygen, nutrition, etc.

6.4 Plant Growth Regulators

6.4.1 Characteristics

Plant growth regulators (PGRs) are small, simple molecules of diverse chemical composition. They could be indole compounds (indole-3-acetic acid, IAA); adenine derivatives (N^6-furfurylamino purine, kinetin), derivatives of carotenoids (abscisic acid, ABA); terpenes (gibberellic acid, GA_3) or gases (ethylene, C_2H_4). Plant growth regulators are variously described as plant growth substances, plant hormones or phytohormones in literature.

PGRs can be broadly divided into two groups based on their functions in a living plant body. One group of PGRs is involved in growth promoting activities, such as cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation. These PGRs are also called plant growth promoters, e.g., auxins, gibberellins and cytokinins. The PGRs of the other group play an important role in plant responses to wounds and stresses of biotic and abiotic origin. They are also involved in various growth inhibiting activities such as dormancy and abscission. The PGR, abscisic acid belongs to this group. The gaseous PGR, ethylene, could fit in either of the groups, but it is largely an inhibitor of growth activities.

6.4.2 The Discovery of Plant Growth Regulators

Interestingly, the discovery of each of the five major groups of PGRs has been accidental. It all started with the observation of Charles Darwin and his son, Francis Darwin, that the coleoptiles of canary grass responded to unilateral illumination by growing towards the light source (phototropism). After a series of experiments, it was concluded that the tip of the coleoptile was the site of transmittable influence that caused the bending of the entire coleoptile (Figure 6.10). Auxin was isolated by F.W. Went from the tips of coleoptiles of oat seedlings.

The 'bakane' (foolish seedling) disease of rice seedlings is caused by a fungal pathogen *Gibberella fujikuroi*. E. Kurosawa reported the appearance of symptoms of the disease in uninfected rice seedlings when they were treated with sterile filtrates of the fungus. The active substances were later identified as gibberellic acid.

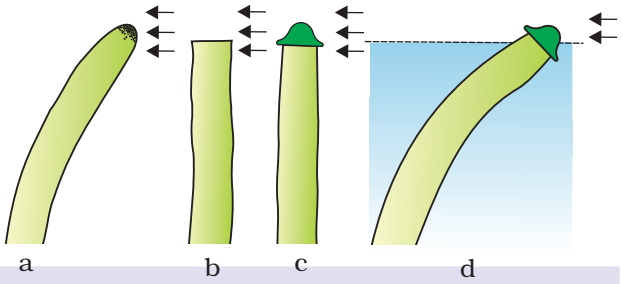


Figure 6.10 Experiment used to demonstrate that the tip of the coleoptile is the source of auxin. Arrows indicate the direction of light

F. Skoog and his co-workers observed that from the internodal segments of tobacco stems the callus (a mass of undifferentiated cells) proliferated only if, in addition to auxins, the nutrients medium was supplemented with one of the following: extracts of vascular tissues, yeast extract, coconut milk or DNA. Skoog and Miller later identified and crystallised the cytokinesis promoting active substance that they termed kinetin.

During the mid-1960s, three independent researches reported the purification and chemical characterisation of three different kinds of inhibitors: inhibitor-B, abscission II and dormin. Later all the three were proved to be chemically identical. It was named abscisic acid (ABA).

Cousins confirmed the release of a volatile substance from ripened oranges that hastened the ripening of stored unripened bananas. Later this volatile substance was identified as ethylene, a gaseous PGR.

Let us study some of the physiological effects of these five categories of PGRs in the following section.

6.4.3 Physiological Effects of Plant Growth Regulators

6.4.3.1 Auxins

Auxins (from Greek 'auxein' : to grow) was first isolated from human urine. The term 'auxin' is applied to indole-3-acetic acid (IAA), and to other natural and synthetic compounds having certain growth regulating properties. Auxins are generally produced by the growing apices of the stems and roots, from where they migrate to the regions of their action. Auxins like IAA and indole butyric acid (IBA) have been isolated from plants. NAA (naphthaleneacetic acid) and 2, 4-D (2, 4-dichlorophenoxyacetic acid) are synthetic auxins. All these auxins have been used extensively in agricultural and horticultural practices.

Auxins help to initiate rooting in stem cuttings, an application widely used for plant propagation in horticulture. Auxins promote flowering e.g. in pineapples. They help to prevent fruit and leaf drop at early stages but promote the abscission of older mature leaves and fruits.

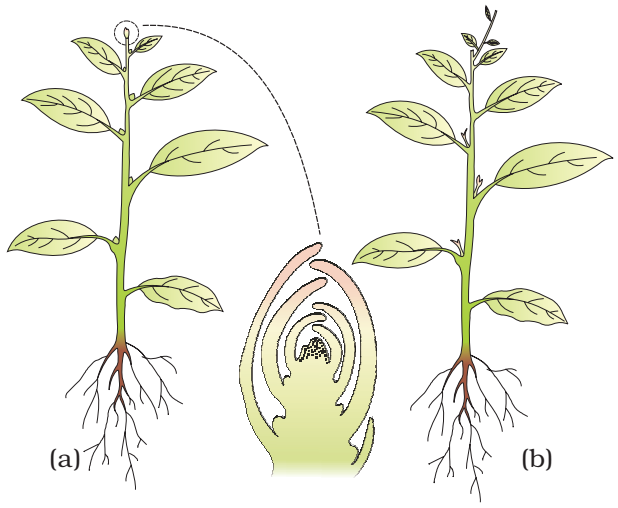


Figure 6.11 Apical dominance in plants :
(a) A plant with apical bud intact
(b) A plant with apical bud removed
Note the growth of lateral buds into branches after decapitation.

In most higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds, a phenomenon called **apical dominance**. Removal of shoot tips (decapitation) usually results in the growth of lateral buds (Figure 6.11). This is widely applied in tea plantations and hedge-making. Can you explain why?

Auxins also induce parthenocarpy, e.g., in tomatoes. They are widely used as herbicides. 2, 4-D, widely used to kill dicotyledonous weeds, does not affect mature monocotyledonous plants. It is used to prepare weed-free lawns by gardeners. Auxins also controls xylem differentiation and help in cell division.

6.4.3.2 *Gibberellins*

Gibberellins are another kind of promotery PGR. There are more than 100 gibberellins reported from widely different organisms such as fungi and higher plants. They are denoted as GA_1, GA_2, GA_3 and so on. However, Gibberellic acid (GA_3) was one of the first gibberellins to be discovered and remains the most intensively studied form. All GAs are acidic. They produce a wide range of physiological responses in plants. Their ability to cause an increase in the length of axis is used to increase the length of grapes' stalks. Gibberellins cause fruits like apple to elongate and improve their shape. They also delay senescence. Thus, fruits can be left on the tree longer so as to extend the market period. GA_3 is used to speed up the malting process in brewing industry.

Sugarcane stores carbohydrate as sugar in their stems. Spraying sugarcane crop with gibberellins increases the length of the stem, thus increasing the yield by as much as 20 tonnes per acre.

Spraying juvenile conifers with GAs hastens the maturity period, thus leading to early seed production. Gibberellins also promote bolting (internode elongation just prior to flowering) in beet, cabbages and many plants with rosette habit.

6.4.3.3 Cytokinins

Cytokinins have specific effects on cytokinesis, and were discovered as kinetin (a modified form of adenine, a purine) from the autoclaved herring sperm DNA. Kinetin does not occur naturally in plants. The search for natural substances with cytokinin-like activities led to the isolation of zeatin from corn-kernels and coconut milk. Since the discovery of zeatin, several naturally occurring cytokinins, and some synthetic compounds with cell division promoting activity have been identified. Natural cytokinins are synthesised in regions where rapid cell division occurs, for example, root apices, developing shoot buds, young fruits etc. They help to produce new leaves, chloroplasts in leaves, lateral shoot growth and adventitious shoot formation. Cytokinins help to overcome the apical dominance. They promote nutrient mobilisation which helps in the delay of leaf senescence.

6.4.3.4 Ethylene

Ethylene is a simple gaseous PGR. It is synthesised in large amounts by tissues undergoing senescence and ripening fruits. Effects of ethylene on plants include horizontal growth of seedlings, swelling of the axis and apical hook formation in dicot seedlings. Ethylene promotes senescence and abscission of plant organs, especially of leaves and flowers. Ethylene is highly effective in fruit ripening. It enhances the respiration rate during ripening of fruits. This rise in the rate of respiration is called respiratory climactic.

Ethylene breaks seed and bud dormancy, initiates germination in peanut seeds and sprouting of potato tubers. Ethylene promotes rapid internode/petiole elongation in deep water rice plants. It helps leaves/upper parts of the shoot to remain above water. Ethylene also promotes root growth and root hair formation, thus helping plants to increase their absorption surface.

Ethylene is used to initiate flowering and for synchronising fruit-set in pineapples. It also induces flowering in mango. Since ethylene regulates so many physiological processes, it is one of the most widely used PGRs in agriculture. The most widely used compound as a source of ethylene is ethephon. Ethephon in an aqueous solution is readily absorbed and transported within the plant and releases ethylene slowly. Ethephon hastens fruit ripening in tomatoes and apples and accelerates abscission in flowers and fruits

(thinning of cotton, cherry, walnut). It promotes female flowers in cucumbers, thereby increasing the yield.

6.4.3.5 *Abscisic acid*

As mentioned earlier, abscisic acid **(ABA)** was discovered for its role in regulating abscission and dormancy. But like other PGRs, it also has other wide ranging effects on plant growth and development. It acts as a general plant growth inhibitor and an inhibitor of plant metabolism. ABA inhibits seed germination. ABA stimulates the closure of stomata in the epidermis and increases the tolerance of plants to various kinds of stresses. Therefore, it is also called the stress hormone. ABA plays an important role in seed development, maturation and dormancy. By inducing dormancy, ABA helps seeds to withstand desiccation and other factors unfavourable for growth. In most situations, ABA acts as an antagonist to GAs.

We may summarise that for every phase of growth, differentiation and development of plants, some PGR or the other has a role to play. Such roles could be complementary or antagonistic. These could be individualistic or synergistic.

Similarly, PGRs may interact with one another and effect a number of events in the life of a plant e.g., dormancy in seeds/buds, abscission, senescence, apical dominance, etc.

Remember, the role of PGR is of only one kind of intrinsic control. Along with genomic control and extrinsic factors, they play an important role in plant growth and development. Many extrinsic factors such as temperature and light control plant growth and development via PGR. Some of such events could be: dormancy, seed germination, vernalisation, flowering, plant movements, etc.

We shall discuss briefly the role of light and temperature (both of them are extrinsic factors) on initiation of flowering.

6.5 Seed Dormancy

Germination of seeds can be defined as the process by which the seedling comes out from the seed. A seed may remain viable but unable to germinate or grow for several reasons. These can be classified into external or internal conditions. An internal situation that is easy to understand is an embryo that has not reached morphological maturity capable of germination (e.g. *Ranunculus*). Only time will allow this maturity to develop. The germination

of seeds of wild plants is often limited in this or some other internal way, but seeds of many domestic plants may be limited only by lack of moisture or warm temperatures. To distinguish between these two different situations, seed physiologists have used two terms: Quiescence is the condition of a seed when it is unable to germinate only because favourable external conditions normally required for growth are not present and dormancy is the condition of a seed when it fails to germinate because of internal conditions, even though external conditions (e.g., temperature, moisture and atmosphere) are suitable. The dormancy of seeds may be caused by hard seed coats that prevent uptake of oxygen or water (e.g. Fabaceae). Such type of dormancy caused by hard seed coats can be broken by scarification, a method by which the hard seed coat is ruptured or weakened. Seeds of certain plants (e.g. tomato) contain chemical compounds which inhibit their germination. Many seeds (e.g. *Polygonum*) will not germinate until they have been exposed to low temperatures in moist conditions in the pressence of oxygen for weeks to months. Rarely, moist seeds respond to high temperatures and several seeds respond best when daily temperatures alternate between high and low. The practice of layering the seeds during winter in layers of moist sand and peat is called stratification or prechilling.

6.6 Photoperiodism

It has been observed that some plants require periodic exposure to light to induce flowering. It is also seen that such plants are able to measure the duration of exposure to light. For example, some plants require the exposure to light for a period exceeding a well defined critical duration, while others must be exposed to light for a period less than this critical duration before flowering is initiated in them. The former group of plants are called **long day plants** while the latter ones are termed **short day plants**. The critical duration is different for different plants. There are many plants, however, where there is no such correlation between exposure to light duration and induction of flowering responses and such plants are called **day-neutral plants** (Figure 6.12). It is now known that not only the duration of light period but the duration of dark period also is of equal importance. Hence, it can be said that flowering in certain plants depends not only on a combination of light and dark exposures but also their relative durations. This response of plants to periods of day/night is termed **photoperiodism**. It is also interesting to note that while shoot apices modify themselves into flowering apices prior to flowering, they (i.e., shoot apices of plants) by themselves cannot percieve photoperiods. The site

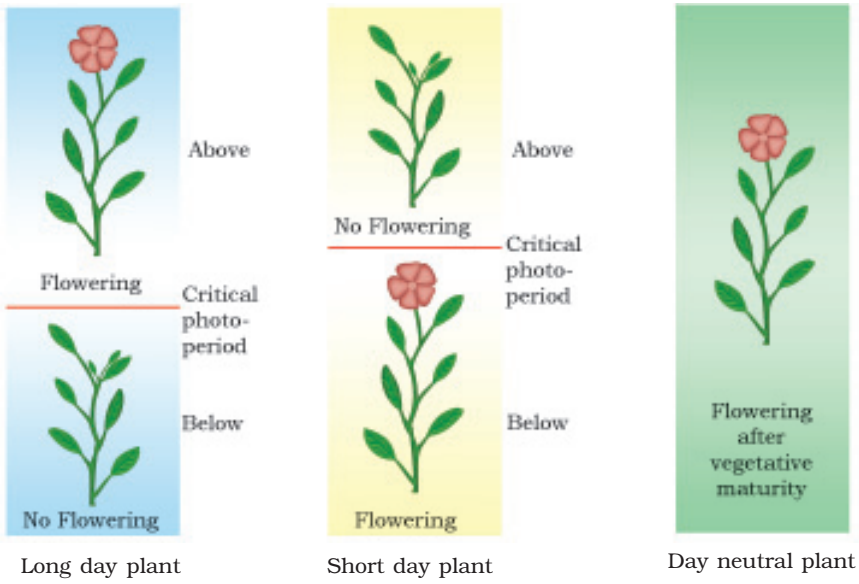


Figure 6.12 Photoperiodism : Long day, short day and day neutral plants

of perception of light/dark duration is the leaves. It has been hypothesised that there is a hormonal substance(s) that is responsible for flowering. This hormonal substance migrates from leaves to shoot apices for inducing flowering only when the plants are exposed to the necessary inductive photoperiod.

6.7 Vernalisation

There are plants for which flowering is either quantitatively or qualitatively dependent on exposure to low temperature. This phenomenon is termed **vernalisation**. It prevents precocious reproductive development late in the growing season, and enables the plant to have sufficient time to reach maturity. Vernalisation refers specially to the promotion of flowering by a period of low temperature. Some important food plants such as wheat, barley and rye have two kinds of varieties: winter and spring varieties. The 'spring' variety is normally planted in the spring and comes to flower and produces grain before the end of the growing season. Winter varieties, however, if planted in spring would normally fail to flower or produce mature grain within the span of a flowering season. Hence, they are planted in autumn. They germinate, and over winter come out as small seedlings, resume growth in the spring, and are harvested usually around mid-summer.

Another example of vernalisation is seen in biennial plants. Biennials are monocarpic plants that normally flower and die in the second season. Sugarbeet, cabbages, carrots are some of the common biennials. Subjecting the growing of a biennial plant to cold treatment stimulates a subsequent photoperiodic flowering response.

SUMMARY

SUMMARY

Growth is one of the most conspicuous events in any living organism. It is an irreversible increase expressed in parameters such as size, area, length, height, volume, cell number etc. It conspicuously involves increased protoplasmic material. In plants, meristems are the sites of growth. Root and shoot apical meristems sometimes along with intercalary meristem, contribute to the longitudinal growth of plant axes. Growth is indeterminate in higher plants. Following cell division in root and shoot apical meristem cells, growth can be arithmetic or geometrical. Growth may not be and generally is not sustained at a high rate throughout the life of cell/tissue/organ/organism. One can define three principle phases of growth – the lag, the log and the senescent phase. When a cell loses the capacity to divide, it leads to differentiation. Differentiation results in development of structures that are commensurate with the function the cells finally have to perform. The general principles for differentiation of cell, tissues and organs are similar. A differentiated cell may dedifferentiate and then redifferentiate. Since differentiation in plants is open, the development could also be flexible, i.e., the development is the sum of growth and differentiation. Plants exhibit plasticity in development.

Plant growth and development are under the control of both intrinsic and extrinsic factors. Intercellular intrinsic factors are the chemical substances, called plant growth regulators (PGR). There are diverse groups of PGRs in plants. They belonging to five main groups: auxins, gibberellins, cytokinins, abscisic acid and ethylene. These PGRs are synthesised in various parts of the plant. They control different differentiation and developmental events. PGRs have diverse physiological effects on plants. Diverse PGRs also manifest similar effects. PGRs may act synergistically or antagonistically. Plant growth and development is also affected by light, temperature, nutrition, oxygen status, gravity and other such external factors.

Flowering in some plants is induced only when the plant is exposed to a certain duration of photoperiod. Depending on the nature of photoperiod requirements, plants are called short day plants, long day plants and day-neutral plants. Certain plants also need to be exposed to low temperature so as to hasten flowering later in life. This treatement is known as vernalisation.

GLOSSARY

Apical Dominance: It is the phenomenon in which a growing apical bud inhibits the growth of lateral (axillary) buds. Auxin synthesized in apical meristem is responsible for apical dominance.

Bolting: It is the sudden elongation of internodes which is followed by flowering.

Defoliation: Removal of leaves from intact plants. It may be either natural or artificial.

Ethephon: It is an ethylene releasing chemical formulation.

Growth: It is an irreversible, permanent increase in size of an organism or its parts or even of an individual cell.

Photoperiodism : The flowering response of plants to periods of day / night is termed photoperiodism.

Plasticity : This is the ability of plants to follow different pathways in response to the environment or phases of life to form different kinds of structures. Heterophylly is an example of plasticity.

Respiratory climactic: The rise in the rate of respiration during the ripening of the fruits is known as respiratory climactic. Ethylene is responsible for it.

Sigmoid curve : 'S' shaped growth curve obtained by plotting the parameter of growth against time is called sigmoid or 'S'-curve.

Vernalisation: It is the method of inducing flowering in plants by pre-chilling treatment.

QUESTIONS

Very Short Answer Type Questions

1. Define plasticity. Give an example
2. What is the disease that formed the basis for the identification of gibberellins in plants? Name the causative fungus of the disease.
3. What is apical dominance? Name the growth hormone that causes it.
4. What is meant by bolting? Which hormone causes bolting?
5. Define respiratory climactic. Name the PGR associated with it.
6. What is ethephon? Write its role in agricultural practices.
7. Which of the PGRs is called stress hormone and why?
8. What do you understand by vernalisation? Write its significance.
9. Define the terms quiescence and dormancy.

Short Answer Type Questions

1. Write a note on agricultural/ horticultural applications of auxins.
2. Write the physiological responses of gibberellins in plants.
3. Write any four physiological effects of cytokinins in plants.
4. What are the physiological processes that are regulated by ethylene in plants?
5. Write short notes on seed dormancy.
6. Which one of the plant growth regulators would you use if you are asked to
   a) Induce rooting in a twig
   b) Quickly ripen a fruit
   c) Delay leaf senescence
   d) Induce growth in axillary buds
   e) 'Bolt' a rosette plant
   f) Induce immediate stomatal closure in leaves
   g) Overcome apical dominance
   h) Kill dicotyledonous weeds

Long Answer Type Questions

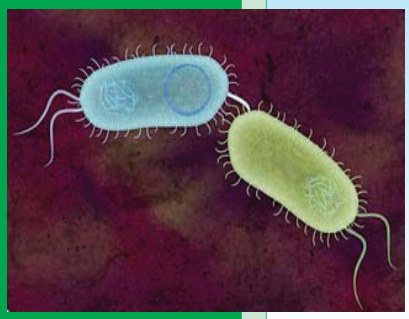
1. Define growth, differentiation, development, dedifferentiation, redifferentiation, determinate growth, meristem and growth rate.
2. Describe briefly
   a) Arithmetic growth
   b) Geometric growth
   c) Sigmoid growth curve
   d) Absolute and relative growth rates.
3. List five natural plant growth regulators. Write a note on discovery, physiological functions and agricultural/ horticultural applications of any one of them.

Exercises

1. Fill in the blanks with appropriate word/ words.
   a. The phase in which growth is most rapid is ___.
   b. Apical dominance as expressed in dicotyledonous plants is due to the presence of more _____ in the apical bud than in the lateral ones.
   c. In addition to auxin, a _____ must be supplied to the culture medium to obtain a good callus in plant tissue culture.
   d. ___ of vegetative plants are the sites of photoperiodic perception.
2. A primary root grows from 5 cm to 19 cm in a week. Calculate the growth rate and relative growth rate over the period.
3. Gibberellins promote the formation of _____ flowers on genetically _____ plants in *Cannabis* whereas ethylene promotes formation of _____ flowers on genetically _____ plants.
4. Classifiy the following plants into long- day plants (LDP), Short day plants (SDP) and Day Neutral Plants (DNP).

   Xanthium, Spinach, Henbane (*Hyoscyamus niger*), Rice, Strawberry, *Bryophyllum*, Sun-flower, Tomato, Maize.
5. A farmer grows cucumber plants in his field. He wants to increase the number of female flowers. Which plant growth regulator can be applied to achieve this?
6. Where are the following hormones synthesized in plants?
   a. IAA
   b. Gibberellins
   c. Cytokinins
7. Light plays an important role in the life of all organisms. Name any three physiological processes in plants which are influenced by light.
8. Growth is one of the characteristics of all living organisms. Do unicellular organisms also grow? If so, what are the parameters?
9. Rice seedlings infected with fungus *Gibberella fujikuroi* are called foolish seedlings. What is the reason?
10. Why isn't any one parameter good enough to demonstrate growth throughout the life of a flowering plant?
11. 'Both growth and differentiation in higher plants are open'. Comment.
12. 'Both a short day plant and a long day plant can produce flowers simultaneously in a given place'. Explain.
13. Would a defoliated plant respond to photoperiodic cycle? Why?

14. What would be expected to happen if:
    a) GA_3 is applied to rice seedlings
    b) Dividing cells stop differentiating
    c) A rotten fruit gets mixed with unripe fruits
    d) You forget to add cytokinin to the culture medium.



UNIT II

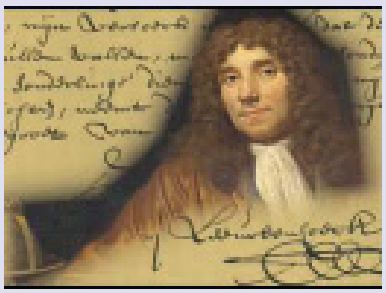
MICROBIOLOGY

Microbiology (Greek, micros (=small, bios(=life), logos(=study) is a branch of biology that deals with the scientific study of microorganisms, (simply, microbes) that are too small to be seen with the naked eye and is concerned with the structure, function, classification and ways of controlling and using their activities.

As already introduced to you in Chapter 1 of your First Year Book, microbes include a diverse group of simple life forms like, Protozoa, microscopic Algae and Fungi (yeasts and molds), Bacteria and Viruses. These are omnipresent in vast numbers and are described as **ubiquitous.** You will study more about bacteria and viruses in the next two chapters.

The work done by **Leeuwenhoek** (1632-1723) and later in the 19th century by **Louis Pasteur** (1822-1893) and **Robert Koch** (1843–1910) formed the foundation for this subject. **Louis Pasteur** developed the techniques of pasteurization and preparation of vaccines.

Microorganisms play a prime role in every field of human endeavor. They affect human life in several ways.



Anton Van Leeuwenhoek (1632-1723)

Leeuwenhoek's Microscope

Anton Van Leeuwenhoek, a Dutch merchant, made small hand–held microscopes as a hobby. Squinting through the lens at specimens held on a pin, he discovered a world of invisible creatures. He called them **animalcules** (small animals). He found them almost everywhere he looked, in water droplets, particles of soil, his teeth scrapings etc.

In 1674 Leeuwenhoek communicated his discoveries to the **Royal Society of London**, sending detailed drawings which were the first representations of **Bacteria and Protozoa**. Because of his pioneering work on these microbes, he is regarded as the **Father of Microbiology**. The most powerful of his microscopes had magnification of 266 x – powerful enough to see an average sized bacterial cell.

Chapter 7

Bacteria

Bacteria are among the most abundant organisms on Earth. The credit for observation and description of bacteria goes to **Anton Van Leeuwenhoek** (1674). Later **Ehrenberg** (1829) coined the term Bacteria for these microscopic creatures. In 1850s **Louis Pasteur's** work showed that bacteria are chemical factories capable of bringing about significant changes in nature. He did extensive research work on bacteria and is regarded as the **Father of Bacteriology**. In 1870s Koch's experiments established their link to infectious disease – the **germ theory of disease**. Due to the efforts of several enthusiastic scientists in the last two centuries to understand the bacterial world, the study of bacteria is now recognized as **Bacteriology**-a new branch of biology.

Can you recall the position of bacteria in Whittaker's Five Kingdom classification?

Bacteria are found everywhere. They are found in soil, water, air and on or inside living organisms. They also occur in a variety of foods. They can withstand extreme cold, heat and drought conditions. They are thus met within much unexpected media - like arctic snow, volcanic ash, hot water and sulphur springs etc. In terms of sheer numbers they greatly exceed every other group of organisms on the planet. Some bacteria are deadly parasites of plants, animals and human beings. Some bacteria make mutually beneficial symbiotic

association with plants. ***Escherichia coli*** *(E. coli)* is a common inhabitant of human intestines.

Can you recollect and name any such symbiotic bacterium and name the special structures in which it is accommodated on roots of plants?

7.1 Morphology of Bacteria

7.1.1 Size

Bacteria are very small organisms which are barely visible under the light microscope. Their size varies according to the species. Majority of the bacteria are in the range of 2.0 to 5.0μm in length and 0.5 to 1.0μm in breadth.

7.1.2 Shape

It is the rigid cell wall that determines the shape of a bacterial cell. Bacterial cells may be spherical (**Cocci**), as elongated rods (**Bacilli**), helical rods (**Spirillum**) or comma shaped (**Vibrios**), while a few are **pleomorphic** – that keep on changing their shape depending upon the type of environment and nutrients available. ***Acetobacter*** is one such pleomorphic bacterium. Some spirillum types of bacteria are flexible and referred to as 'Spirochaetes'. Some bacteria are in the form of a thread of filament (long chains) e.g. *Beggiotoa*.

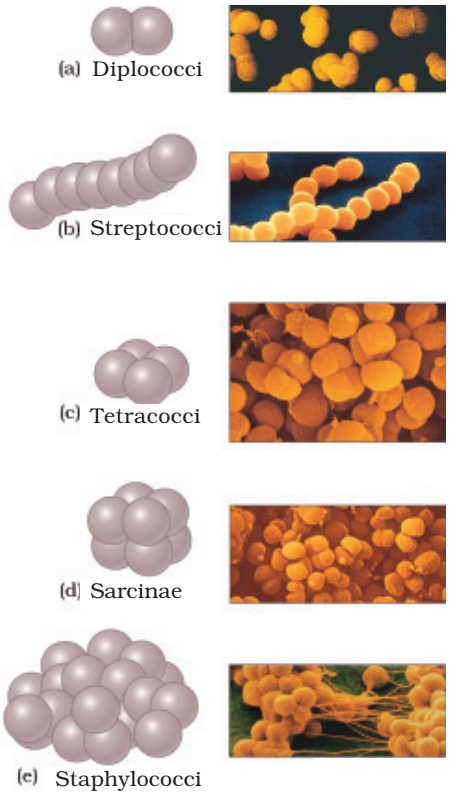


Figure 7.1 Arrangements in Coccal Forms of Bacteria

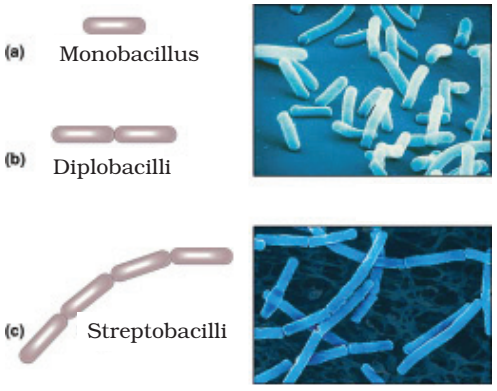


Figure 7.2 Arrangements of Bacillus forms

Bacteria may occur individually (e.g. Spirillum) or in groups. The arrangement of bacteria in groups depends upon adherence of cells together after cell division. The arrangement of cells is more complex in Cocci than in Bacilli. Based on the number of cells adhering together and their arrangement, the coccal forms are named as **Monococcus** (a single cell), **Diplococcus** (a pair of cells), **Tetracocci** (a group of four cells), **Streptococcus** (a linear chain of cells arranged in a single row), **Staphylococci** (irregular pattern of arrangement of cells producing bunches) and **Sarcinae** (cells arranged in cubes of eight).

In the same way, the bacillus forms exist as **Monobacillus** (a single elongated cell), **Diplobacillus** (paired cells of bacilli),and **Streptobacillus** (chains of bacilli appearing like straws).

Spiral forms may be **Vibrioid** (cells having less than one complete twist) or **Spirillum** (cells that have more than one complete twist – a distinct helical shape) or **Spirochete** (slender, long and cork-screw shaped).

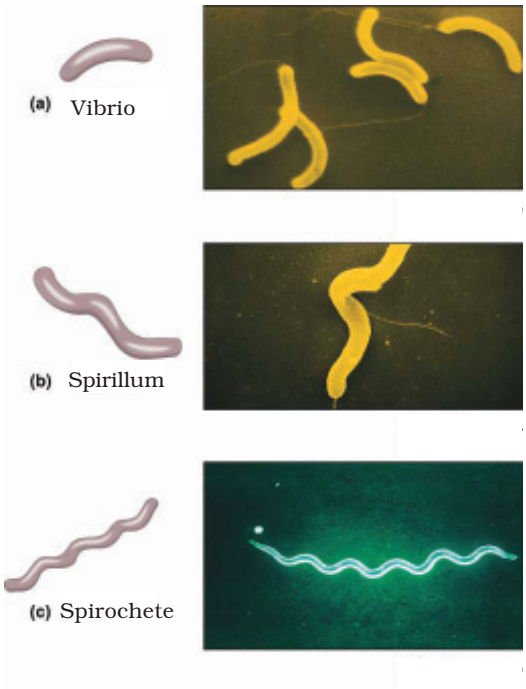


Figure 7.3 Spiral forms of Bacteria

7.2 Bacterial cell structure

With the development of the Electron Microscope in the 1940s, bacteria revealed themselves as more than simple spheres and rods. Scientists uncovered a wealth of microscopic and sub-microscopic details of bacteria and showed how very minute bacteria can be as complex as very large visible organisms. *Recollect the prokaryotic cell structure you have already studied in your first year.* However, we will discuss two variant components of bacterial cell here. These are:

7.2.1 Flagella

Some species of bacteria are endowed with flagella and show rapid wavelike movement. One advantage of motility is that it enables a bacterium to move toward a favourable environment or away from an adverse one.

Bacterial cells have four arrangements of flagella: **monotrichous** (a single polar flagellum), **amphitrichous** (a single flagellum at each end of the cell, **lophotrichous** (two or more flagella at one pole of the cell), and **peritrichous** (flagella distributed over the entire cell).

7.2.2 Plasmids

In addition to the "bacterial chromosome", or **genophore** which is the main genetic material, bacteria often contain small circular, double stranded DNA molecules called **plasmids**. Plasmids usually contain fewer genes than the genophore and often confer protective traits such as resistance to drugs and production of toxins and enzymes.They may be gained or lost without harming

the bacterial cell. Because plasmids can be readily manipulated in the laboratory and transferred from one bacterial cell to another, these are used as agents (vectors) in modern genetic engineering technique (described in Unit V).

7.3 Nutrition

The main determinants of a bacterial nutritional type are its sources of carbon and energy. Four major nutritional groups of bacteria are described following this criterion.

Photoautotrophs are photosynthetic, that is, they capture light energy and transform it into chemical energy and obtain carbon from atmospheric carbon dioxide (e.g. *Chromatium, Chlorobium*). **Chemoautotrophs** have an unusual nutritional adaptation that requires neither sunlight nor organic nutrients. These bacteria derive energy from the oxidation of inorganic substances and carbon from carbon dioxide. Chemoautotrophic bacteria such as *Nitrosomonas, Nitrobacter, Beggiotoa and Methanogens* play an important role in recycling inorganic nutrients.

Rhodospirillum, Rhodopseudomonas are **photoheterotrophs** which obtain energy from light but carbon is derived from organic sources. In contrast, **chemoheterotophs** derive both carbon and energy from organic compounds. Processing these organic molecules by respiration or fermentation releases energy in the form of ATP. Chemoheterotophs are categorized into two groups based on how they obtain their organic nutrients: **Saprophytes** are free living microorganisms that feed primarily on organic detritus from dead organisms (E.g. *Bacillus sps.*). **Parasites** derive nutrients from the cells or tissues of a host (E.g. *Xanthomonas, Salmonella*). Most microbes of biomedical importance belong to these two categories.

Bdellovibrio bacteriovorus grows as a parasite on some harmful bacteria, and their abundance is supposed to be responsible for the microbial purity of Ganges waters.

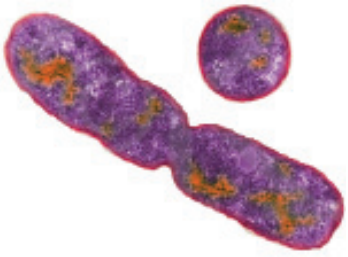


Figure 7.4 Bacterial Cell undergoing Binary Fission

7.4 Reproduction

*Do you remember that we studied in first year that Bacteria reproduces commonly by a kind of cell division called **binary fission**?* In this process, the dividing bacterial cell produces two genetically identical daughter cells. The interval of time between successive binary fissions of a

cell is known as generation time (or doubling time) which may be as little as 20 minutes in some bacteria.

7.4.1 Sexual Reproduction

True sexual reproduction is absent in bacteria. However, the exchange of genetic material (genetic recombination) which is the essence of sexual reproduction takes place in three ways. They are:

7.4.1.1 Conjugation

In this process, two live bacteria come together and the donor cell directly transfers DNA to the recipient cell. This process was first observed in 1946 by Lederberg and Tatum in *Escherichia coli*.

Figure 7.4 Bacterial cells in conjugation

The process of conjugation requires a special conjugation apparatus called the conjugation tube or pilus or sex pilus. For cell-to-cell contact, the **donor** cell designated F^+ produces the pilus that makes contact with the **recipient** cell, known as an F^- cell. The donor cell is called F+ because it contains a plasmid called an F plasmid. The F- cell lacks an F plasmid. Once contact is established, the pilus shortens to bring the two bacteria close together. The F plasmid then begins replicating and the replicated DNA passes through the bridge formed by the pilus to the recipient cell. Conjugation is a very conservative process, in that the donor bacterium generally retains a copy of the genetic material being transferred.

7.4.1.2 Transformation

This mode of bacterial genetic recombination was discovered by **Frederick Griffith** (1928) in *Streptococcus pneumoniae*. Transformation is uptake of naked DNA fragments from the surrounding environment and the expression of that genetic information in the recipient cell, that is, the recipient cell has now acquired a characteristic that it previously lacked. The discovery of DNA as the genetic material was based on transformation experiments (transforming principle).

7.4.1.3 Transduction

The transfer of genetic material from one bacterium to another through bacteriophage is known as transduction. It was discovered in 1951 by **Lederberg** and **Zinder** in *Salmonella typhimurium*.

7.5 The importance of Bacteria to Humans

Bacteria are known to cause plant, animal and human diseases. Yet many bacteria are directly or indirectly beneficial to humans. Hence, they are considered both as '**Friends and foes of man**'.

Some bacteria that cause human diseases are:

Clostridium tetani	Tetanus
Clostridium botulinum	Botulism
Vibrio cholera	Cholera
Salmonella typhi	Typhoid
Corynebacterium diphtheriae	Diphtheria
Mycobacterium tuberculosis	Tuberculosis
Diplococcus pneumonia	Pneumonia
Mycobacterium leprae	Leprosy
Neisseria gonorrhoea	Gonorrhoea
Treponema pallidum	Syphilis

Bacteria also cause certain plant diseases like **Blight of rice** (*Xanthomonas oryzae*), **citrus canker** (*X. axonopodis pv. citri*) and **crown gall** of apples and pear (*Agrobacterium tumefaciens*).

Microbes are now used in extracting valuable metals like uranium from rocks. The process is known as Bio-mining. The use of microbes in mining reduces the cost of production by more than 50%. DNA components from bacteria are used as **Biosensors** that can detect biologically active toxic pollutants. They also find application in medical diagnostics, food and fermentation operations. The most important development in Biotechnology depends on the possibility of altering the genetic makeup of bacteria through **genetic engineering** about which you will study in Chapter 11 & 12. Some of the more common uses of bacteria are presented in detail in Chapter 14.

SUMMARY

SUMMARY

Bacteria are an important group of microbes which are omnipresent. Though small, they are endowed with the ability to perform certain acts of living things i.e., they take food, grow and reproduce. Some bacteria are deadly parasites while a few form symbiotic associations with plants, animals and humans. The cell wall provides shape and protection to bacteria. Bacteria occur in several shapes – cylindrical (Bacillus), spherical (Cocci), spiral (Spirillum). Bacilli and cocci also occur in a number of cellular arrangements. One or more flagella occur on many bacilli and provide for cellular motility. Saprophytic and parasitic bacteria have biomedical importance. Bacteria normally reproduce by binary fission. Bacterial plasmids can be manipulated in the laboratory and are used as vectors in rDNA technology. Genetic exchange among bacteria takes place by conjugation, transformation and transduction. Conjugation is genetic transfer through direct contact between two bacterial cells and is facilitated by conjugative plasmids. Some bacteria can take up DNA from their environment and incorporate it into their genome to undergo transformation. Transduction is mediated by bacteriophages.

GLOSSARY

Antibiotic: An antimicrobial agent produced naturally by a bacterium or fungus.

Germ theory of disease: The principle that microorganisms cause disease.

Pasteurization: The process of mild heating to kill particular spoilage microorganisms or pathogens.

Symbiosis: The living together of two different organisms or populations.

Vaccine: is a biological preparation that improves immunity to a particular disease.

QUESTIONS

Very Short Answer Type Questions

1. Write briefly on the occurrence of microorganisms.
2. Define Microbiology.
3. Name the bacteria which is a common inhabitant of human intestine.How is it used in biotechnology?
4. What are pleomorphic bacteria? Give an example.
5. What is sex pilus?What is its function?
6. What is a genophore ?
7. What is a plasmid? What is its significance?
8. What is conjugation ?Who discovered it and in which organism?
9. What is transformation ?Who discovered it and in which organism?
10. What is transduction?Who discovered it and in which organism?

Short Answer Type Questions

1. Explain the importance of Microbiology.
2. How are bacteria classified on the basis of morphology?
3. How are bacteria classified on the basis of number and distribution of flagella?
4. What are the nutritional groups of bacteria based on their source of energy and carbon.
5. Write briefly about chemoheterotrophs and their significance.
6. Explain the conjugation in bacteria.

Long Answer Type Questions

1. Explain different methods of sexual reproduction in bacteria.
2. "Bacteria are friends and foes of man"-Discuss.

Exercises

1. Many people believe that bacteria do little more than cause human illness and infectious diseases. How does the information in this chapter help you correct that misconception?
2. Humans produce about 50 grams of feces per day. Scientists estimated in broad terms about one-third of human feces is composed of bacteria. If one *E. coli* cell weighs $1x10^{-12}$ g, how many bacteria are there in a day's feces? How can this be possible?
3. An organism is described as a peritrichous bacillus. How might you translate this bacteriological language into a description of the organism.

Chapter 8

Viruses

Viruses are a unique group of "**biological entities**" known to infect every type of cell, including those of bacteria, algae, fungi, protozoa, plants and animals.

Remember that you have already gone through an introductory account of viruses in your first year course.

Viruses are very different from other organisms. They are not composed of cells and cannot be seen under a light microscope. A virus particle contains a single type of nucleic acid, either DNA or RNA. The nucleic acid is enclosed in a protein coat and the coat is sometimes covered in an envelope of lipids, proteins and carbohydrates. Viruses do not exhibit most of the life processes of a cell, but maintain genetic continuity through multiplication and undergo mutations. Thus, they are certainly more than inert lifeless molecules. Viruses are best described as **infectious particles**.

Viruses are **obligate intracellular parasites** i.e. they cannot multiply unless they invade a specific host cell and instruct its genetic and metabolic machinery to make and release daughter or progeny viruses. In this process, they destroy the host cells causing serious damage and diseases in humans, plants and animals. The study of viruses is known as **Virology**.

8.1 Discovery

Viruses have been victimizing mankind from ancient times, causing many diseases in humans and also in economically useful plants and animals. An identifiable agent responsible for these diseases was not known, even after the proposition of the ***germ theory of disease***. In 1892 for the first time, the Russian pathologist **Dmitri Iwanowski**, while studying **tobacco mosaic disease**, filtered the '***sap of diseased tobacco leaf***' through filter which was designed to retain bacteria. However the 'infectious agent' passed through the pores of the filter. After injecting the filtered sap into a healthy plant, he found the development of symptoms of mosaic disease in it . Unable to see any microorganism in the clear sap, Iwanowski reported that a **filterable agent** was responsible for the disease. Later, **Martinus Beijerinck** repeated Iwanowski's experiments and concluded that the disease causing agent was a 'contagious living fluid' (***contagium vivum fluidum***), rather than a discrete entity.

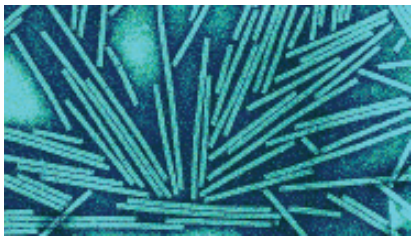


Figure 8.1 Crystallized forms of 'TMV' obtained by Stanley

W.M. Stanley (1935) purified the sap and announced that the virus causing mosaic disease in tobacco could be crystallized. It was named Tobacco Mosaic Virus (TMV). **Fraenkel Conrat** (1956) confirmed that the genetic material of the TMV is **RNA**. Utilizing the techniques of *ultracentrifugation*, *X-ray crystallography* and *electron microscopy*, a number of new viruses were reported and their ultrastructure was elucidated.

8.2 Classification of Viruses

At present the **International Committee on Taxonomy of Viruses** (**ICTV**) regulates the norms of classification and nomenclature of viruses. The ICTV scheme has only three hierarchial levels – the **Family** (including some sub-families), **Genus** and **Species**. The family names end with the suffix 'viridae' while the genus names with 'virus' and the species names are common English expressions describing their nature. Viruses are named after the disease they cause (e.g. polio virus). Using the ICTV system, the virus that causes **Acquired Immune Deficiency Syndrome** (**AIDS**) in human beings is classified as: Family: Retroviridae, Genus: Lentivirus, Species: Human Immune deficiency Virus (HIV).

8.3 Structure of Viruses

Viruses range in size from 300 nanometers (nm) as in TMV to 20 nm as in **parvoviruses**. At the upper end of the spectrum, viruses approximate the size of the smallest bacterial cells such as mycoplasmas and at the lower end, they have about the same diameter as that of a 'ribosome'. Viruses may be classified into several different morphological types. **Helical viruses** resemble long rods that may be rigid or flexible like rabies virus and tobacco mosaic virus. Many animal, plant and bacterial viruses are **polyhedral** (many-sided). Herpes simplex and polio viruses exist in this form. The capsid of some viruses is covered by an envelope and such **enveloped** viruses are roughly spherical. Influenza virus is an enveloped virus. **Bacteriophages**, the viruses which infect bacteria, have complicated structures and are called **complex viruses**. They have '**polyhedral symmetry**' in the 'head' and 'helical symmetry' in the 'tail sheath'. In some viruses such as the measles virus, the envelope contains '**glycoprotein**' projections known as **spikes**. The spikes function in attaching the virus to receptors on susceptible host cells.

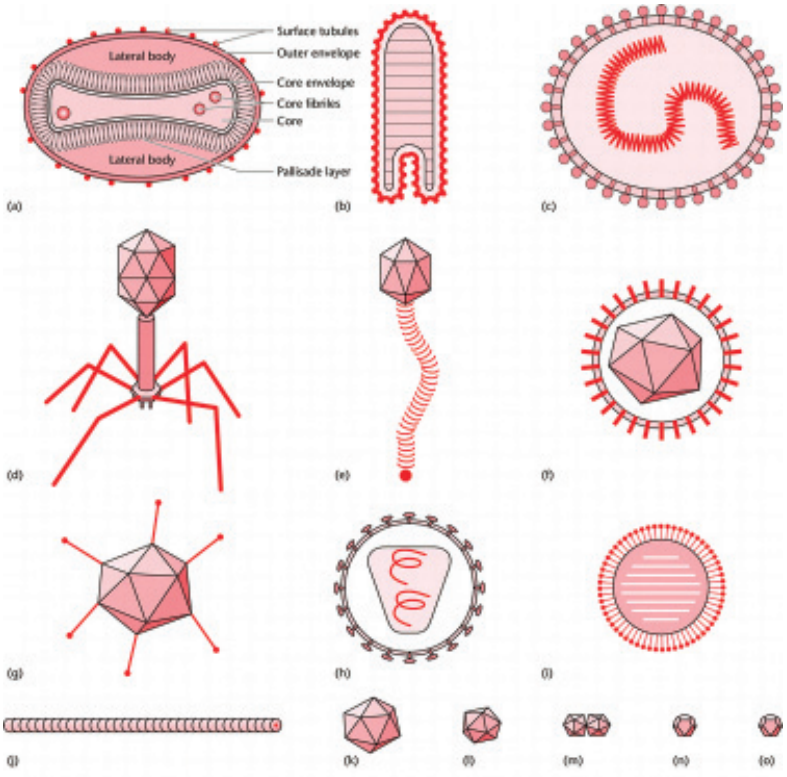


Figure 8.2 (a) Vaccinia Virus (b) Rabies Virus (c) Mumps Virus (d) Bacteriophage (e) Lambda Phage (f) Rubella Virus (g) Adeno Virus (h) HIV Virus (i) Influenza Virus (j) TMV (k) Pailloma Virus (l) Papova Virus (m) Turnip Yellow Mosaic Virus (n) Wound tumor Virus.

All viruses consist of two basic components: a **core** of nucleic acid that forms the **genome** and the surrounding coat of protein known as **capsid.** The capsid gives shape to the virus and provides a protective covering for the genome. It is made up of protein subunits called **capsomeres**. The number of capsomeres is characteristic for each type of virus.

A virus contains its genetic information in either a double stranded (ds) DNA, or single stranded (ss) DNA, i.e., **dsDNA** or **ssDNA**. In general, viruses that infect plants have **ssRNA** and viruses that infect animals have **dsDNA**. Bacteriophages are usually **dsDNA** viruses. Viral nucleic acid molecules are either circular or linear. Most viruses have a single nucleic acid molecule, but a few have more than one (e.g. **HIV**– which has two identical molecules of RNA, representing its **genomic copies**).

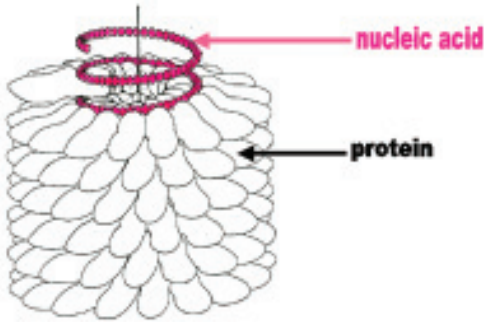


Figure 8.3 Tobacco Mosaic Virus – Helical Symmetry

Let us look at the detailed structure of two typical viruses - the TMV and T_4 phage.

The tobacco mosaic virus is about 300 nm long and 18 nm in diameter, with a molecular weight of 39 x10^6 Daltons. The capsid is made of 2,130 protein subunits of identical size, called capsomeres. The capsomeres are arranged in a helical manner around a central hollow space of 4 nm (40Å). Each protein subunit is made up of a single polypeptide chain which possesses 158 amino acids. Inside the protein capsid there is a single stranded spirally coiled RNA molecule consisting of 6,500 nucleotides.

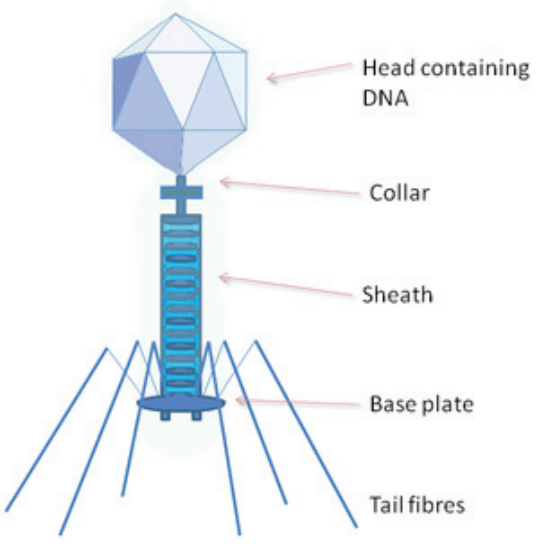


Figure 8.4 Bacteriophage- A complex virus

The body of a typical bacteriophage such as T_4 can be distinguished into head and tail regions joined by a collar. The tail region includes a tail sheath, a base plate, pins and tail fibres which help the virus attach to host cell. The tail sheath aids in injecting viral DNA into the host cell.

8.4 Multiplication of Bacteriophages

For a virus to multiply or reproduce or replicate, it must invade a host cell and take over the host's metabolic machinery. Though the method by which a virus enters and exits a host cell may vary, the basic mechanism of viral multiplication is similar for all viruses. The best understood viral life cycles are those of the bacteriophages. Phages can multiply by two alternative mechanisms: the **lytic cycle** and the **lysogenic cycle.** The lytic cycle ends with the 'lysis' or death of the host cell, whereas the host cells remains alive in the lysogenic cycle.

T-even phages that attack the bacterium *E. coli* cause lysis of the cells and are called **virulent** phages. They show **lytic cycle**, which is a five-step process involving - *attachment*, *penetration*, *biosynthesis*, *maturation* and *release*.

After a chance contact between phage particles and bacteria, **attachment** or **adsorption** occurs. The phages use tail fibres for attachment to the *complementary receptor sites* on the bacterial cell wall. Attachment is followed by the process of **penetration,** during which the tail sheath of phage contracts, and the tail core is driven in through the bacterial cell wall. When the tip of the core reaches the plasma membrane, the DNA from the bacteriophage head passes through the tail core through the plasma membrane and enters the bacterial cell. The capsid remains outside the bacterial cell and is referred to as **ghost**. Thus, the phage particle functions like a hypodermic syringe and injects its DNA into the bacterial cell.

Once the phage DNA reaches the cytoplasm of the host cell, many copies of phage DNA, enzymes and capsid proteins are synthesized, using the cellular machinery of the host cell. For several minutes following infection, complete phages cannot be found in the host cell but the individual DNA and protein components can be detected. The next in the sequence of events is the process of 'maturation'. In this process, bacteriophage DNA and capsids are assembled into complete **virions.** This period of time between the infection by a virus and the appearance of the mature virus within the cell is called the **eclipse period**.

The final stage of viral multiplication is the **lysis phase** of the host cell and the release of virions from the host cell. The plasma membrane of the host cell gets dissolved or ***lysed*** due to the viral enzyme, called **lysozyme**, an enzyme synthesized within the cell and the bacterial cell wall breaks releasing the newly produced **phage particles** / **virions**.

The number of newly synthesized phage particles released from a single cell is referred to as the **"burst size"**, and it usually ranges from about 50 to 200. The released phage particles infect other susceptible cells in the vicinity and the multiplication cycle is repeated.

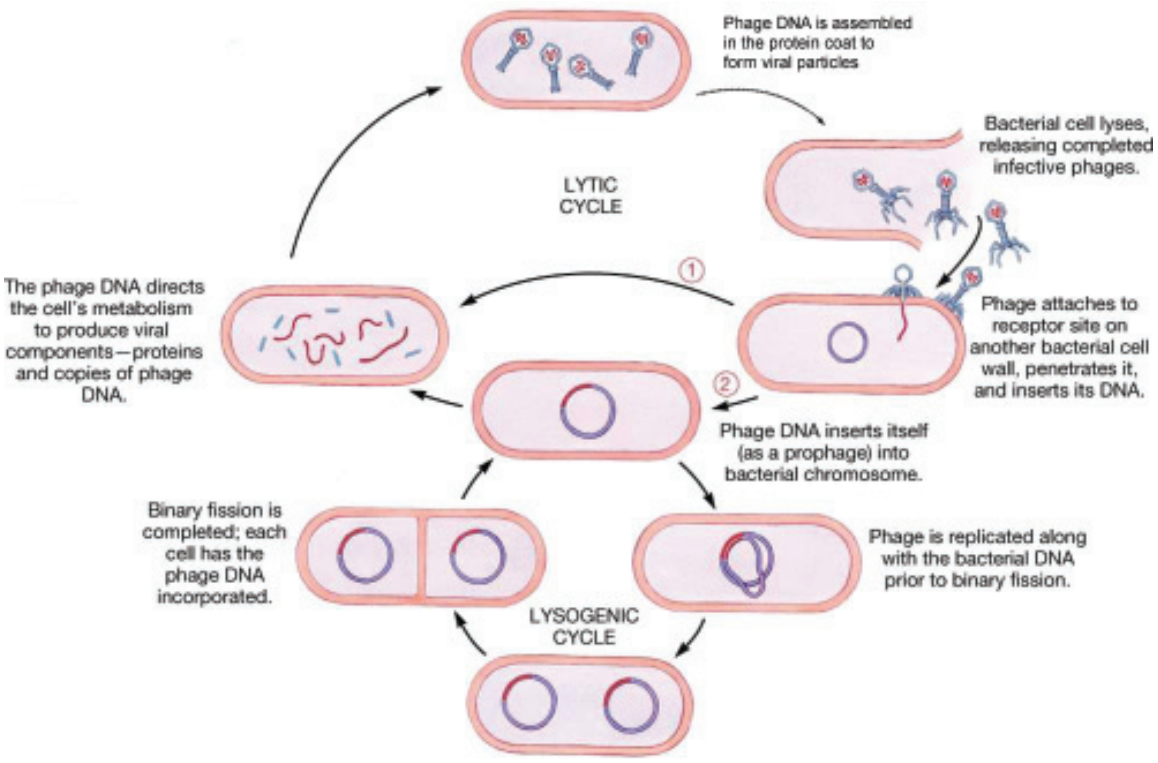


Figure 8.5 Viral multiplication cycles

8.4.1 The Lysogenic Cycle

Some *bacteriophages* such as λ (**Lambda**) phages do not cause lysis and death of the host cell when they multiply. Instead, the phage DNA upon penetration into an *E. coli* cell gets **integrated** in to the circular bacterial DNA, becomes part of it and remains **latent** (inactive). Such phages are called **temperate** phages. The inserted phage DNA is now called **prophage.** Every time the bacterial genetic material replicates, the **prophage** also undergoes replication. The prophage remains latent within the progeny cells. However, in some rare spontaneous events or when the host cell is exposed to UV light or some chemicals, the phage DNA separates from the bacterial genetic material, leading to the initiation of the lytic cycle.

*Which of the two - **lytic** or **lysogenic**-cycles facilitates the phenomenon of '**transduction**'?*

8.5 Viral diseases in Plants

Viruses, being obligate parasites, cause many plant diseases while growing inside them. Plant diseases caused by viruses are mostly systemic in nature (affect the whole plant). The leaves generally exhibit characteristic symptoms. The usual symptoms of viral diseases are **chlorosis** (e.g. peach yellowing disease), **mosaic** (e.g. tobacco mosaic disease), **vein clearing** (e.g. bhendi vein clearing), **malformation** (e.g. swollen shoot of cocoa) and **breaking of flowers** (e.g. tulip mosaic break) etc.

Usually virions are infectious and cause diseases. A virion is a completely assembled virus outside its host cell. Unusually a tiny fragment of nucleic acid with 300-400 nucleotides and without a protein coat, called **viroid,** can cause plant diseases on a variety of economically important plants (e.g. tomatos, potatos, cucumbers, citrus).

8.6 Viral diseases in Humans

Viruses cause widespread diseases in humans such as common cold, hepatitis, chickenpox , influenza, herpes, warts, polio etc. Although most viral infections do not result in death, some such as Rabies, AIDS and Ebola have high mortality rate, others such as polio and neonatal rubella can lead to long-term debility (physical weakness).

Do you know, some viruses such as Epstein-Barr virus and Human Papilloma virus cause cancers in animals and humans!

Chronic hepatitis B (caused by Hepatitis B virus) may lead to cancer. Such cancer causing viruses are called Oncoviruses. Not only viruses, but also "*proteinaceous infectious particles*" called **prions**, cause some serious animal diseases such as mad cow disease (Bovine Spongiform Encephalitis) in cows and scrapie disease in sheep. The 'mad cow disease' causing prion may reach man through beef and cause '**Creutzfeldt-Jakob disease**' in him .

SUMMARY

Viruses are very small, infectious, obligate intracellular parasites. They are acellular and lack metabolism of their own. Structurally they are very simple, with a nucleic acid core protected by a protein capsid. The complete virus particle is called a virion and its genome comprises of either DNA or RNA. Viruses can be crystallized. Viruses occur in several morphological forms. Viruses are grouped according to their shared properties and are classified by ICTV system of classification. Viruses multiply in host cells which they attack. The viral genome is replicated and directs the synthesis of other virion components by cellular systems of other virion components.

Viruses that attack bacteria are called bacteriophages. Virulent phages follow lytic cycle of replication whereas temperate phages follow lysogenic cycle of replication. In this process viruses cause several human, plant and animal diseases. Some of the viruses can cause cancer and they are called oncogenic viruses. There exist infectious agents that are simpler than viruses-viroids and prions which also can cause diseases.

SUMMARY

GLOSSARY

Burst size: The number of newly synthesized bacteriophage particles released from a single affected host cell.

Latent infection: A condition in which a pathogen remains in the host for long periods without causing any disease symptoms.

QUESTIONS

Very Short Answer Type Questions

1. Mention the living and non-living characters of viruses.
2. What is the shape of T_4 phage? What is its genetic material?
3. What are virulent phages. Give an example
4. What is lysozyme and what is its function ?
5. Define 'lysis' and 'burst size' with reference to viruses and their effects on host cells .
6. What is a prophage
7. What are temperate phages ? Give one example.
8. Mention the differences between virulent phages and temperate phages.

Short Answer Type Questions

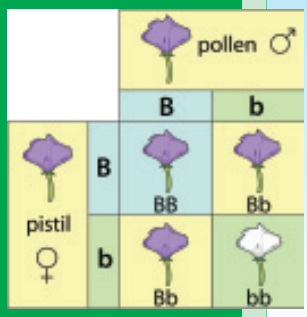
1. What is ICTV? How are viruses named?
2. Explain the chemical structure of viruses.
3. Write briefly about the symmetry of viruses.
4. Explain the structure of TMV.
5. Explain the structure of T-even bacteriophages.
6. Explain the lytic cycle with reference to certain viruses .
7. Explain how temperate phages play a role in transduction.
8. Mention the differences between lytic and lysogenic cycles.

Long Answer Type Questions

1. Write about the discovery and structural organization of viruses.
2. Describe the process of multiplication of viruses.

Exercises

1. When discussing the multiplication of viruses, Virologists prefer to call the process as replication, rather than reproduction. Why?
2. In dealing with public health, the approach to deal with bacterial diseases is treatment. Can you guess the nature of the general public health approach to viral diseases? What example do you cite to support your answer?



UNIT III

GENETICS

Chapter 9 : Principles of Inheritance and Variation

The work of Mendel and others who followed him gave us an idea of inheritance patterns. However the nature of those 'factors' which determine the phenotype was not very clear. As these 'factors' represent the genetic basis of inheritance, understanding the structure of genetic material and the structural basis of genotype and phenotype conversion became the focus of attention in biology for the next century. The entire body of molecular biology was a consequent development with major contributions from Watson, Crick, Nirenberg, Khorana, Kornbergs (father and son), Benzer, Monod, Brenner, etc. In this unit the basic principles of inheritance have been examined and explained.

Gregor Johann Mendel
(1822-1884)

Gregor Johann Mendel, an Austrian monk, discovered the basic principles of heredity through experiments on the pea plant.

Johann Mendel was born on July 22, 1822, and spent his early youth in a rural setting, until age 11, when a local schoolmaster who was impressed with his aptitude for learning, recommended that he be sent to secondary school. Mendel excelled in his studies and in 1840 graduated from the school with honors.

Following his graduation, Mendel enrolled in a two-year program at the Philosophical Institute of the University of Olmütz. There, he again distinguished himself academically, particularly in the subjects of physics and math, and tutored in his spare time to make ends meet. Mendel joined the Augustinian order at the St. Thomas Monastery in Brno, and was given the name Gregor.

In 1853, upon completing his studies at the University of Vienna, Mendel returned to the monastery in Brno and was given a teaching position at a secondary school, where he stayed for more than a decade. It was during this time that he began the experiments for which he is best known.

Around 1850, Mendel began to research the transmission of hereditary traits in plant hybrids. He published the results of his studies under the title "Experiments on Plant Hybrids" The observations he made while growing peas in his monastery's garden became the foundation of modern genetics and the study of heredity.

CHAPTER 9

Principles of Inheritance and Variation

Have you ever wondered why an elephant always gives birth only to a baby elephant and not to some other animal? Or why a mango seed forms only a mango plant and not any other plant?

Given that they do, are the offspring identical to their parents? Or do they show differences in some of their characteristics? Have you ever wondered why siblings sometimes look very similar to each other? Or sometimes even so different?

These and several related questions are dealt with, scientifically, in a branch of biology known as Genetics. This subject deals with the inheritance, as well as the variation of characters from parents to offspring. Inheritance is the process by which characters are passed on from parent to progeny; it is the basis of heredity. Variation is the degree by which progeny differ from their parents as well as among themselves.

Humans knew from as early as 8000-1000 B.C. that one of the causes of variation was hidden in sexual reproduction. They exploited the variations that were naturally present in the wild populations of plants and animals to

selectively breed and choose organisms that possessed desirable characters. For example, through artificial selection and domestication from ancestral wild cows, we have well-known Indian breeds, e.g., Sahiwal cows in Punjab and 'Ongole' bulls in Andhra Pradesh. We must, however, recognise that though our ancestors knew about the inheritance of characters and variation, they had very little idea about the scientific basis of these phenomena.

9.1 Mendel's Experiments

It was during the mid-nineteenth century that headway was made in the understanding of inheritance. Mendel conducted hybridisation experiments on garden peas for seven years (1856-1863) and proposed the laws of inheritance in living organisms. During Mendel's investigations into inheritance patterns, statistical analysis and mathematical logic were applied to problems in biology for the first time. Mendal's experiments had a large sampling size, which gave greater credibility to the data that he collected. Also, the confirmation of his inferences from experiments on successive generations of his test plants proved that his results pointed to general rules of inheritance and were not unsubstantiated ideas. Mendel investigated characters in the garden pea plant that manifested as two opposing traits, e.g., tall or dwarf plants, yellow or green seeds. This allowed him to set up a basic framework of rules governing inheritance, which was expanded by later scientists to account for all the diverse natural observations and the complexity inherent in them.

Mendel conducted artificial pollination/cross pollination experiments using several true-breeding pea lines. A truebreeding line is one that, having undergone continuous self-pollination, shows the stable trait inheritance and expression for several generations. Mendel selected 14 true-breeding pea plant varieties, as pairs which were similar except for one character with contrasting traits. Some of the contrasting traits selected were smooth or wrinkled seeds, yellow or green seeds, smooth or inflated pods, green or yellow pods and tall or dwarf plants (Table 9.1).

Table 9.1 Contrasting Traits studied by Mendel in Pea

S.No.	Characters	Constrasting Traits
1.	Stem height	Tall/dwarf
2.	Flower colour	Violet/white
3.	Flower position	Axial/terminal
4.	Pod shape	Inflated/constricted
5.	Pod colour	Green/ yellow
6.	Seed shape	Round/ wrinkled
7.	Seed colour	Yellow/ green

Advantages of selecting garden pea plant by Mendel for his hybridization experiments are:

1. It is an annual plant that has well defined characteristics.
2. It can be grown and crossed easily.

3. It has bisexual flowers containing both female and male parts.
4. It can be self-fertilized conveniently.
5. It has a short life cycle and produces large number of offsprings.

9.2 Inheritance of one gene (Monohybrid Cross)

Let us take the example of one such hybridisation experiment carried out by Mendel where he crossed tall and dwarf pea plants to study the inheritance of one gene (Figure 9.1). He collected the seeds produced as a result of this cross and grew them to generate plants of the first hybrid generation. This generation is also called the **First Filial progeny** or the **F_1**. Mendel observed that all the F_1 progeny plants were tall, like one of its parents; none were dwarf (Figure 9.2). He made similar observations for the other pairs of traits – he found that the F_1 always resembled either of the parents, and that the trait of the other parent was not seen in them.

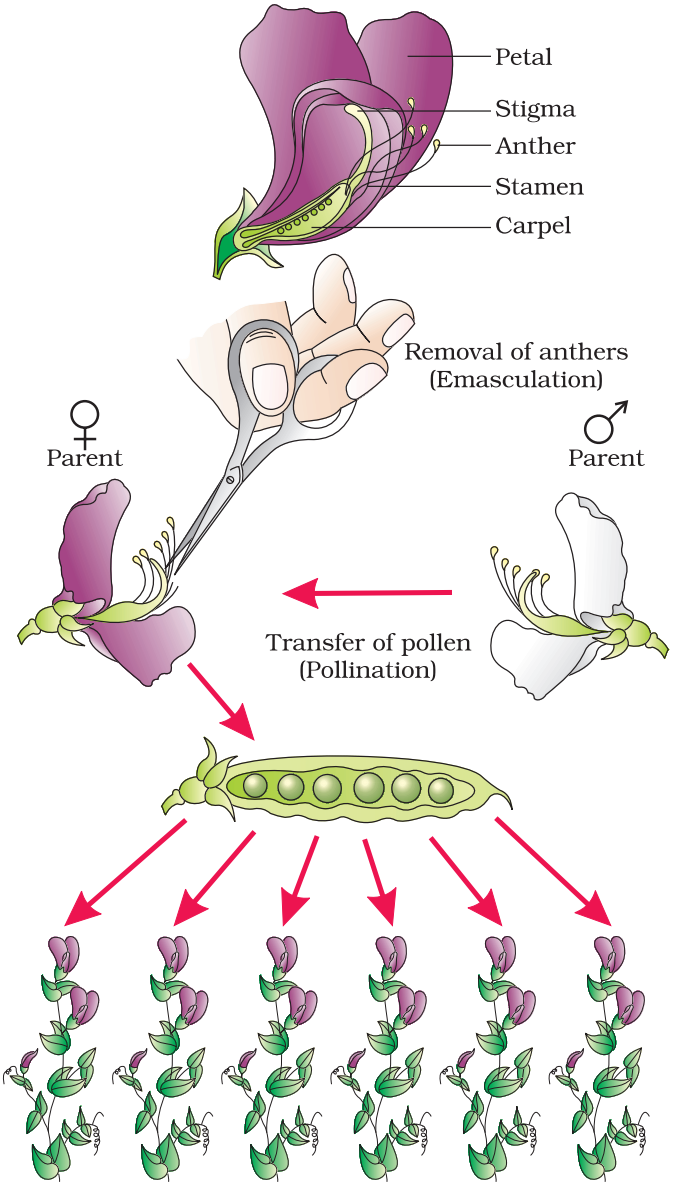


Figure 9.1 Steps in making a cross in pea

Mendel then self-pollinated the tall F_1 plants and to his surprise found that in the **Second Filial generation** or **F_2** some of the offspring were 'dwarf'; the character that was not seen in the F_1 generation was now expressed. The proportion or probability of plants that were dwarf were $1/4^{th}$ of the F_2 plants while $3/4^{th}$ of the F_2 plants were tall. The tall and dwarf traits were identical to their parental types and did not show any blending, that is all the offspring were either tall or dwarf, none were of in-between height (Figure 9.2).

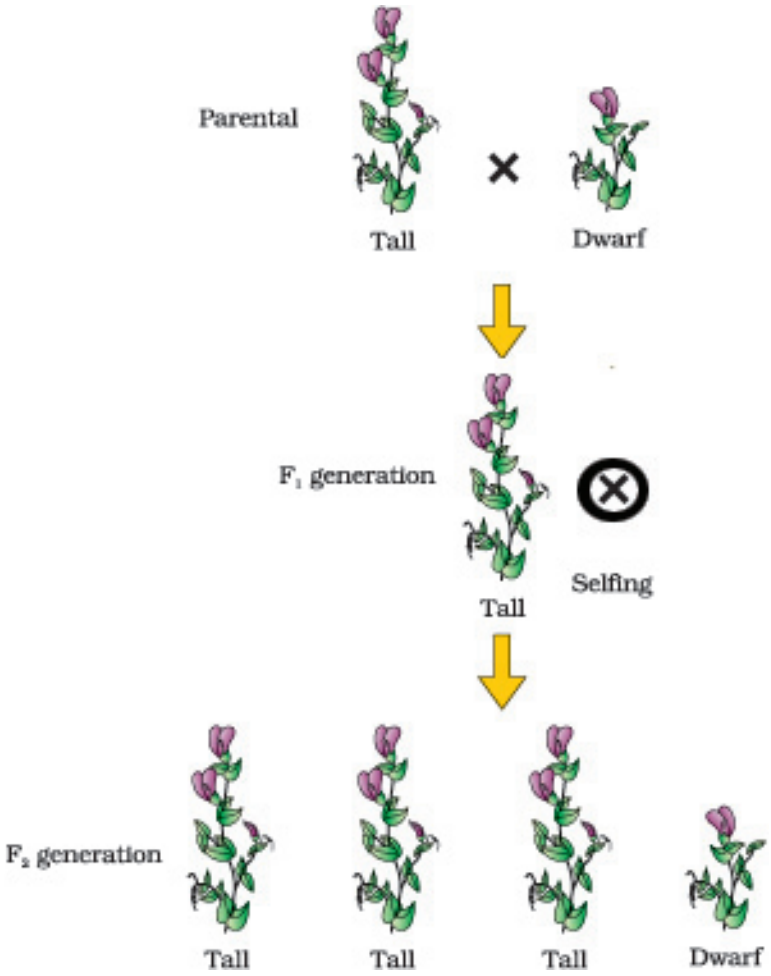


Figure 9.2 Diagrammatic representation of monohybrid cross

Similar results were obtained with the other traits that he studied: only one of the parental traits was expressed in the F_1 generation while at the F_2 stage both the traits were expressed in the proportion 3:1. The contrasting traits did not show any blending at either F_1 or F_2 stage.

Based on these observations, Mendel proposed that something was being stably passed down, unchanged, from parent to offspring through the gametes, over successive generations. He called these things as 'factors'. Nowadays, we call them **genes**. Genes, therefore, are the units of inheritance. They contain the information that is required to express a particular trait in an organism. Genes which code for a pair of contrasting traits are known as **alleles**, i.e., they are slightly different forms of the same gene.

If we use alphabetical symbols for each gene, then the capital letter is used for the trait expressed at the F_1 (or hybrid) stage and the small alphabet for the other trait. For example, in case of the character of height, ***T*** is used for the Tall trait and ***t*** for the 'dwarf', and ***T*** and ***t*** are alleles of each other. Hence, in plants the possible pairs of alleles for height would be ***TT***, ***Tt*** or ***tt***. Mendel also proposed that in a true breeding tall or dwarf pea variety, the allelic pair of genes for height are identical or **homozygous**, ***TT*** and ***tt***, respectively. ***TT*** and ***tt*** are called the **genotype** of the plant while the descriptive terms **tall** and **dwarf** are the **phenotype**.

*What would be the phenotype of a plant that had a genotype **Tt**?*

As Mendel found the phenotype of the F_1 **heterozygote *Tt*** to be exactly like the ***TT*** parent in appearance, he proposed that in a pair of dissimilar factors, one dominates the other (as in the F_1) and hence is called the **dominant** factor while the other factor is **recessive**. In this case ***T*** (for tallness) is dominant over ***t*** (for dwarfness), that is recessive. He observed identical behaviour for all the other characters/trait-pairs that he studied (Figure 9.3).

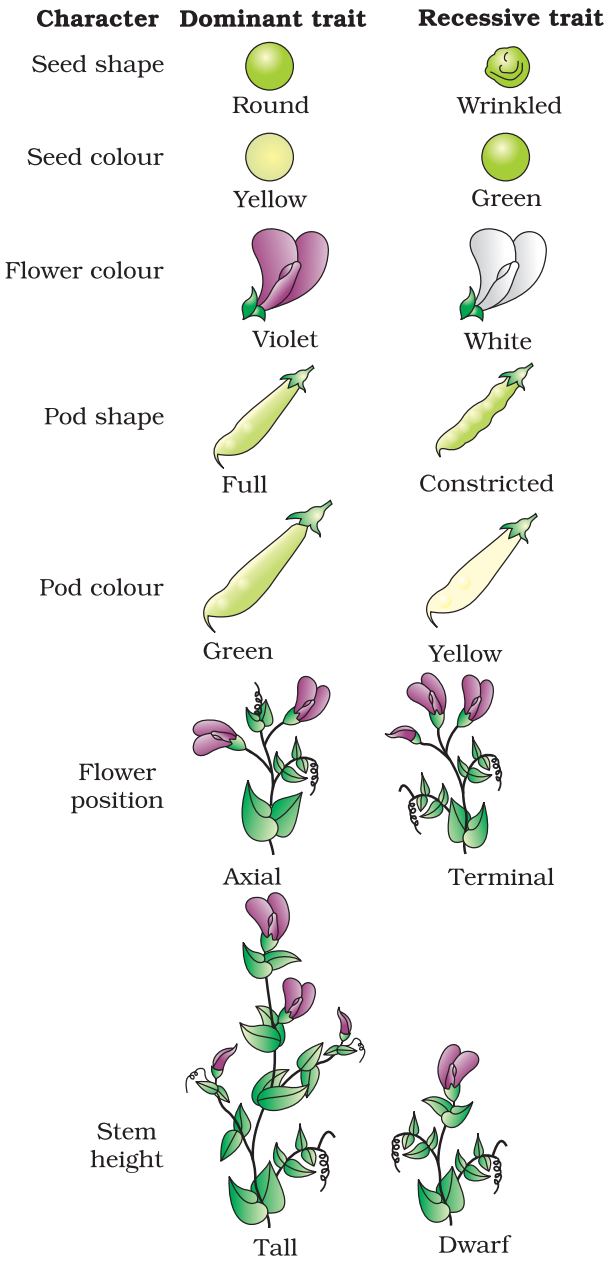
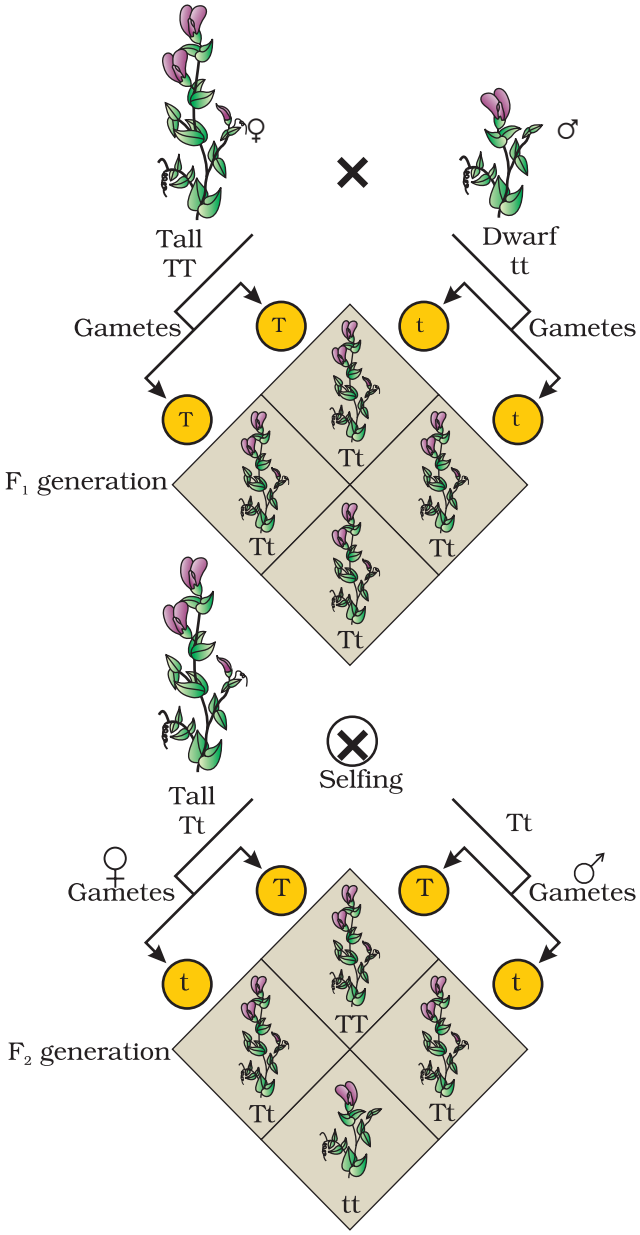


Figure 9.3 Seven pairs of contrasting traits in pea plant studied by Mendel

It is convenient (and logical) to use the capital and lower case of an alphabetical symbol to remember this concept of dominance and recessiveness. (Do not use ***T*** for tall and ***d*** for dwarf because you will find it difficult to remember whether ***T*** and ***d*** are alleles of the same gene/character or not). Alleles can be similar as in the case of homozygotes ***TT*** and ***tt*** or can be dissimilar as in the case of the heterozygote ***Tt***. Since the ***Tt*** plant is heterozygous for genes controlling one character (height), it is a **monohybrid** and the cross between ***TT*** and ***tt*** is a **monohybrid cross**.

From the observation that the recessive parental trait is expressed without any blending in the F_2 generation, we can infer that when the tall and dwarf plants produce gametes, by the process of meiosis, the alleles of the parental pair separate or **segregate** from each other and only one allele is transmitted to a gamete. This segregation of alleles is a random process and so there is a 50 per cent chance (0.5 probability) of a gamete containing either allele, as has been verified by the results of the crossings. In this way the gametes of the tall ***TT*** plants have the allele ***T*** and the gametes of the dwarf ***tt*** plants have the allele ***t***. During fertilisation the two alleles, ***T*** from one parent say, through the pollen, and ***t*** from the other parent,



Phenotypic ratio : tall : dwarf
3 : 1
Genotypic ratio : TT : Tt : tt
1 : 2 : 1

Figure 9.4 A Punnett square used to understand a typical monohybrid cross between true-breeding tall plants and true-breeding dwarf plants conducted by Mendel

then through the egg, are united to produce zygotes that have one ***T*** allele and one ***t*** allele. In other words the hybrids have ***Tt***. Since these hybrids contain alleles which express contrasting traits, the plants are **heterozygous**. The types of gametes produced by the parents, the formation of the zygotes and the progeny can be represented in a diagram called **Punnett Square** (commonly called checker board) as shown in Figure 9.4. It was developed by a British geneticist, Reginald C. Punnett. It is a graphical representation to indicate all possible unions of gametes during fertilization and to calculate the probability of all possible genotypes of offspring in a genetic cross. The possible gametes are written on two sides, usually the top row and left columns. All possible combinations of gametes during fertilization are represented in boxes below in the squares, which generates a square output form.

The figure 9.4 shows the parental tall **TT** (male) and dwarf **tt** (female) plants, the gametes produced by them and the F_1 **Tt** progeny.The F_1 plants of genotype ***Tt*** are self-pollinated. The symbols ♀and ♂ are used to denote the female (eggs) and male (pollen) gametes of the F_1 generation, respectively. The F_1 plant of the genotype ***Tt*** produces gametes of the genotype ***T*** and ***t*** in equal proportion. When fertilisation takes place, the pollen grains of genotype ***T*** have a 50 per cent chance to pollinate eggs of the genotype ***T***, as well as of genotype ***t***. Also pollen

grains of genotype ***t*** have a 50 per cent chance of pollinating eggs of genotype ***T***, as well as of genotype ***t***. As a result of random fertilisation, the resultant zygotes can be of the genotypes ***TT***, ***Tt*** or ***tt.***

Can you identify the difference in the representation of genotypes of gametes and genotypes of zygotes in the Punnet Square?

From the Punnet square it is easily seen that $1/4^{th}$ of the random fertilisations (i.e with a probability of 0.25) lead to ***TT***, 1/2 (= probability 0.5) lead to ***Tt*** and $1/4^{th}$ (probability 0.25) to ***tt***. Though the F_1 have a genotype of ***Tt***, the phenotypic character seen is 'tall'. At F_2, $3/4^{th}$ of the plants are tall, where some of them are ***TT*** while others are ***Tt***. Externally it is not possible to distinguish between the plants with the genotypes ***TT*** and ***Tt***. Hence, in the two genotypes ***TT*** and ***Tt***, only tall '***T*** is expressed. Hence the character 'tall' or allele **T** is said to dominate over the other allele **t** or 'dwarf' character. It is thus due to this dominance of one character over the other that all the F_1 are tall (though the genotype is ***Tt***) and in the F_2 3/4th of the plants are tall (though genotypically 1/2 are ***Tt*** and only $1/4^{th}$ are ***TT***). This leads to a phenotypic ratio of 3 tall : 1 dwarf, but a genotypic ratio is 1:2:1(= probabilities 0.25 : 0.5 : 0.25).

The 1/4 : 1/2 : 1/4 ratio of ***TT***: ***Tt***: ***tt*** is mathematically condensable to the form of the binomial expression $(ax + by)^2$, that has the gametes bearing genes ***T*** or ***t*** in equal frequency of ½. The expression is expanded as given below :

$(1/2T + 1/2\ t)^2 = (1/2T + 1/2t)\ X\ (1/2T + 1/2t) = 1/4\ TT + 1/2Tt + 1/4\ tt$

Mendel self-pollinated the F_2 plants and found that dwarf F_2 plants continued to generate dwarf plants in F_3 and F_4 generations. He concluded that the genotype of the dwarfs was homozygous – ***tt***.

What do you think he would have got had he self-pollinated a tall F_2 plant?

9.2.1 Back cross and Test cross

From the preceeding paragraphs it is clear that the genotypic ratios can be calculated using mathematical probability, but by simply looking at the phenotype of a dominant trait, it is not possible to know the genotypic composition. That is, for example, whether a tall plant from F_1 or F_2 has ***TT*** or ***Tt*** composition, cannot be predicted. Therefore, to determine the genotype of a tall plant at F_2, Mendel crossed the tall plant from F_1 with a dwarf plant. This he called a **test cross**. In a typical test cross, an organism (pea plants here) showing a dominant phenotype (and whose genotype is to be determined) is crossed with the recessive parent instead of self-fertilisation. The progenies of such a cross can easily be analysed to predict the genotype of the test organism. Figure 9.5 shows the results of a typical test cross where the violet colour of a flower (W) is dominant over the white colour (w) (Figure 9.5).

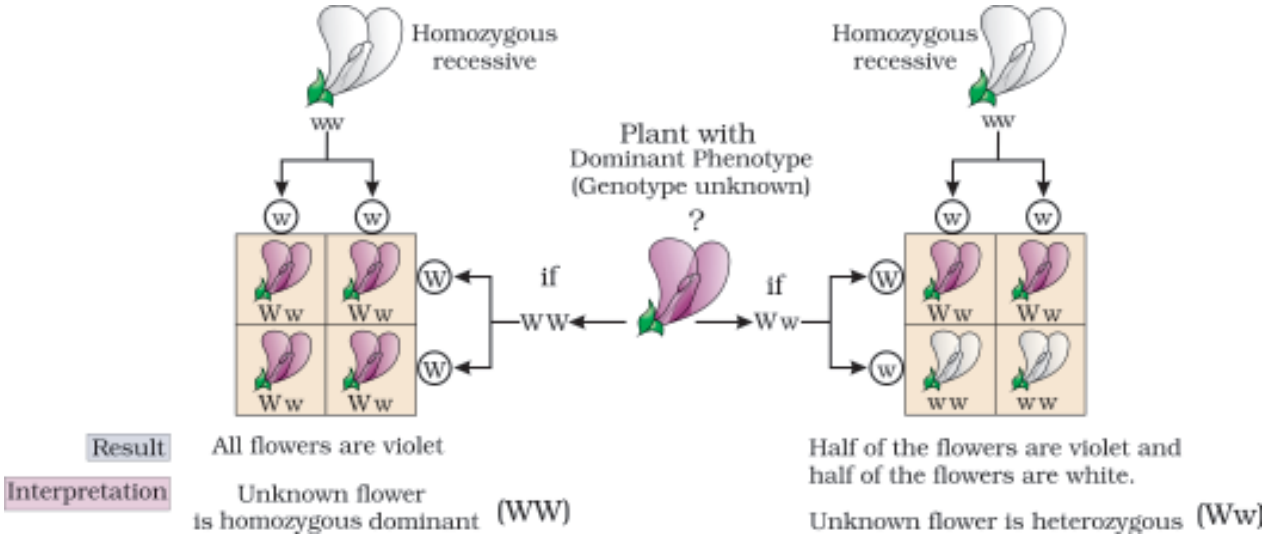


Figure 9.5 Diagrammatic representation of a test cross

Using the Punnett square, try to find out the nature of offspring of a test cross for tall and dwarf characters. What ratio did you get?

Can you give a general definition for a test cross?

If the F_1 hybrid (or in general, a heterozygote) is crossed with the parental type having dominant trait in homozygous condition, no recessive individuals are obtained in the progeny. Although all the plants show phenotypically dominant character, they show a genotypic ratio of 1:1 (Look at the left half of figure 9.5).

When the F_1 individuals (or heterozygotes) are crossed with any one of its parents or organisms that are phenotypically and genotypically similar to the parents, it is generally called Back cross. Test cross is one kind of back cross.

Based on his observations on monohybrid crosses, Mendel proposed two general rules to consolidate his understanding of inheritance in monohybrid crosses. Today these rules are called the **Principles or Laws of Inheritance:** the First Law or **Law of Dominance** and the Second Law or **Law of Segregation**.

9.2.1 Law of Dominance

(i) Characters are controlled by discrete units called **factors**.

(ii) Factors occur in pairs.

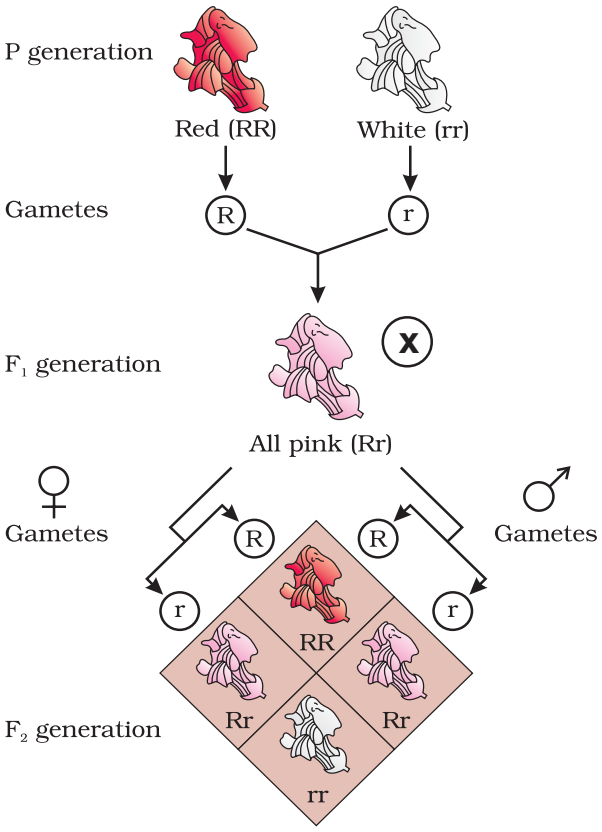
(iii) In a dissimilar pair of factors pertaining to a character one member of the pair dominates (dominant) the other (recessive).

The law of dominance is used to explain the expression of only one of the parental characters in a monohybrid cross in the F_1 and the expression of both in the F_2. It also explains the proportion of 3:1 obtained at the F_2.

9.2.2 Law of Segregation or Law of purity of gametes

Mendel's Law of Segregation states that "the two alleles of a gene when present together in a heterozygous state, do not fuse or blend in any way, but remain distinct and segregate during meiosis or in the formation of gametes so that each meiotic product or gamete will carry only one of them".

This law is based on the fact that the alleles do not show any blending and that both the characters are recovered as such in the F_2 generation though one of these is not seen at the F_1 stage. Though the parents contain two alleles during gamete formation, the factors or alleles of a pair segregate from each other such that a gamete receives only one of the two factors. Of course, a homozygous parent produces all gametes that are similar while a heterozygous one produces two kinds of gametes in equal proportions, each having one allele. Segregation of genes is a universal phenomenon in all organisms reproducing by normal sexual method.



Phenotypic ratio : red : pink : white
1 : 2 : 1

Genotypic ratio : RR : Rr : rr
1 : 2 : 1

9.3 Deviations from Mendelian concept of dominance

9.3.1 Incomplete Dominance

When experiments on peas were repeated using other traits in other plants, it was found that sometimes the F_1 had a phenotype that did not resemble either of the two parents and was in between the two. The inheritance of flower colour in the dog flower (snapdragon or *Antirrhinum sp.*) is a good example to understand incomplete dominance. In a cross between true-breeding (homozygous) red-flowered (***RR***) and truebreeding (homozygous) white-flowered plants (***rr***), the F1 (***Rr***) was pink (Figure 9.6). When the F_1 was self-

Figure 9.6 Results of monohybrid cross in the plant Snapdragon, where one allele is incompletely dominant over the other allele

pollinated, the F_2 resulted in the following ratio 1 ***(RR)*** Red: 2 ***(Rr)*** Pink: 1 ***(rr)*** White. Here the genotypic ratios were exactly as we would expect in any mendelian monohybrid cross, but the phenotypic ratio had changed from the 3:1 of dominant : recessive ratio. What happened was that R was not completely dominant over r and this made it possible to distinguish Rr as pink from RR (red) and rr (white). Thus, the phenotypic and genotypic ratios in F_2 progeny are the same, that is, 1:2:1.

9.3.2 Co-dominance

Till now we were discussing crosses where the F_1 resembled one of the two parents (dominance) or was in-between (incomplete dominance). But, in the case of co-dominance the F_1 generation resembles both parents. Good examples are different types of red blood cells that determine ABO blood grouping in human beings and seed coat pattern and size in lentil plants.

Lentil [*Lens culinaris* ssp. culinaris (Medik.) Williams] is a major grain legume (pulse) crop in North America. A cross between pure-breeding spotted (having few big irregular patches) lentils and pure-breeding dotted (having several small circular dots) lentils produce heterozygotes that are both spotted and dotted (Figure 9.7). These F_1 hybrids illustrate a second significant departure from complete dominance. They show the phenotypic features of both parents, which means that neither the "spotted" nor the "dotted" allele is dominant or recessive to the other. Because both traits show up equally in the heterozygote's phenotype, the alleles are termed **codominant.** Self-pollination of the spotted/ dotted F_1 generation produces F_2 progeny in the ratio of **1 spotted : 2 spotted & dotted:1 dotted**. The Mendelian **1:2:1** ratio among these F_2 progeny establishes that the spotted and dotted traits are determined by alternative alleles of a single gene. Once again, because the heterozygotes can be distinguished from both homozygotes, the phenotypic and genotypic ratios coincide.

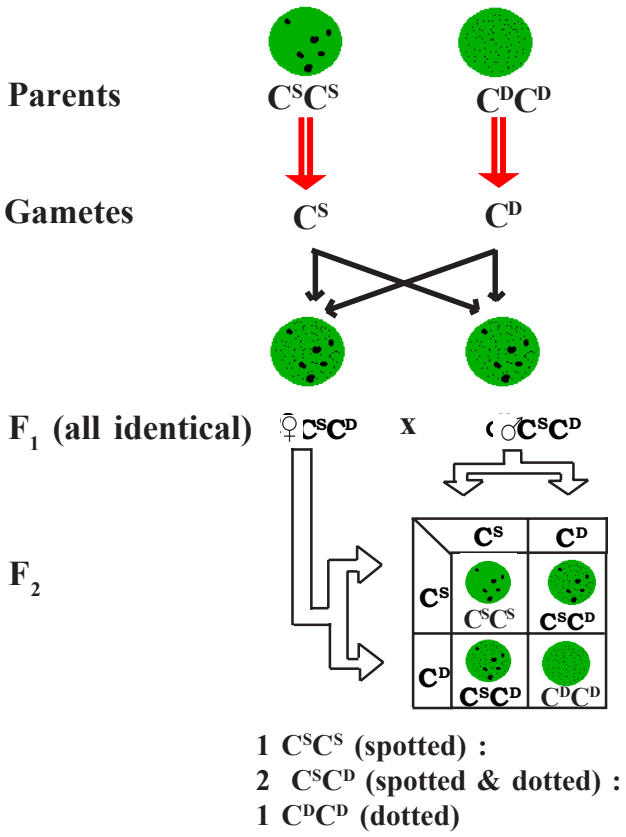


Figure 9.7 Codominant lentil seed coat patterns

Occasionally, a single gene product may produce more than one effect so that a single gene may be related to more than one character. Such phenomenon is called **Pleiotropy**. For example, starch synthesis in pea seeds is controlled by one gene. It has two alleles **B** and **b**. Starch is synthesised effectively by **BB** homozygotes and therefore, large starch grains are produced. In contrast, **bb** homozygotes have lesser efficiency in starch synthesis and produce smaller starch grains. After maturation of the seeds, **BB** seeds are round where **bb** seeds are wrinkled. Heterozygotes produce round seeds, and so **B** seems to be the dominant allele. But, the starch grains produced are of intermediate size in **Bb** seeds. So if starch grain size is considered as the phenotype, then from this angle, the alleles show **incomplete dominance**.

Therefore, dominance is not an autonomous feature of a gene or the product that it has information for. It depends as much on the gene product and the production of a particular phenotype from this product as it does on the particular phenotype that we choose to examine, in case more than one phenotype is influenced by the same gene.

9.3.3 Explanation of the concept of dominance

What exactly is dominance? Why are some alleles dominant and some recessive? To tackle these questions, we must understand what a gene does. Every gene, as you know by now, contains the information to express a particular trait. In a diploid organism, there are two copies of each gene, i.e., as a pair of alleles. Now, these two alleles need not always be identical, (eg. a heterozygote). One of them may be different due to some changes that it has undergone (about which you will read further on, and in the next chapter) which modifies the information that the particular allele contains.

Let's take an example of a gene that contains the information for producing an enzyme. Now there are two copies of this gene, the two allelic forms. Let us assume (as is more common) that the normal allele produces the normal enzyme that is needed for the transformation of a substrate S. Theoretically, the modified allele could be responsible for the production of –

(i) the normal/less efficient enzyme, or

(ii) a non-functional enzyme, or

(iii) no enzyme at all

In the first case, the modified allele is equivalent to the unmodified (normal) allele, i.e., it will produce the same phenotype/trait, i.e., result in the transformation of substrate S. Such equivalent allele pairs are very common. But, if the allele produces a less functional enzyme or non-functional enzyme

or no enzyme, the phenotype may be effected. The phenotype/trait will only be dependent on the functioning of the unmodified allele. The unmodified (functioning) allele, which represents the original phenotype is the dominant allele and the modified allele is generally the recessive allele. Hence, in the example above the recessive trait is seen due to non-functional enzyme or because no enzyme is produced.

9.4 Inheritance of two genes (Di-hybrid Cross)

Mendel also worked with and crossed pea plants that differed in two characters, as is seen in the cross between a pea plant that has seeds with yellow colour and round shape and one that had seeds of green colour and wrinkled shape (Figure 9.8). Mendel found that the seeds resulting from the crossing of the parents, had yellow coloured and round shaped seeds. *Here can you tell which of the characters in the pairs yellow/ green colour and round/wrinkled shape were dominant?*

Thus, yellow colour was dominant over green and round shape dominant over wrinkled. These results were identical to those that he got when he made separate monohybrid crosses between yellow and green seeded plants and between round and wrinkled seeded plants.

Let us use the genotypic symbols ***Y*** for dominant yellow seed colour and ***y*** for recessive green seed colour, ***R*** for round shaped seeds and ***r*** for wrinkled seed shape. The genotype of the parents can then be written as ***RRYY*** and ***rryy***. The cross between the two plants can be written down as in Figure 9.8 showing the genotypes of the parent plants. The gametes ***RY*** and ***ry*** unite on fertilisation to produce the F_1 hybrid ***RrYy***. When Mendel self pollinated the F_1 plants, he found that $3/4^{th}$ of F_2 plants had yellow seeds and $1/4^{th}$ had green. The yellow and green colour segregated in a 3:1 ratio. Round and wrinkled seed shape also segregated in a 3:1 ratio; just like in a monohybrid cross.

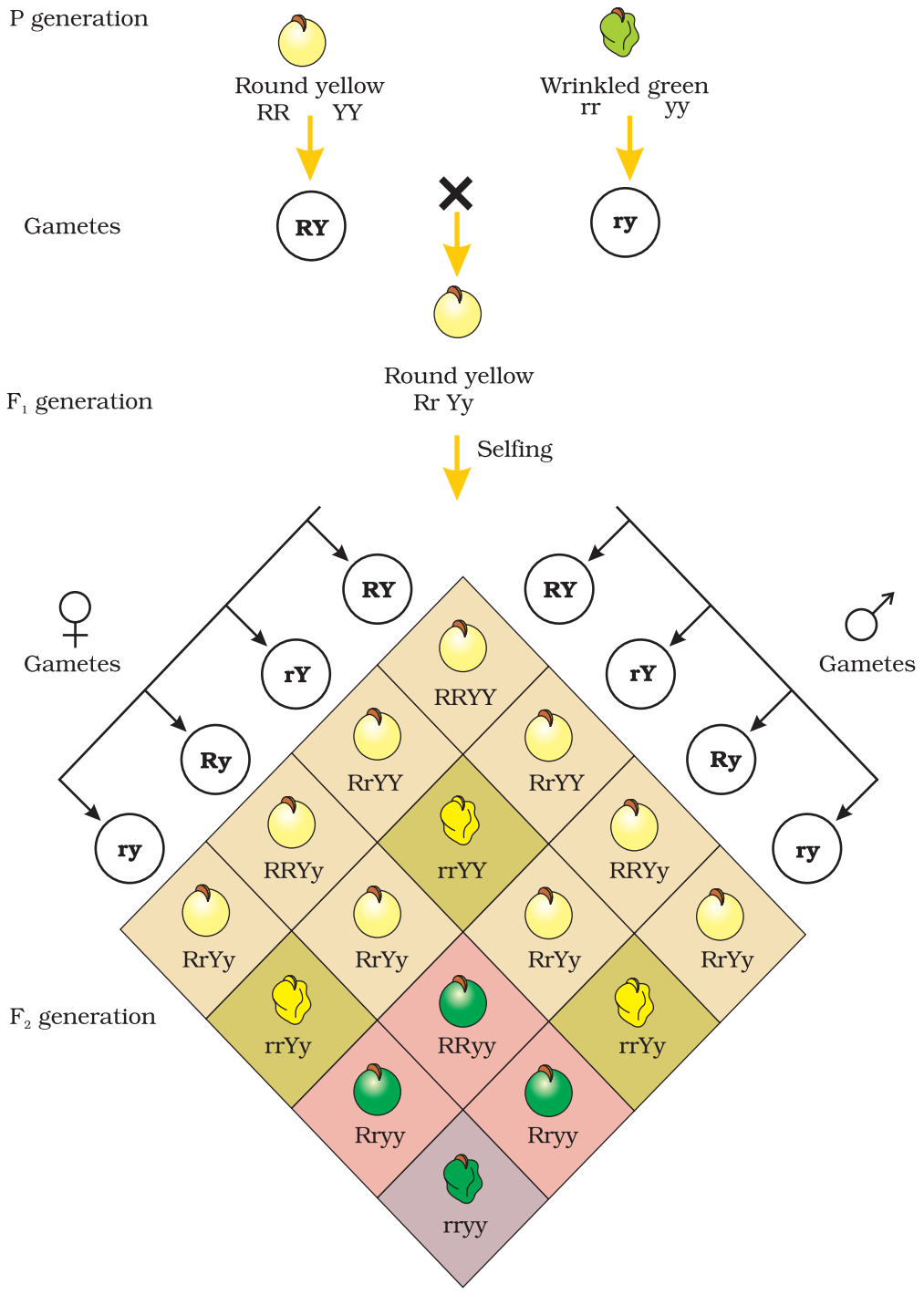
9.4.1 Law of Independent Assortment

In the dihybrid cross (Figure 9.8), the phenotypes round, yellow; wrinkled, yellow; round, green; and wrinkled, green appeared in the ratio 9:3:3:1. Such a ratio was observed for several pairs of characters that Mendel studied.

The ratio of 9:3:3:1 can be derived as a combination series of 3 yellow: 1 green, with 3 round : 1 wrinkled. This derivation can be written as follows:

(3 Round : 1 Wrinkled) (3 Yellow : 1 Green) = 9 Round, Yellow : 3 Wrinkled, Yellow: 3 Round, Green : 1 Wrinkled, Green

In otherwords, the probability of round yellow is $3/4 \times 3/4 = 9/16$, wrinkled yellow is $1/4 \times 3/4 = 3/16$, round green is $3/4 \times 1/4 = 3/16$, green wrinkled is $1/4 \times 1/4 = 1/16$.



Phenotypic ratio : round yellow : round green : wrinkled yellow : wrinkled green
9 3 3 1

Figure 9.8 Results of a dihybrid cross where the two parents differed in two pairs of contrasting traits: seed colour and seed shape

Based upon such observations on **dihybrid crosses** (crosses between plants differing in two traits) Mendel proposed a second set of generalisations that we call Mendel's Law of Independent Assortment. The law states that 'when two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent of the other pair of characters'.

The Punnett square can be effectively used to understand the independent segregation of the two pairs of genes. For example, look at the genotype of the F_1 plant which is a double heterozygote (***RrYy***). Consider the segregation of one pair of genes ***R*** and ***r***. Fifty per cent of the gametes have the gene ***R*** and the other 50 per cent have ***r***. Now besides each gamete having either ***R*** or ***r***, it should also have the allele ***Y*** or ***y***. The important thing to remember here is that segregation of 50 per cent ***R*** and 50 per cent *r* is *independent* of the segregation of 50 per cent ***Y*** and 50 per cent ***y***. Therefore, 50 per cent of the ***r*** bearing gamete has ***Y*** and the other 50 per cent has ***y***. Similarly, 50 per cent of the ***R*** bearing gamete has *Y* and the other 50 per cent has ***y***. Thus there are four genotypes of gametes (four types of pollen and four types of eggs). The four types are ***RY***, ***Ry***, ***rY*** and ***ry*** each with a frequency of 25 percent or ¼th of the total gametes produced. When you write down the four types of eggs and pollen on the two sides of a Punnett square, it is very easy to derive the composition of the zygotes that give rise to the F_2 plants (Figure 9.8). *Although there are 16 squares how many different types of genotypes and phenotypes are formed?* Note them down in the format given.

Can you, using the Punnett square data, work out the genotypic ratio at the F_2 stage and fill in the format given? Is the genotypic ratio also 9:3:3:1?

S.No.	Genotypes found in F_2	Their expected Phenotypes

Can you work out the result of a dihybrid test cross !

9.5 Chromosomal Theory of Inheritance

Mendel published his work on inheritance of characters in 1865 but for several reasons, it remained unrecognised till 1900. Firstly, communication was not as easy in those days as it is now and his work could not be widely publicised. Secondly, his concept of **genes** (or **factors**, in Mendel's words) as stable and discrete units that controlled the expression of traits and, of the pair of alleles which did not 'blend' with each other, was not accepted by his contemporaries as an explanation for the apparently continuous variation seen in nature. Thirdly, Mendel's approach of using mathematics to explain biological phenomena was totally new and unacceptable to many of the biologists of his time. Finally, though Mendel's work suggested that factors (genes) were discrete

units, he could not provide any physical proof for the existence of factors or say what they were made of.

In 1900, three Scientists (de Vries, Correns and von Tschermak) independently rediscovered Mendel's results on the inheritance of characters. Also, by this time due to advancements in microscopy that were taking place, scientists were able to carefully observe cell division. This led to the discovery of structures in the nucleus that appeared to double and divide just before each cell division. These were called **chromosomes** (*colored bodies*, as they were visualised by staining). By 1902, the chromosome movement during meiosis had been worked out. Walter Sutton and Theodore Boveri noted that the behaviour of chromosomes was parallel to the behaviour of genes predicted by Mendel and they used chromosome movement (Figure 9.9) to explain Mendel's laws (Table 9.2). Recall that you have studied the behaviour of chromosomes during mitosis

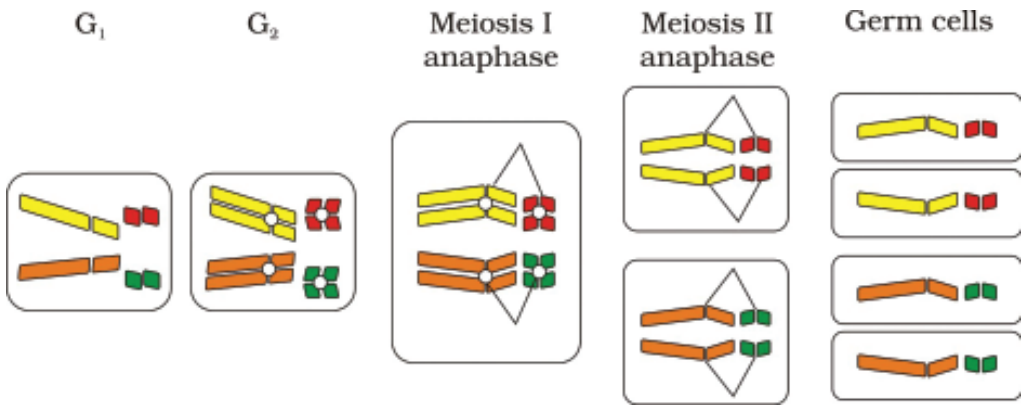


Figure 9.9 Meiosis and germ cell formation in a cell with four chromosomes. Can you see how chromosomes segregate when germ cells are formed?

Table 9.2 A Comparison between the Behaviour of Chromosomes and Genes

A	B
Occur in pairs	Occur in pairs
Segregate at the time of gamete formation such that only one of each pair is transmitted to a garnete	Segregate at gamete formation and only one of each pair is transmitted to a gamete
Independent pairs segregate independently of each other	One pair segregates independently of another pair
Can you tell which of these columns A or B represent the chromosome and which represents the gene? How did you decide?	

(equational division) and during meiosis (reduction division). The important thing to remember is that chromosomes as well as genes occur in pairs. The two alleles of a gene pair are located on homologous sites on homologous chromosomes.

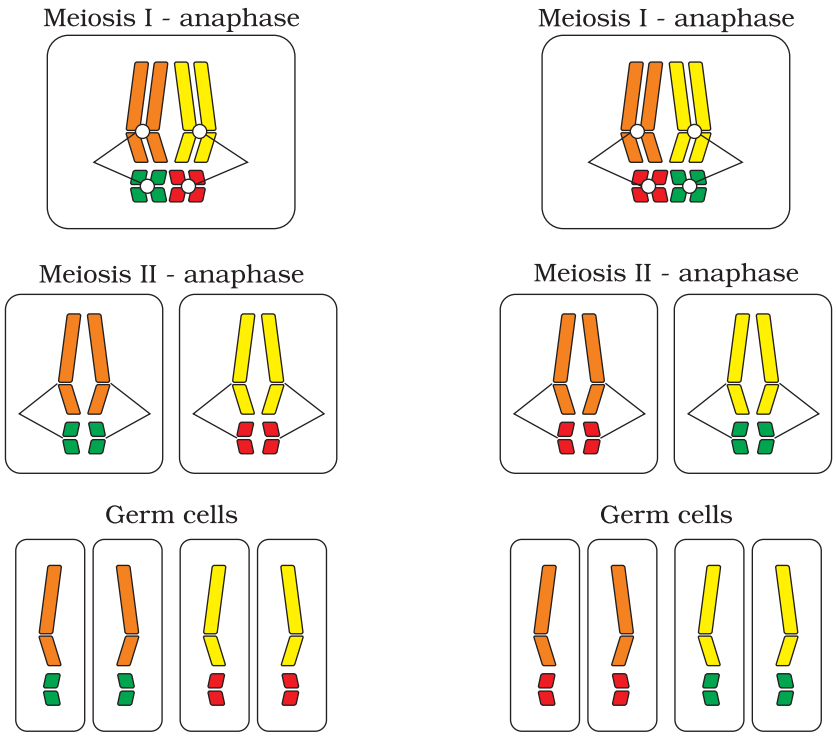


Figure 9.10 Independent assortment of chromosomes

During Metaphase of meiosis I, the two chromosome pairs can align at the metaphase plate independently of each other (Figure 9.10). To understand this, compare the chromosomes of four different colours in the left and right columns. In the left column (Possibility I) orange and green are segregating together. But in the right hand column (Possibility II) the orange chromosome is segregating with the red chromosomes.

Sutton and Boveri argued that the pairing and separation of a pair of chromosomes would lead to the segregation of a pair of factors they carried. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it the **chromosomal theory of inheritance**.

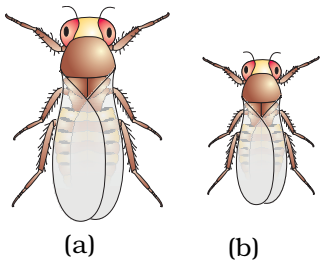


Figure 9.11 *Drosophila melanogaster* (a) Male (b) Female

This synthesis of ideas was followed by the experimental verification of the chromosomal theory of inheritance by Thomas Hunt Morgan and his colleagues. It led to discovering the basis for the variation that sexual reproduction produced. Morgan worked with the tiny fruit flies, ***Drosophila melanogaster*** (Figure 9.11), which were found very suitable for such studies. They could be grown on simple synthetic medium in the laboratory. They complete their life cycle in about two weeks, and a single mating could produce a large number of progeny flies. Also, there was a clear differentiation of the sexes – the male and female

flies are easily distinguishable. Also, the fruit fly has many types of hereditary variations that could be seen with low power microscope.

9.6 Linkage and Recombination

Several dihybrid crosses in *Drosophila* were carried out by Morgan in the same way as the dihybrid crosses carried out by Mendel in peas. For example, Morgan hybridised yellow-bodied, white-eyed females to brown-bodied, red-eyed males and intercrossed their F1 progeny. However, he observed that the two genes did not segregate independently of each other and the F2 ratio deviated very significantly from the 9:3:3:1 ratio (as expected if the two genes were independent).

Morgan and his group knew that the genes were located on the X chromosome and saw quickly that when the two genes in a dihybrid cross were situated on the same chromosome, the proportion of parental gene combinations was much higher than the non-parental type. Morgan attributed this due to the physical association or linkage of the two genes and coined the term **linkage** to describe this physical association of genes on a chromosome and the term **recombination** to describe the generation of non-parental gene combinations (Figure 9.12). Morgan and his group also found that even when genes were grouped on the same chromosome, some genes were very tightly linked (showed very low recombination) (Figure 9.12, Cross A) while others were loosely linked (showed higher recombination) (Figure 9.12, Cross B). For example he found that the genes white and yellow were very tightly linked and showed only 1.3 per cent recombination while white and miniature wing showed 37.2 per cent recombination. His student, **Alfred Sturtevant**, used the frequency of recombination between gene pairs on the same chromosome as a measure of the distance between genes and 'mapped' their position on the chromosome. Today genetic maps are extensively used as a starting point in the sequencing of whole genomes as was done in the case of the Human Genome Sequencing Project.

9.7 Mutations

Mutation is a phenomenon which results in alteration of genes (=DNA sequences) and consequently results in changes in the genotype and the phenotype of an organism. In addition to recombination, mutation is another phenomenon that leads to variation in DNA. Mutations were first noticed by Hugo de Vries in the plant *Oenothera lamarckiana* (Lamarck's evening primrose).

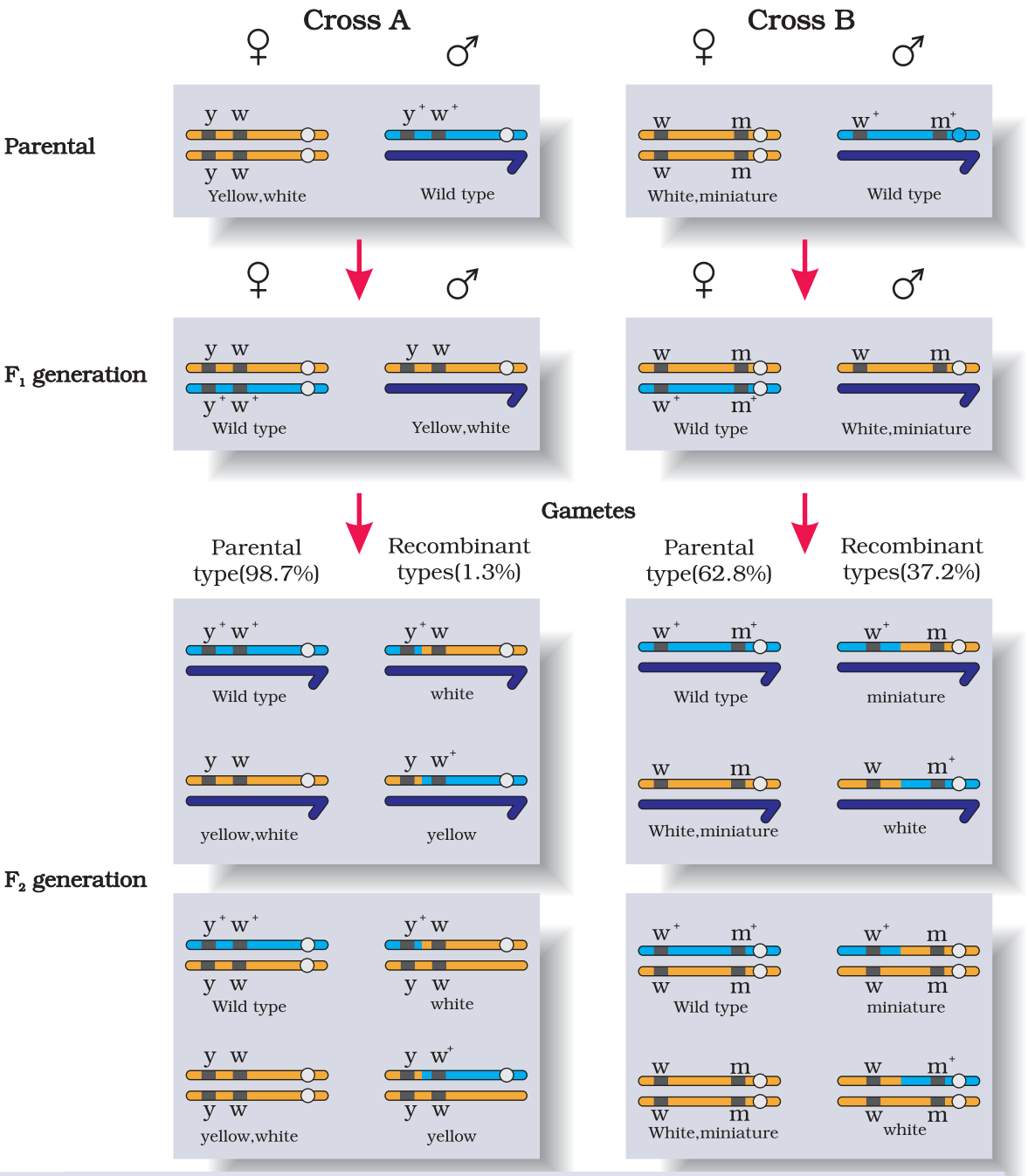


Figure 9.12 Linkage: Results of two dihybrid crosses conducted by Morgan. Cross A shows crossing between gene y and w; Cross B shows crossing between genes w and m. Here dominant wild type alleles are represented with (+) sign in superscript

Note : The strength of linkage between y and w is higher than w and m.
Wild type : has dominant phenotypes for both characters (brown body & red eye)
White : had dominant phenotype (wild character) for body colour but recessive phenotype for eye colour.
Yellow : had recessive phenotype for body colour (yellow) but dominant phenotype (wild character) for eye colour (red)

One DNA helix runs continuously from one end to the other in each chromatid in a highly supercoiled form. Therefore loss (deletions) or gain (insertion/ duplication) of a segment of DNA results in alteration in chromosomes. Since genes are known to be located on chromosomes, alteration in chromosomes results in abnormalities or aberrations. Chromosomal aberrations are commonly observed in cancer cells.

In addition to the above, mutations also arise due to change in a single base pair of DNA. This is known as point mutation. A classical example of such a mutation is sickle cell anemia. Deletions and insertions of base pairs of DNA cause frame -shift mutations.

The mechanism of mutation is beyond the scope of discussion at this level. However, there are many chemical and physical factors that induce mutations. These are referred to as mutagens. UV radiation can cause mutations in organisms- it is a mutagen.

9.7.1 Significance of mutations

Mutations generate a large amount of variability in a population from which a breeder can select the desirable types; hence improved varieties of crop plants with several desirable characters can be obtained after careful selection and hybridization.

SUMMARY

SUMMARY

Genetics is a branch of biology which deals with the principles of inheritance and its practices. Progeny resembling the parents in morphological and physiologycal features has attracted the attention of many biologists. Mendel was the first to study this phenomenon systematically. While studying the pattern of inheritance in pea plants with contrasting characters, Mendel proposed the principles of inheritance, which are today referred to as 'Mendel's Laws of Inheritance'. He proposed that the 'factors' (later named as genes) regulating the characters are found in pairs known as alleles. He observed that the expression of the characters in the offspring follow a definite pattern in different generations–first generations (F1), second (F2) and so on. Some characters are dominant over others. The dominant characters are expressed when factors are either in homozygous or in heterozygous condition (Law of Dominance). The recessive characters are only expressed in homozygous conditions. The characters never blend in heterozygous condition. A recessive character that was not expressed in heterozygous conditon may be expressed again when it becomes homozygous. Hence, characters segregate during formation of gametes (Law of Segregation). Not all characters show true dominance. Some characters show incomplete dominance and some show co-dominance. When Mendel studied the inheritance of two characters together, it was found that the factors independently assort and combine in all permutations and combinations (Law of Independent Assortment). Different combinations of gametes are theoretically represented in a square tabular form known as 'Punnett Square'. The factors (now known as genes) on chromosomes regulating the characters are called the genotype and the physical expression of the chraracters is called phenotype. After knowing that the genes are located on the chromosomes, a good correlation was drawn between Mendal's laws and segregation and assortment of chromosomes during meiosis. Mendel's laws were extended in the form of 'Chromosomal Theory of Inheritance'. Later, it was found that Mendel's law of independent assortment did not hold true for the genes that were located on the same chromosomes. These genes are called 'linked genes'. Closely located genes assorted together, and distantly located genes, due to recombination, assorted independently. Mutations involve changes in chromosomes and / or genes. They help to increase variability which might be useful in crop improvement.

GLOSSARY

Alleles: The alternative forms of a gene.

Backcross: If the F_1 progeny (or heterozygote) are mated back to one of their parents (or individuals with a genotype identical to that of either of their parents), the mating is termed backcross.

Codominance: The phenomenon where heterozygotes have features of both the homozygotes, that is, an allele is neither dominant nor recessive to the other.

Dihybrid cross: The cross made between individuals differing in two characters.

Dominance: It is the phenomenon where a character is expressed phenotypically in both homozygotes and heterozygotes.

Gene: It is the unit of inheritance and contains the information required to express a character.

Genotype: It is the genetic makeup of an individual.

Heterozygote: An individual having two different alleles for a single character. Consequently it will produce two different types of gametes with reference to a gene.

Homozygote: An individual having two similar or identical alleles for a single character. Hence it will produce only one kind of gametes with reference to a gene.

Incomplete dominance: It is the condition when one allele of a gene is not completely dominant over the other allele and results in the heterozygotes having phenotype different from the dominant and recessive homozygotes.

Linkage: is the presence of two or more genes on a chromosome as a result of which the genes tend more often to be inherited together.

Monohybrid cross: The cross made between two individuals differing in one character.

Mutagen: Any substance or factor that can induce mutations.

Mutation: A sudden and heritable change in the genetic material.

Phenotype: The physical or external appearance of a character. Sometimes it may require special tests for its identification such as determination of respiratory quotient or serological tests for blood grouping.

Pleiotropy: The phenomenon where a single gene influences several characters.

Recessive: The character which is not expressed phenotypically in heterozygous condition.

Test cross: The cross between F1 progeny (or heterozygotes) and their recessive homozygous parent (or individuals with recessive homozygous genotype).

QUESTIONS

Very Short Answer Type Questions

1. What is the cross between the F_1 progeny and the homozygous recessive parent called? How is it useful?
2. Do you think Mendel's laws of inheritance would have been different if the characters that he chose were located on the same chromosome?
3. Who proposed the Chromosome Theory of Inheritance?
4. Define true breeding. Mention its significance.
5. Explain the terms phenotype and genotype.
6. What is point mutation? Give an example.
7. A person has to perform crosses for the purpose of studying inheritance of a few traits / characters. What should be the criteria for selecting the organisms?
8. In order to obtain the F_1 generation, Mendel pollinated a pure-breeding tall plant with a pure breeding dwarf plant. But, to get the F_2 generation, he simply self-pollinated the tall F_1 plants. Why?
9. How are alleles of a particular gene differ from each other? Explain its significance.
10. In a monohybrid cross between red and white flowered plants, a geneticist got only red flowered plants. On self-pollinating these F_1 plants, he got both red and white flowered plants in 3:1 ratio. Explain the basis of using RR and rr symbols to represent the genotype of plants of parental generation.
11. What is the genetic nature of wrinkled phenotype of pea seeds?

Short Answer Type Questions

1. In a Mendelian monohybrid cross, the F_2 generation shows identical genotypic and phenotypic ratios. What does it tell us about the nature of alleles involved? Justify your answer.
2. Mention the advantages of selecting pea plant for experiment by Mendel.
3. Differentiate between the following:
   (a) Dominant and Recessive
   (b) Homozygous and Heterozygous
   (c) Monohybrid and Dihybrid.
4. Explain the Law of Dominance using a monohybrid cross.
5. Define and design a test-cross.
6. Using a Punnett Square, work out the distribution of phenotypic features in the first filial generation after a cross between a homozygous female and a heterozygous male for a single locus.
7. When a cros is made between tall plant with yellow seeds (TtYy) and a tall plant with green seeds

(Ttyy), what proportion of phenotype in the offspring is expected to be
(a) tall and green?
(b) dwarf and green?

8. Explain the following terms with examples
(a) Co-dominance
(b) Incomplete dominance
9. Write a brief note on chromosomal mutations and gene mutations.
10. How was it concluded that genes are located on chromosomes?
11. A plant with red flowers was crossed with one having yellow flowers. If F_1 showed all flowers in orange colour, explain the inheritance.
12. Define Law of Segregation and Law of Independent Assortment.
13. In peas, tallness is dominant over dwarfness and violet colour of flowers is dominant over the white colour. When a tall plant bearing violet flowers was pollinated with a dwarf plant bearing white flowers, different phenotypic groups were obtained in the progeny in numbers mentioned against them:

Tall, Violet	= 138
Tall, White	= 132
Dwarf, Violet	= 136
Dwarf, White	= 128

Mention the genotypes of the two parents and of the four offspring types.

14. How do genes and chromosomes share similarity from the view point of genetical studies?
15. With the help of an example differentiate between incomplete dominance and co-dominance.

Long Answer Type Questions

1. In a plant, tallness is dominant over dwarfness and red flower is dominant over white. Starting with the parents work out a dihybrid cross. What is standard dihybrid ratio? Do you think the values would deviate if the two genes in question are interacting with each other?

Exercises

1) What will be the phenotypic ratio in the offsprings obtained from the following crosses.
a) Aa × aa b) AA × aa
c) Aa × Aa d) Aa × AA
Note: Gene 'A' is dominant over gene 'a'
2) In garden pea, the gene T for tall is dominant over its allele for dwarf. Give the genotypes of the parents in the following crosses.
a) tall × dwarf producing all tall plants.
b) tall × tall producing 3 tall and 1 dwarf plants
c) tall × dwarf producing half tall and half dwarf number of plants.
3) Mendel crossed pea plants producing round seeds with those producing wrinkled seeds. From a total of 7324 F_2 seeds, 5474 were round and 1850 were wrinkled. Using the symbols R and r for genes, predict the :

a) the parental (p) genotypes
b) the gametes
c) F_1 progeny
d) the cross between F_1 hybrids
e) genotypes, phenotypes, genotypic frequency, phenotypic ratio of F_2 progeny

4) The following data was obtained from an experiment on peas. The grey coloured seed is dominant over white coloured seed. Use the letter G for grey and g for white traits. Predict genotypes of the parents in each of the following crosses.

	Parent	**Progeny**	
		Grey	white
a)	Grey × white	164	156
b)	Grey × grey	59	19
c)	White × white	0	100
d)	Grey × grey	180	0

5) In tomatoes red fruit colour (R) is dominant to yellow (r). Suppose a tomato plant homozygous for red is crossed with one homozygous for yellow. Determine the appearance of the following:
(a) the F_1, **(b)** F_2, **(c)** the off spring of a cross of the F_1 back to the red parent, **(d)** the offspring of a cross of the F_1 back to the yellow parent.

6) In pea, axillary position of flowers (T) is dominant over its terminal position (t). Coloured flowers (C) are dominant to white flowers (c). A true breeding plant with coloured flowers in axils is crossed to one with white terminal flowers. Give the phenotyes, genotyes and expected ratios of F_1, F_2, back cross and test cross progenies. What genotypic ratio is expected in the F_2 progeny?

7) In summer squash, a plant with white flowers and disc-shaped fruits is crossed to a plant with yellow flowers and sphere-shaped fruits. The F_1 hybrids had white flowers and disc-shaped fruits. Which phenotypes are dominant? Give the genotypes of the parents and the hybrids. If these hybrids were selfed and 256 progeny were obtained, what what would be the frequencies of the various phenotypes?

8) Give the ratios of the following:
a) Monohybrid test cross,
b) Dihybrid test cross
c) F_2 phenotypic ratio of mono-hybrid cross
d) F_2 phenotypic ratio of dihybrid cross
e) F_2 Genotypic ratio of monohybrid cross and
f) F_2 genotypic ratio of dihybrid cross

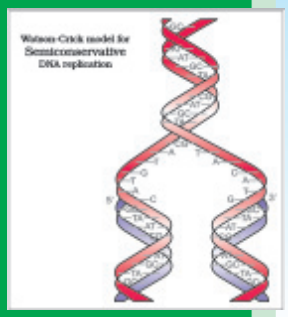
9) A diploid organism is heterozygous for 4 loci. How many types of gametes can it produce?

10) What is crossing over? In which stage of cell division crossing over occurs? What is its significance?

11) "Genes contain the information that is required to express a particular trait." Explain.

12) For the expression of traits genes provide only the potentiality and the environment provides the opportunity. Comment on the veracity of the statement.

13) Two heterozygous parents are crossed. If the two loci are linked what would be the distribution of phenotypic features in F1 generation for a dibybrid cross?



UNIT IV

MOLECULAR BIOLOGY

Chapter 10 : Molecular Basis of Inheritance

Molecular biology is relatively a young discipline among life sciences. It is about the study of macromolecules and their mechanisms in living things such as gene replication, mutation, and expression. Warren Weaver recognized quite early the importance of the physical and chemical approaches to biology, and introduced the term Molecular Biology to uncover the minute details of certain life processes. The field of molecular biology arose from the convergence of work by geneticists, physicists, and structural chemists on the structure and function of the gene. The classifical period of Molecular Biology began in 1953 with James Watson and Francis Crick's discovery of the double helical structure of DNA.

James Watson
Francis Crick

James Dewey Watson was born in Chicago on 6 April 1928. In 1947, he received B.Sc. degree in Zoology. During these years his interest in bird-watching had matured into a serious desire to learn genetics. This became possible when he received a Fellowship for graduate study in Zoology at Indiana University, Bloomington, where he received his Ph.D. degree in 1950 on a study of the effect of hard X-rays on bacteriophage multiplication.

He met Crick and discovered their common interest in solving the DNA structure. Their first serious effort was unsatisfactory. Their second effort based upon more experimental evidence and better appreciation of nucleic acid literature resulted in the proposal of the complementary double-helical configuration in 1953.

Francis Harry Compton Crick was born on 8 June 1916, at Northampton, England. He studied physics at University College, London and obtained a B.Sc. in 1937. He completed Ph.D. in 1954 on a thesis entitled "X-ray Diffraction: Polypeptides and Proteins".

A critical influence in Crick's career was his friendship with J. D. Watson, then a young man of 23, leading in 1953 to the proposal of the double-helical structure for DNA and the replication scheme. Crick was made an F.R.S. in 1959.

The honours to Watson with Crick include: the John Collins Warren Prize of the Massachusetts General Hospital, in 1959; the Lasker Award, in 1960; the Research Corporation Prize, in 1962 and above all, the Nobel Prize in 1962.

Chapter 10

Molecular Basis of Inheritance

In the previous chapter, you learnt about inheritance patterns and the genetic basis of such patterns. During Mendel's time, the nature of those 'factors' regulating the pattern of inheritance was not clear. Over the next hundred years, the nature of the putative genetic material was investigated, culminating in the realisation that DNA – deoxyribonucleic acid – is the genetic material, at least for the majority of organisms. In First Year you learnt that nucleic acids are polymers of nucleotides.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two types of nucleic acids found in living systems. DNA acts as the genetic material in most of the organisms. Though RNA also acts as a genetic material in some viruses, it mostly functions as a messenger and has additional roles as well. It functions as adapter, structural and in some cases as a catalytic molecule. You have already learnt the structures of nucleotides and the way these monomer units are linked to form nucleic acid polymers. In this chapter we are going to discuss the structure of DNA, its replication, the process of making RNA from DNA (transcription), the genetic code that determines the sequences of amino acids in proteins, the process of protein synthesis (translation) and the elementary basis of their regulation. The determination of the complete

nucleotide sequence of the human genome during last decade has heralded a new era of **genomics**.

Let us begin our discussion by first understanding the structure of the most interesting molecule in the living system, that is, the DNA. In subsequent sections, we will learn why it is the most abundant genetic material and its relationship with RNA.

10.1 The DNA

DNA is a long polymer of deoxyribonucleotides. The length of DNA is usually defined in terms of number of nucleotides or nucleotide pairs or base pairs present in it. This also is the characteristic of an organism. For example, a bacteriophage known as ϕ×174 has 5386 nucleotides, Bacteriophage lambda has 48502 base pairs (bp), *Escherichia coli* has 4.6×10^6 bp, and haploid content of human DNA is 3.3×10^9 bp. Let us discuss the structure of such a long polymer.

10.1.1 Structure of Polynucleotide Chain

Let us recapitulate the chemical structure of a polynucleotide chain (DNA or RNA). A nucleotide has three components – a nitrogenous base, a pentose sugar (ribose in case of RNA, and deoxyribose for DNA), and a phosphate group. There are two types of nitrogenous bases – Purines (Adenine and Guanine), and Pyrimidines (Cytosine, Uracil and Thymine; Fig. 10.1). Purines

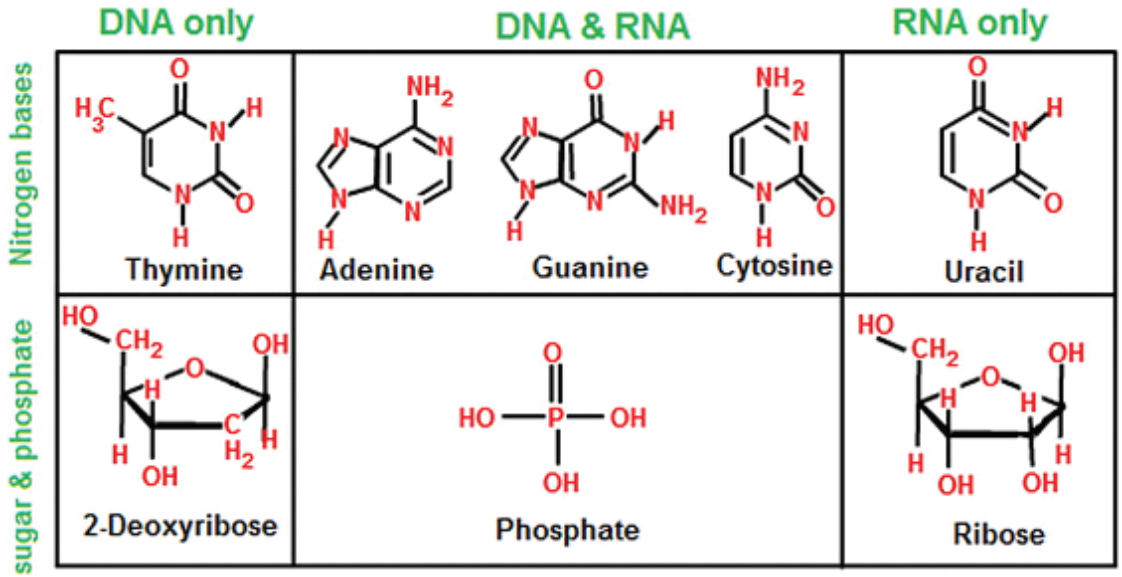


Figure 10.1 Molecular structures of the components of nucleic acids

and Cytosine are common to both DNA and RNA whereas Thymine is present only in DNA. Uracil is present in RNA at the place of Thymine. A nitrogenous base is linked to the pentose sugar through a N-glycosidic linkage to form a nucleoside, such as adenosine or deoxyadenosine, guanosine or deoxyguanosine, cytidine or deoxycytidine and thymidine or deoxythymidine. When a phosphate group is linked to 5′-OH of a nucleoside through phosphoester linkage, a corresponding nucleotide (or deoxynucleotide depending upon the type of sugar present) is formed. Two nucleotides are linked through 3′→5′ phosphodiester linkage to form a dinucleotide. More nucleotides can be joined in such a manner to form a polynucleotide chain. A polymer thus formed has at one end a free phosphate moiety (at 5′-end of the sugar), which is referred to as the 5'-end of polynucleotide chain. Similarly, at the other end of the polymer, the sugar has a free 3′-OH group which is referred to as 3'- end of the polynucleotide chain. The backbone in a polynucleotide chain is formed due to sugar and phosphates. The nitrogenous bases are linked to sugar moiety project from the backbone (Figure 10.2).

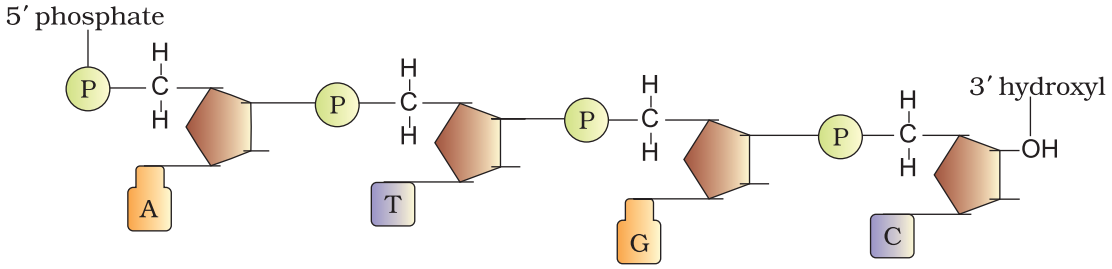


Figure 10.2 A Polynucleotide chain

In RNA, every nucleotide residue has an additional –OH group present at 2′-position in the ribose. Also, in RNA the uracil is found at the place of thymine (5-methyl uracil, another chemical name for thymine).

DNA as an acidic substance present in nucleus was first identified by **Friedrich Meischer** in 1869. He named it '**Nuclein**'. However, due to technical limitation in isolating such a long polymer intact, the elucidation of structure of DNA remained elusive for a very long period of time. It was only in 1953 that **James Watson** and **Francis Crick**, based on the X-ray diffraction data produced by **Maurice Wilkins** and **Rosalind Franklin**, proposed a very simple but famous **Double Helix** model for the structure of DNA. One of the hallmarks of their proposition was base pairing between the two strands of polynucleotide chains. However, this proposition was also based on the observation of **Erwin Chargaff** that for a double stranded DNA, the ratio between **Adenine** and **Thymine** and that between **Guanine** and **Cytosine** are constant and each equals one.

The base pairing confers a unique property to the polynucleotide chains. The chains are complementary to each other, and therefore if the sequence of bases in one strand is known then the sequence in other strand can be predicted. Also, if each strand from a DNA (let us call it parental DNA) acts as a template for synthesis of a new strand, the two double stranded DNA (let us call them daughter DNA) thus, produced would be identical to the parental DNA molecule. Because of this, the genetic implications of the structure of DNA became very clear.

The salient features of the Double-Helix structure of DNA are as follows:

(i) It is made of two polynucleotide chains, where the backbone is constituted by sugar-phosphate, and the bases project inside.

(ii) The two chains have anti-parallel polarity. This means that if one chain has the polarity 5′→3′, the other has 3′→5′.

(iii) The bases in two strands are paired through hydrogen bonds (H-bonds) forming base pairs (bp). **Adenine** forms two hydrogen bonds with **Thymine** from the opposite strand and vice-versa. Similarly, **Guanine** is bonded with **Cytosine** with three H-bonds. As a result, a Purine always comes opposite to a Pyrimidine. This generates approximately uniform distance (20 A°) between the two strands of the helix (Figure 10.3 a & b).

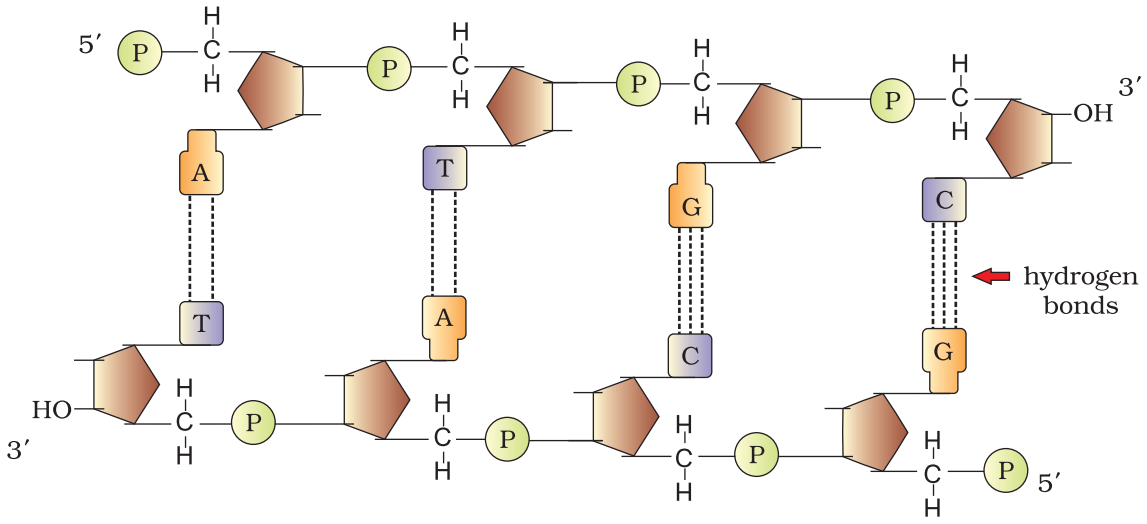


Figure 10.3 a Schematic representation of a Double stranded polynucleotide chain

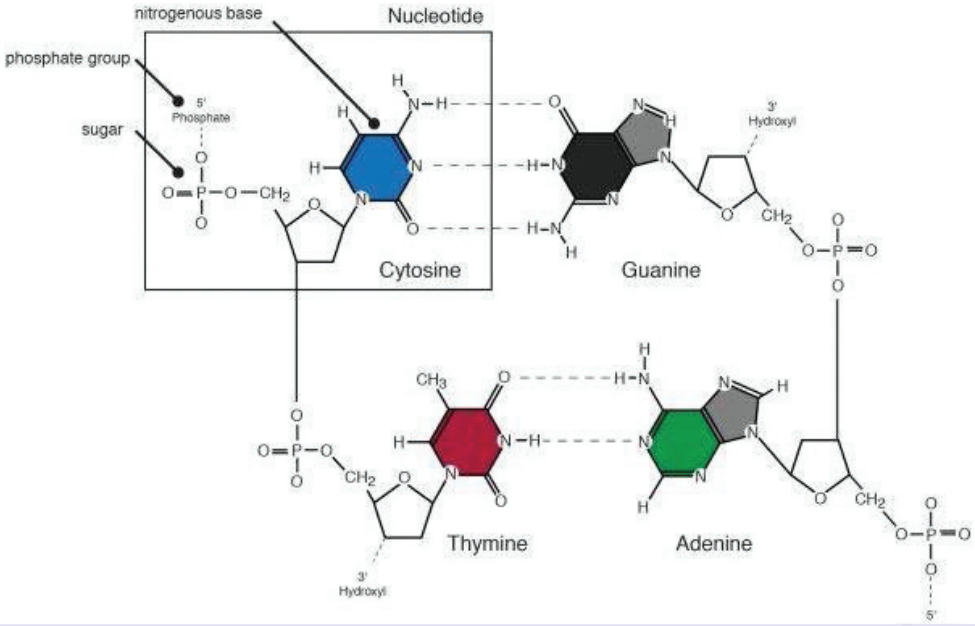


Figure 10.3b A portion of figure 10.2 showing positions and bonds in DNA double helix

(iv) The two chains are coiled in a right-handed fashion. The pitch of the helix is 3.4 nm (a nanometre is one billionth of a metre, that is 10^{-9} m) and there are roughly 10 bp in each turn. Consequently, the distance between two successive base pairs (bp) in a helix is approximately equal to 0.34 nm.

(v) The plane of one base pair stacks over the other in a double helix. This, in addition to H-bonds, confers stability of the helical structure (Figure 10.4).

Compare the structure of purines and pyrimidines. Can you find out why the distance between two polynucleotide chains in DNA remains almost constant?

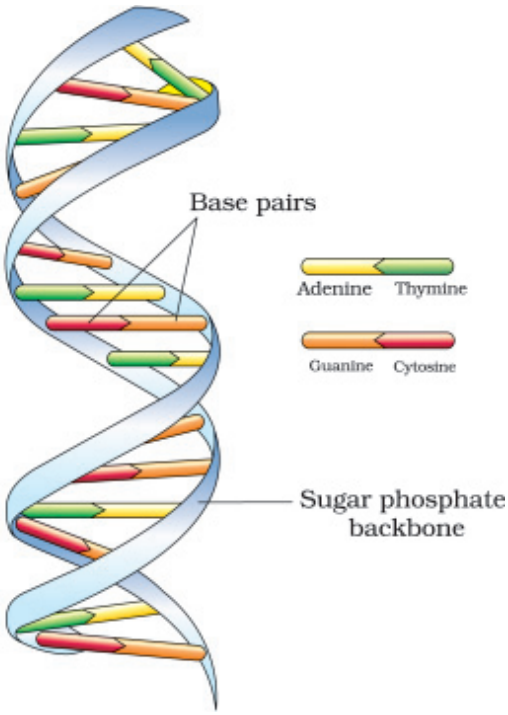


Figure 10.4 DNA double helix

The proposition of a double helix structure for DNA and its simplicity in explaining the genetic implication became revolutionary. Very soon Francis Crick proposed the Central dogma in molecular

biology, which states that genetic information flows from

DNA → RNA → Protein (Fig. 10.5)

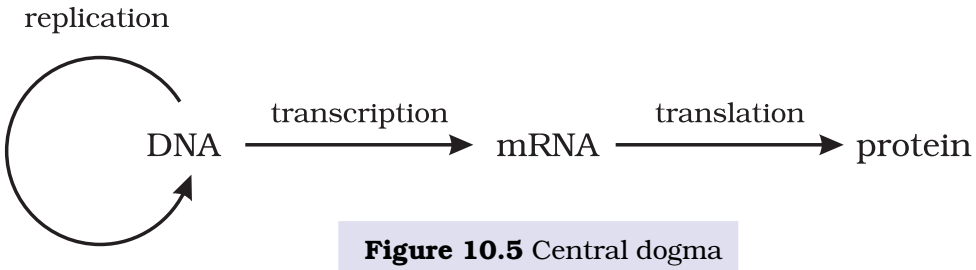


Figure 10.5 Central dogma

In some viruses called Retro viruses which contain RNA as genetic meterial (eg. HIV) the flow of information is in the reverse direction, that is, from RNA to DNA.

Can you suggest a simple name for the process?

10.1.2 Packaging of DNA Helix

Taking the distance between two consecutive base pairs as 0.34 nm (0.34×10^{-9} m), the length of DNA double helix in a typical mammalian cell, if calculated (simply by multiplying the total number of bp with distance between two consecutive bp, that is, 6.6×10^{9} bp $\times$ 0.34×10^{-9}m/bp), Turns out to be approximately 2.2 metres. This length is far greater than the dimension of a typical nucleus (approximately 10^{-6} m). How is such a long polymer packaged in a cell?

If the length of E. coli DNA is 1.36 mm, can you calculate the number of base pairs in E.coli? Scientists usually describe the length of DNA in kb or kbp (= 1000 bp).

In prokaryotes, such as, *E. coli*, though they do not have a defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region termed as 'nucleoid'. The DNA in the nucleoid is organised in large loops held by proteins.

The DNA of prokaryotes is almost circular and lacks chromatin organisation. This is referred to as *genophore.*

In eukaryotes, this organisation is much more complex. There is a set of positively charged basic proteins called **histones**. A protein acquires charge depending upon the abundance of amino acid residues with charged side chains. Histones are rich in the basic amino acid residues lysines and arginines. Both the amino acid residues carry positive charges in their side chains.

Histones are organised to form a unit of eight molecules called **histone octamer**. The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called **nucleosome** (Figure 10.6a). A typical nucleosome contains 200 bp of DNA helix. Nucleosomes constitute the repeating unit of a structure in nucleus called **chromatin**. The nucleosomes in chromatin are seen as 'beads-on-string' when viewed under a electron microscope (EM) (Figure 10.6b).

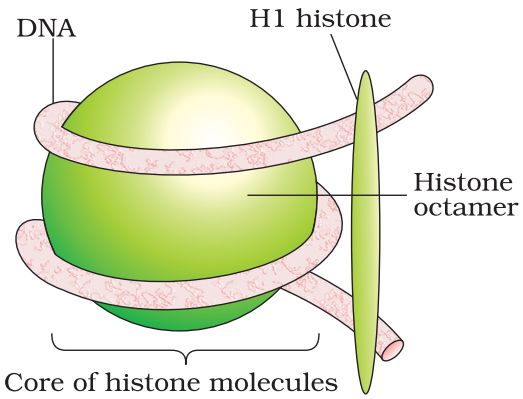


Figure 10.6a Nucleosome

The beads-on-string structure in chromatin is packaged to form chromatin fibers that are further coiled and condensed at metaphase stage of cell division to form chromosomes. The packaging of chromatin at a higher level requires additional set of proteins that collectively are referred to as **Non-histone Chromosomal (NHC) proteins**. In a typical nucleus, some regions of chromatin are loosely packed (and stain light) and are referred to as **euchromatin**. The chromatin that is more densely packed and stains dark is called as **Heterochromatin**. Euchromatin is transcriptionally active chromatin, whereas heterochromatin is inactive.

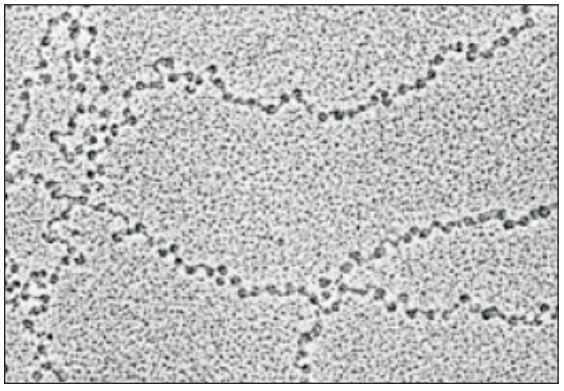


Figure 10.6b EM picture-'Beads-on-String'

10.2 The Search for Genetic Material

Even though the discovery of nuclein by Meischer and the proposition for principles of inheritance by Mendel were made almost at the same time, it took a longtime to discover and prove that DNA acts as a genetic material. By 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level. Previous discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan and numerous other scientists had narrowed the search to the chromosomes located in the nucleus of most cells. But the question of what molecule was actually the genetic material, had not been answered.

Transforming Principle

In 1928, Frederick Griffith, in a series of experiments with *Streptococcus pneumoniae* (bacterium responsible for pneumonia), witnessed a miraculous transformation in the bacteria. During the course of his experiment, he found that a living organism (bacteria) could change in physical form.

When *Streptococcus pneumoniae* (*Pneumococcus*) bacteria were grown on a culture plate, some produced smooth shiny colonies (S) while others produced rough colonies (R). This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not. Mice infected with the S strain (virulent) die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain → Injected into mice Mice die (Living S strain cells recovered from the body of dead mice)

R strain Injected into mice Mice live (Living R strain cells recovered from the body of mice)

Griffith was able to kill bacteria by heating them. He observed that heat-killed S strain bacteria injected into mice did not kill them. When he injected a mixture of heat killed S and live R bacteria, the mice died. Moreover, he recovered living S bacteria from the dead mice.

S strain (heat killed) Injected into mice Mice live (S or R strain cells not found in the body of mice)

S strain (heat killed) + R strain (live) injected into mice Mice die (Living S strain cells found in the body of dead mice)

He concluded that the R strain bacteria had somehow been **transformed** by the heat killed S strain bacteria. Some 'transforming principle', transferred from the heat killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent. This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material was not defined from his experiments.

Biochemical Characterisation of Transforming Principle

Prior to the work of **Oswald Avery**, **Colin MacLeod** and **Maclyn McCarty** (1933-44), the genetic material was thought to be a protein. These scientists worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.

They purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material, but not all biologists were convinced.

Can you think of any difference between DNAs and DNase?

10.2.1 The Genetic Material is DNA

The unequivocal proof that DNA is the genetic material came from the experiments of **Alfred Hershey** and **Martha Chase** (1952). They worked with viruses that infect bacteria, bacteriophages.

The bacteriophage attaches to the bacterium and its genetic material then enters the bacterial cell. The bacterial cell treats the viral genetic material as if it was its own and subsequently manufactures more virus particles. Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.

They grew some viruses on a medium that contained radioactive phosphorus and some others on a medium that contained radioactive sulfur. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA but not radioactive protein because DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulfur contained radioactive protein but not radioactive DNA because DNA does not contain sulfur.

Radioactive phages were allowed to attach to *E. coli* bacteria. Then, as the infection proceeded, the viral coats were removed from the bacteria by agitating them in a blender. The virus particles were separated from the bacteria by spinning them in a centrifuge.

Bacteria which were infected with viruses that had radioactive DNA were radioactive, indicating that DNA was the material that passed from the virus to the bacteria. Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did not enter the bacteria from the viruses. DNA is therefore the genetic material that is passed from virus to bacteria (Figure 10.7).

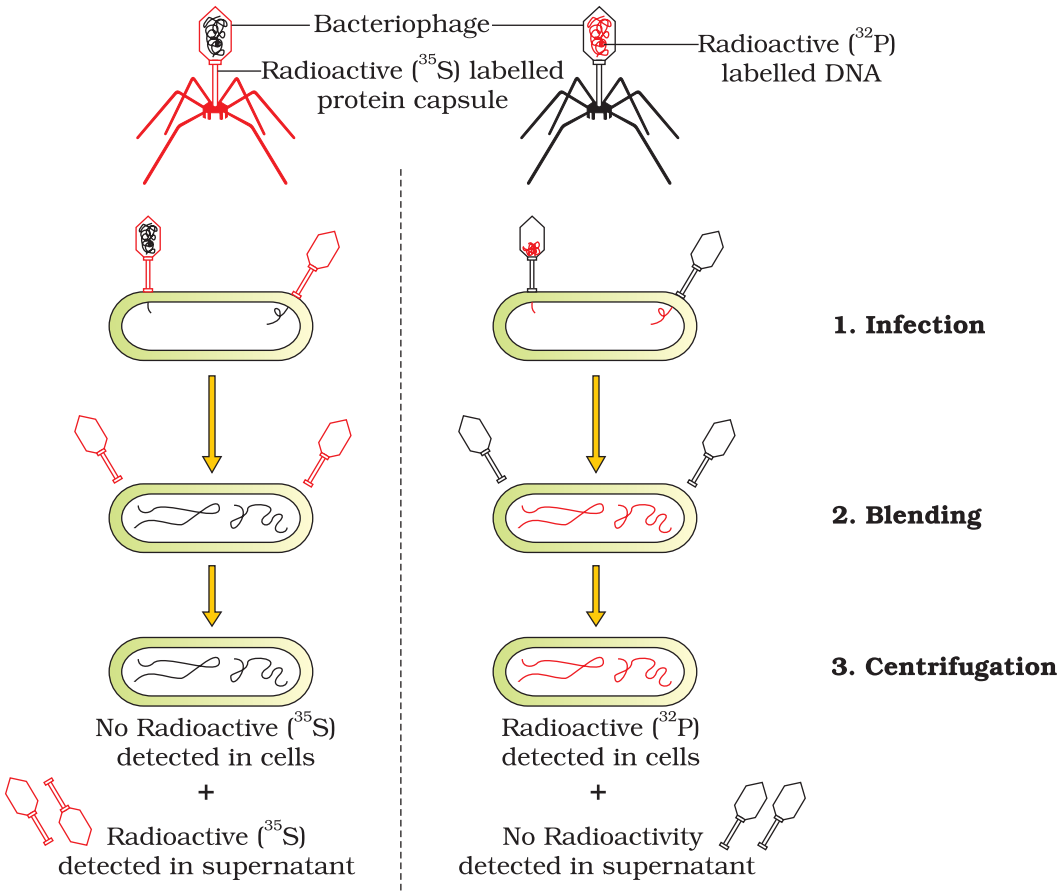


Figure 10.7 The Hershey-Chase experiment

10.2.2 Properties of Genetic Material (DNA versus RNA)

From the foregoing discussion, it is clear that the debate between proteins versus DNA as the genetic material was unequivocally resolved by the **Hershey-Chase** experiment. It became an established fact that it is DNA that acts as genetic material. However, it subsequently became clear that in some viruses, RNA is the genetic material (for example, Tobacco Mosaic viruses, QB bacteriophage, HIV etc.). The answers to some questions such as, why DNA is the predominant genetic material whereas RNA performs dynamic functions of messenger and adapter can be found from the differences between the chemical structures of the two nucleic acid molecules.

Can you recall the two chemical differences between DNA and RNA?

A molecule that can act as a genetic material must fulfill the following criteria:

(i) It should be able to generate its replica (Replication).

(ii) It should chemically and structurally be stable.

(iii) It should provide scope for slow changes (mutation) that are required for evolution.

(iv) It should be able to express itself in the form of 'Mendelian Characters'.

If one examines each requirement one by one, because of the rule of base pairing and complementarity, both the nucleic acids (DNA and RNA) have the ability to direct their duplications. The other molecules in the living system, such as proteins fail to fulfill the first criterion itself.

The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of the properties of genetic material was clearly evident in Griffith's 'transforming principle'. Heat, which killed the bacteria, at least did not destroy some of the properties of the genetic material. This now can easily be explained in the light of DNA. The two strands, being complementary, if separated by heating, come together when appropriate conditions are provided. Further, 2′-OH group present at every nucleotide in RNA is a reactive group and makes RNA labile and easily degradable. RNA is also now known to be catalytic, hence reactive. Therefore, DNA is chemically less reactive and structurally more stable when compared to RNA. Therefore, between the two nucleic acids, DNA is a better genetic material.

In fact, the presence of thymine at the place of uracil also confers additional stability to DNA. (Detailed discussion about this requires understanding of the process of repair in DNA, and you will study these processes in higher classes.)

Both DNA and RNA are able to mutate. In fact, RNA being unstable, mutates at a faster rate. Consequently, viruses having RNA genome and having shorter life span mutate and evolve faster.

RNA can directly code for the synthesis of proteins, hence can easily express the characters. DNA, however, is dependent on RNA for the synthesis of proteins. The protein synthesising machinery has evolved around RNA. The above discussion indicates that both RNA and DNA can function as genetic material, but DNA, being more stable, is preferred for storage of genetic information. For the transfer of genetic information, RNA is better.

10.3 RNA World

From the foregoing discussion, an immediate question becomes evident –which is the first genetic material? It shall be discussed in detail elsewhere under chemical evolution, but briefly, we shall highlight some of the facts.

RNA was the first genetic material. There is now enough evidence to suggest that essential life processes (such as metabolism, translation, splicing, etc.), evolved around RNA. RNA used to act as a genetic material as well as a catalyst (there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes). Catalytic RNA or RNA enzymes are known as **Ribozymes**.

But RNA, being a catalyst, was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that made it more stable. DNA, being double stranded and having complementary strands, further resists changes by evolving a process of repair.

10.4 Replication

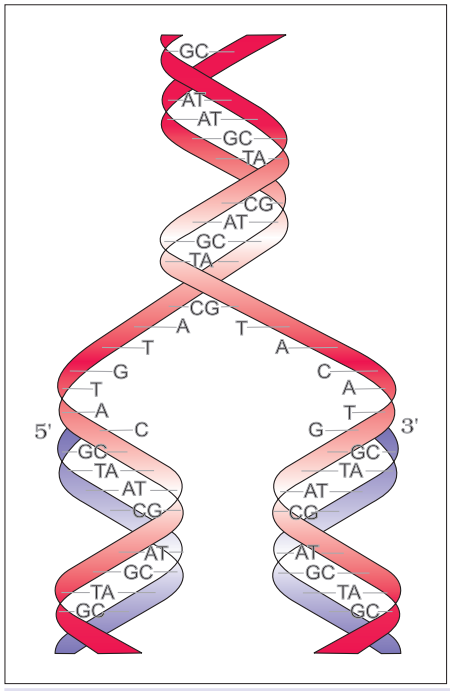


Figure 10.8 Watson-Crick model for semiconservative DNA replication

While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).

The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This scheme was termed as **semiconservative** DNA replication (Figure 10.8).

10.4.1 The Experimental Proof

It is now proven that DNA replicates semiconservatively. It was shown first in *Escherichia coli* and subsequently in higher organisms such as plants and human cells. Matthew Meselson and Franklin Stahl performed the following experiment in 1958:

(i) They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Note that ^{15}N is not a radioactive isotope, and it can be separated from ^{14}N based on densities only).

(ii) Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA. The results are shown in Figure 10.9.

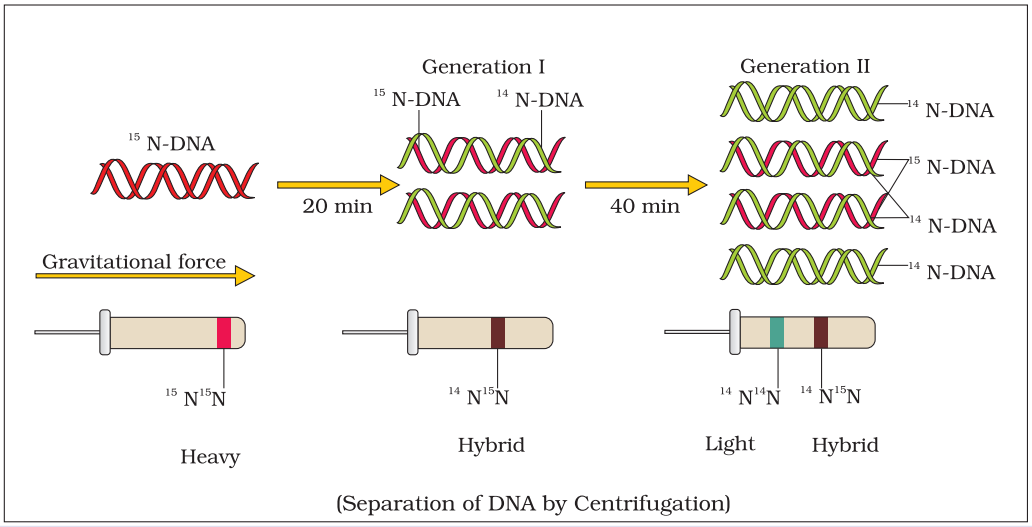


Figure 10.9 Meselson and Stahl's Experiment

Can you recall what centrifugal force is, and think why a molecule with higher mass/density would sediment faster?

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; *E. coli* divides in 20 minutes] had a hybrid or intermediate density.

DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of 'light' DNA.

If E. coli was allowed to grow for 80 minutes then what would be the proportions of DNA molecules with light and hybrid densities ?

Very similar experiments involving use of radioactive thymidine to detect distribution of newly synthesised DNA in the chromosomes was performed on *Vicia faba* (faba beans) by **Taylor** and his colleagues in 1958. The experiments proved that the DNA in chromosomes also replicates semiconservatively.

10.4.2 The Machinery and the Enzymes

In living cells, such as *E. coli*, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-dependent **DNA polymerase**, since it uses a DNA template to catalyse the polymerisation of deoxynucleotides. These enzymes are highly efficient enzymes as they have to catalyse polymerisation of a large number of nucleotides in a very short time. *E. coli* that has only 4.6×10^6 bp (compare it with humans' whose diploid content is 6.6×10^9 bp), completes the process of replication within 38 minutes; that means the average rate of polymerisation has to be approximately 2000 bp per second.

Not only do these polymerases have to be fast, but they also have to catalyse the reaction with a high degree of accuracy. Any mistake during replication would result in mutations. Furthermore in terms of energy, replication is a very expensive process. Deoxyribonucleoside triphosphates serve a dual purpose. In addition to acting as substrates, they provide energy for polymerisation reaction (the two terminal phosphates in a deoxynucleoside triphosphates are high-energy phosphates, as in the case of ATP).

In addition to DNA-dependent DNA polymerases, many additional enzymes are required to complete the process of replication with a high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated throughout the entire length in one stretch (due to very high energy requirement), the replication occur within a small opening of the DNA helix, referred to as **replication fork**. The DNA-dependent DNA polymerases catalyse polymerisation only in one direction, that is $5'\rightarrow3'$.

This creates some additional complications at the replicating fork. Consequently, on one strand (the template with polarity $3'\rightarrow5'$) the replication is **continuous**, (this strand is known as leading stand) while on the other (the

template with polarity 5′→3′), it is **discontinuous** (it is the lagging strand). The discontinuously synthesised fragments (known as Okazaki fragments) are later joined by the enzyme **DNA ligase** (Figure 10.10).

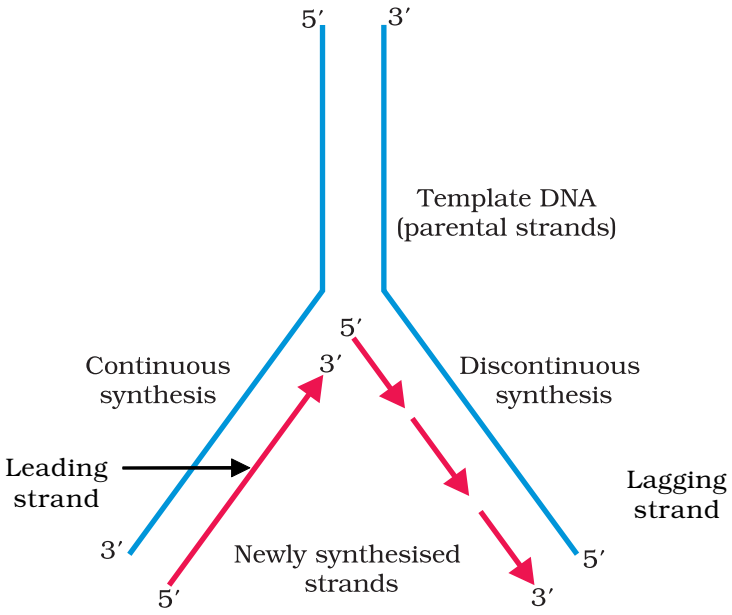


Figure 10.10 Replicating Fork

The DNA polymerases on their own cannot initiate the process of replication. A small stretch of RNA (called a primer) is required for initiation. It is used as an anchor for the addition of new DNA neucleotides. The primer is removed later. Also the replication does not initiate randomly at any place in the DNA. Rather, there is a definite region in *E. coli* DNA where the replication originates. Such a region is termed as the **origin of replication** (*Ori*). It is because of the requirement of this origin of replication that a piece of DNA, if needed to be propagated during recombinant DNA procedures, requires a vector. The vectors provide the origin of replication.

Further, not every detail of replication is understood well. In eukaryotes, the replication of DNA takes place at the S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results in polyploidy (a chromosomal anomaly). You will learn the detailed nature of the origin and the processes occurring at this site, in higher classes.

10.5 Transcription

The process of copying genetic information from one strand of DNA into RNA is termed **transcription**. Here also, the principle of complementarity governs the process of transcription, except that the adenosine now base pairs with **uracil** instead of **thymine**. However, unlike in the process of replication in which once set in, the total DNA of an organism gets duplicated, in transcription only a segment of DNA and only one of the strands is copied into RNA. This necessitates defining the boundaries that would demarcate the region and the strand of DNA that would be transcribed.

Why both the strands are not copied during transcription has a simple answer. First, if both strands act as a template, they would code for RNA molecules with different sequences (remember complementarity does not mean being identical), and in turn, if they code for proteins, the sequence of amino acids in the proteins would be different. Hence, one segment of the DNA would be coding for two different proteins, and this would complicate the genetic information transfer machinery. Second, the two RNA molecules, if produced simultaneously, would be complementary to each other, hence would form a double stranded RNA. This would prevent RNA from being translated into protein and the exercise of transcription would become a futile one.

10.5.1 Transcription Unit

A transcription unit in DNA is defined primarily by the three regions in the DNA:

(i) A Promoter

(ii) The Structural gene

(iii) A Terminator

There is a convention in defining the two strands of the DNA in the structural gene of a transcription unit. Since the two strands have opposite polarity and the **DNA-dependent RNA polymerase** (or transcriptase) also catalyses the polymerisation in only one direction, that is, 5′→3′, the strand that has the polarity 3′→5′ acts as a template, and is also referred to as **template strand**. The other strand, which has the polarity (5′→3′) and the sequence same as RNA (except **thymine** at the place of **uracil**), is displaced during transcription. Strangely, this strand (which, does not code for anything) is referred to as the **coding strand**. All points while defining a transcription unit are made with reference to the coding strand. To explain the point, a hypothetical sequence from a transcription unit is represented below:

3′-ATGCATGCATGCATGCATGCATGC-5′ Template Strand
5′-TACGTACGTACGTACGTACGTACG-3′ Coding Strand

Can you now write the sequence of RNA transcribed from the above DNA?

The **promoter** and **terminator** flank the **structural gene** in a transcription unit. The promoter is said to be located towards 5′-end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides the binding site for RNA polymerase, and it is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with that of the terminator, the definition of coding and template strands can be reversed. The terminator is located towards 3′-end (downstream) of the coding strand and it usually defines the end of the process of transcription (Figure 10.11). There are additional regulatory sequences that may be present further upstream or downstream of the promoter. Some of the properties of these sequences shall be discussed while dealing with the regulation of gene expression.

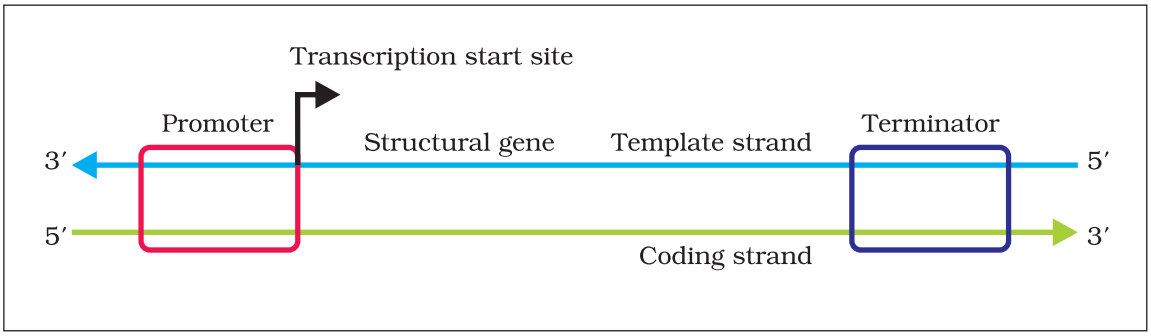


Figure 10.11 Schematic structure of a transcription unit

10.5.2 Transcription Unit and the Gene

A gene is defined as the functional unit of inheritance. Though there is no ambiguity that the genes are located on the DNA, it is difficult to literally define a gene in terms of DNA sequence. The DNA sequence coding for tRNA or rRNA molecule also define a gene. However by defining a **cistron** as a segment of DNA coding for a polypeptide, the structural gene in a transcription unit can be said to be **monocistronic** (mostly in eukaryotes) or **polycistronic** (mostly in bacteria or prokaryotes). In eukaryotes, the monocistronic structural genes have interrupted coding sequences – the genes in eukaryotes are split. The coding sequences or expressed sequences are defined as **exons**. Exons are said to be those sequence that appear in mature or processed RNA. The exons

are interrupted by **introns**. Introns or intervening sequences do not appear in mature or processed RNA. The split-gene arrangement further complicates the definition of a gene in terms of a DNA segment.

Inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as regulatory genes, even though these sequences do not code for any RNA or protein.

10.5.3 Types of RNA and the process of Transcription

In bacteria, there are three major types of RNAs: mRNA (messenger RNA), tRNA (transfer RNA), and rRNA (ribosomal RNA). All three RNAs are needed to synthesise a protein in a cell. The mRNA provides the template, tRNA reads the genetic code and brings aminoacids and rRNAs play a structural and catalytic role during translation. There is single DNA-dependent RNA polymerase that catalyses transcription of all types of RNA in bacteria. RNA polymerase binds to the promoter and initiates transcription (**Initiation**). It uses nucleoside triphosphates as substrate and polymerises in a template dependent fashion following the rule of complementarity. It somehow also facilitates opening of the helix and continues **elongation.** Only a short stretch of RNA remains bound to the enzyme. Once the polymerase reaches the terminator region, the nascent RNA falls off, so also the RNA polymerase. This results in **termination** of transcription.

An intriguing question is that how is the RNA polymerase is able to catalyse all the three steps, initiation, elongation and termination. The RNA polymerase is only capable of catalysing the process of elongation. It associates transiently with **initiation-factor** (σ) and **termination-factor** (ρ) to initiate and terminate the transcription, respectively. Association with these factors alter the specificity of the RNA polymerase to either initiate or terminate (Figure 10.12).

In bacteria, since the mRNA does not require any processing to become active, and also since transcription and translation take place in the same compartment (there is no separation of cytosol and nucleus in bacteria), many times the translation can begin much before the mRNA is fully transcribed. Consequently, the transcription and translation can be coupled in bacteria.

In eukaryotes, there are two additional complexities –

(i) There are at least three RNA polymerases in the nucleus (in addition to the RNA polymerase found in the organelles). There is a clear cut division of labour. The RNA polymerase I transcribes **rRNAs** (28S, 18S, and 5.8S), the RNA polymerase II transcribes the precursor of mRNA, the **heterogeneous nuclear RNA** (**hnRNA**), whereas the RNA polymerase III is responsible for transcription of **tRNA**, **5srRNA**, and **snRNAs** (**small nuclear RNAs**).

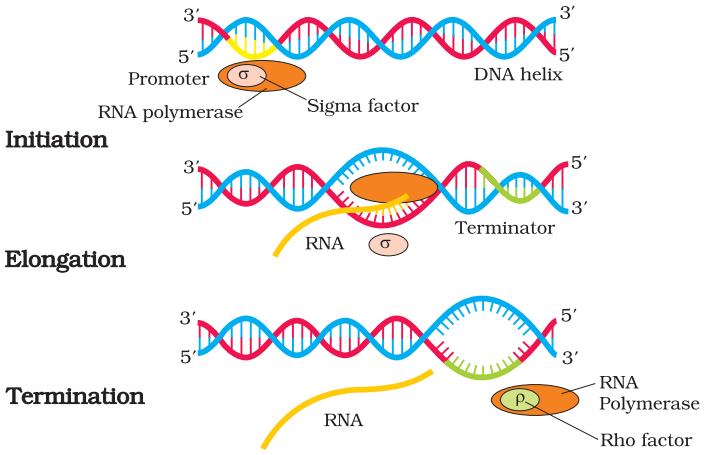


Figure 10.12 Process of Transcription in Bacteria

(ii) The second complexity is that the primary transcripts contain both exons and introns and are non-functional. Hence, they are subjected to a process called **splicing** where the introns are removed and exons are joined in a defined order. hnRNA undergoes additional processing called capping and tailing. In **capping** an unusual nucleotide (methyl guanosine triphosphate) is added to the 5′-end of hnRNA. In **tailing**, adenylate residues (200-300) are added at 3′-end in a template independent manner. It is the fully processed hnRNA, now called mRNA, that is transported out of the nucleus for translation (Figure 10.13).

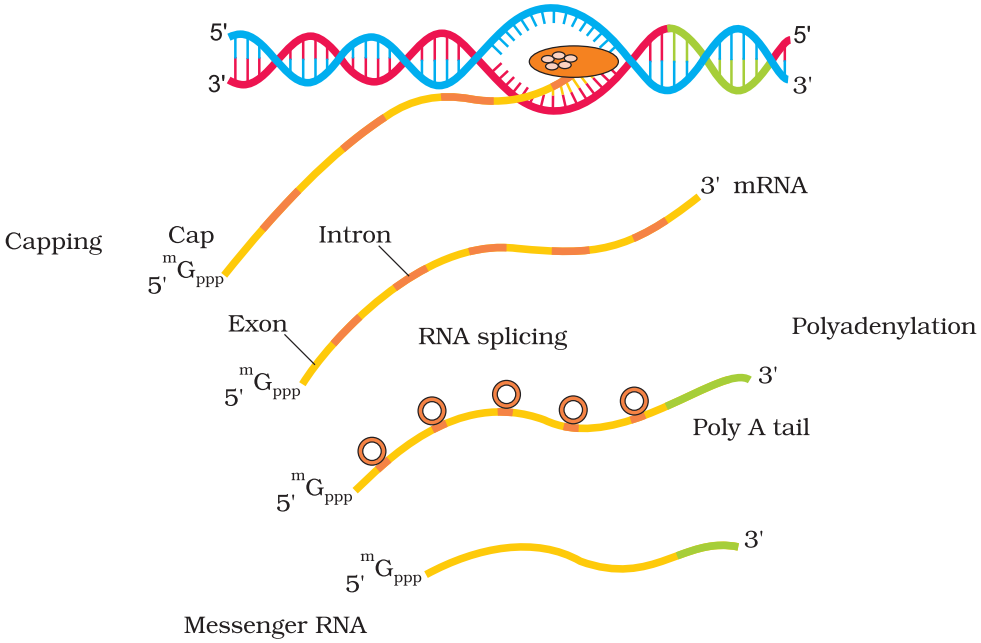


Figure 10.13 Process of Transcription in Eukaryotes

The significance of such complexities is now beginning to be understood. The split-gene arrangements represent probably an ancient feature of the genome. The presence of introns is reminiscent of antiquity, and the process of splicing represents the dominance of **RNA-world**. In recent times, the understanding of RNA and RNA-dependent processes in the living system have assumed more importance.

10.6 Genetic Code

During both replication and transcription, a nucleic acid is copied to form another nucleic acid. Hence, these processes are easy to conceptualise on the basis of complementarity. The process of translation requires transfer of genetic information from a polymer of nucleotides to a polymer of amino acids. Neither does any complementarity exist between nucleotides and amino acids, nor could any be drawn theoretically. There existed ample evidences to support the notion that change in nucleic acids (genetic material) was responsible for change in amino acids in proteins. This led to the proposition of a genetic code that could direct the sequence of amino acids during synthesis of proteins.

If determining the biochemical nature of genetic material and the structure of DNA was very exciting, the proposition and deciphering of genetic code were most challenging. In a very true sense, it required involvement of scientists from several disciplines – physicists, organic chemists, biochemists and geneticists. It was George Gamow, a physicist, who argued that since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases. He suggested that in order to code for all the 20 amino acids, the code should be made up of three nucleotides. This was a very bold proposition, because a permutation and combination of 4^3 (4 × 4 × 4) would generate 64 codons, generating many more codons than required.

Providing proof that the codon was a triplet, was a more daunting task. The chemical method developed by Har Gobind Khorana was instrumental in synthesising RNA molecules with defined combinations of bases (homopolymers such as UUU and copolymers such as UUC, CCA). Marshall Nirenberg's cell-free system for protein synthesis finally helped the code to be deciphered. The enzyme polynucleotide phosphorylase (Ochoa's enzyme; named after Severo Ochoa) was also helpful in polymerising RNA with defined sequences in a template independent manner (enzymatic synthesis of RNA). Finally a checker-board for genetic code was prepared which is given in Table 10.1.

Table 10.1 The Codons for the Various Amino Acids

First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

The salient features of the genetic code are as follows:

(i) The codon is triplet. 61 codons code for amino acids and 3 codons do not code for any amino acids, hence they function as stop codons.

(ii) One codon codes for only one amino acid, hence, it is **unambiguous** and **specific**.

(iii) Some amino acids are coded by more than one codon, hence the code is **degenerate**.

(iv) The codon is read in mRNA in a contiguous fashion. There are no punctuations.

(v) The code is nearly **universal**: for example, from bacteria to human, UUU would code for Phenylalanine (Phe). Some exceptions to this rule have been found in mitochondrial codons, and in some protozoans.

(vi) AUG has dual functions. It codes for Methionine (Met) , and also acts as the **initiator** codon.

If following is the sequence of nucleotides in mRNA, predict the sequence of amino acid coded by it (take the help of the checkerboard):

-AUG UUU UUC UUC UUU UUU UUC-

(There are no gaps in the actual arrangement of the nucleotides)

Now try the opposite. Following is the sequence of amino acids coded by an mRNA. Predict the nucleotide sequence in the RNA:

Met-Phe-Phe-Phe-Phe-Phe-Phe

Do you face any difficulty in predicting the opposite?

Can you now correlate this with two properties of genetic code you have learnt?

10.6.1 Mutations and Genetic Code

The relationships between genes and DNA are best understood by mutation studies. You have studied about mutation and its effect in Chapter 9. Effects of large deletions and rearrangements in a segment of DNA (or in a chromosome)

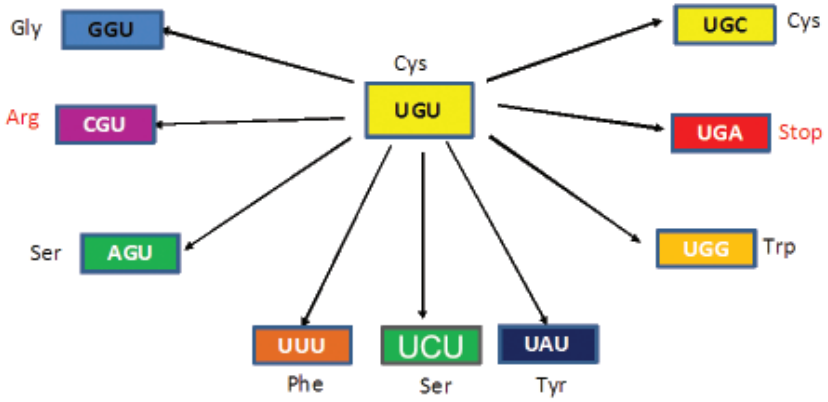


Figure 10.14 - Results of a nucleotide change in a codon

are easy to comprehend. It may result in loss or gain of a gene and so a function. The effect of point mutations will be explained here. A classical example of point mutation is a change of single base pair in the gene for beta globin chain (in human haemoglobin) that results in the change of amino acid residue glutamate to valine. It results in a diseased condition called **sickle cell anemia**. How a change in a single nucleotide in a codon alters the amino acids in a polypeptide chain is given in Fig. 10.14. Effect of point mutations that insert or delete a base in structural gene can be better understood by following a simple example.

Consider a statement that is made up of the following words each having three letters like a genetic code.

RAM HAS RED CAP

If we insert a letter B in between HAS and RED and rearrange the statement, it would read as follows:

RAM HAS BRE DCA P

Similarly, if we now insert two letters at the same place, say BI', it would read
RAM HAS BIR EDC AP

Now we insert three letters together, say BIG, the statement would read
RAM HAS BIG RED CAP

The same exercise can be repeated, by deleting the letters R, E and D, one by one and rearranging the statement to make a triplet word.

RAM HAS EDC AP

RAM HAS DCA P

RAM HAS CAP

The conclusion from the above exercise is obvious. Insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion. Insertion or deletion of three or its multiple bases insert or delete one or multiple codons - hence one or multiple amino acids, and reading frame remains unaltered from that point onwards. Such mutations are referred to as **frame-shift, insertion** or **deletion mutations** depending on the change. This forms the genetic basis of proof that codon is a triplet and is read in a contiguous manner.

10.6.2 tRNA– the Adapter Molecule

From the very beginning of the proposition of code, it was clear to Francis Crick that there has to be a mechanism to read the code and also to link it to the amino acids, because amino acids have no structural specialities to read the code uniquely. Crick postulated the presence of an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acids. The tRNA, then called sRNA (soluble RNA), was known before the genetic code was postulated. However, its role as an adapter molecule was assigned much later.

tRNA has an **anticodon loop** that has bases complementary to the code, and it also has an **amino acid acceptor end** with which it binds to amino acids. tRNAs are specific for each amino acid (Figure 10.15) . For initiation, there is another specific tRNA that is referred to as **initiator tRNA**. There are no tRNAs for stop codons. In Figure 10.15, the secondary structure of tRNA has been depicted that looks like a clover-leaf. In actual structure, the tRNA is a compact molecule which looks like an inverted L.

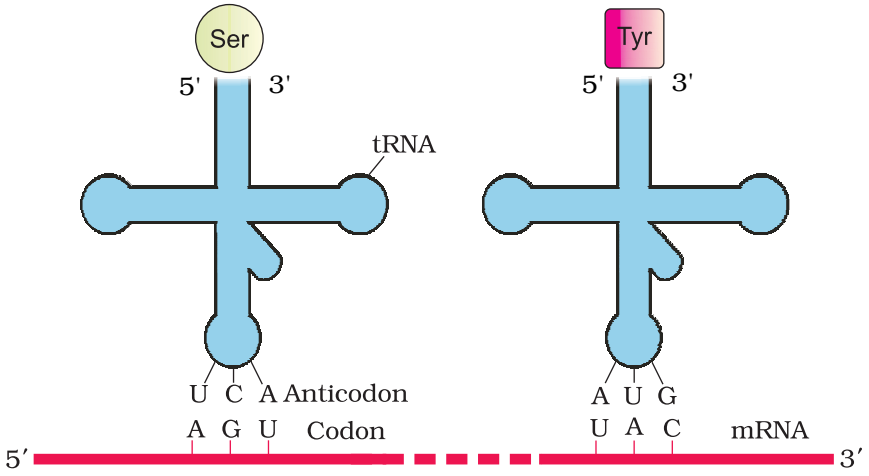


Figure 10.15 tRNA - the adapter molecule

10.7 Translation

Translation refers to the process of polymerisation of amino acids to form a polypeptide (Figure 10.16). The order and sequence of amino acids are defined

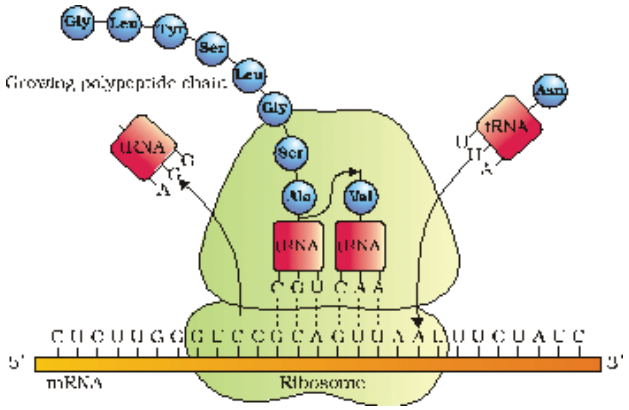


Figure 10.16 Translation

by the sequence of bases in the mRNA. The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy. Therefore, in the first phase itself, amino acids are activated in the presence of ATP and linked to their cognate tRNA–a process commonly called as **charging of tRNA** or **aminoacylation of tRNA**, to be more specific. If two such charged tRNAs are brought close enough, the formation of a peptide bond between them would be favoured energetically. The presence of a catalyst would enhance the rate of peptide bond formation.

The cellular factory responsible for synthesising proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins. In its inactive state, it exists as two subunits; a large subunit and a small subunit. When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. There are two sites in the large subunit for subsequent amino acids to bind to and, thus, be close enough to each other for the formation of a peptide bond. The ribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) for the formation of a peptide bond.

A translational unit in mRNA is the sequence of RNA that is flanked by the start codon (AUG) and the stop codon and codes for a polypeptide. An mRNA also has some additional sequences that are not translated and are referred to as **untranslated regions** (**UTR**). The UTRs are present at both 5′-end (before start codon) and at 3′-end (after stop codon). They are required for efficient translation process.

For initiation, the ribosome binds to the mRNA at the start codon (AUG) that is recognised only by the initiator tRNA. The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes composed of an amino acid linked to tRNA sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into polypeptide sequences dictated by DNA and represented by mRNA. At the end, a **release factor** binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.

10.8 Regulation of Gene Expression

Regulation of gene expression refers to a very wide sequence of events that may occur at various levels. Considering that gene expression results in the formation of a polypeptide, it can be regulated at several levels. In eukaryotes, the regulation could be exerted at

(i) transcriptional level (formation of primary transcript)

(ii) processing level (regulation of splicing)

(iii) transport of mRNA from the nucleus to the cytoplasm

(iv) translational level

The genes in a cell are expressed to perform a particular function or a set of functions. For example, an enzyme called beta-galactosidase is synthesised by *E. coli*. It is used to catalyse the hydrolysis of a disaccharide, lactose into

galactose and glucose; bacteria use them as a source of energy. Hence, if the bacteria do not have lactose around them to be utilised for energy source, they would no longer require the synthesis of the enzyme beta-galactosidase. Therefore, in simple terms, it is the metabolic, physiological or environmental conditions that regulate the expression of genes. The development and differentiation of embryo into adult organisms is also a result of the coordinated regulation of expression of several sets of genes.

In prokaryotes, control of the rate of transcriptional initiation is the predominant target site for control of gene expression. In a transcription unit, the activity of RNA polymerase at a given promoter is, in turn, regulated by interaction with accessory proteins, which affects the ability to recognise start sites. These regulatory proteins can act both positively (activators) and negatively (repressors). The accessibility of promoter regions of prokaryotic DNA is in many cases regulated by the interaction of proteins with sequences termed **operators**. The operator region is adjacent to the promoter elements in most operons and in most cases the sequences of the operator bind a repressor protein. Each operon has its specific operator and specific repressor. For example, *lac* operator is present only in the *lac* operon and it interacts specifically with *lac* repressor only.

10.8.1 The *Lac* operon

The elucidation of the *lac* operon was also a result of a close association between a geneticist, **Francois Jacob** and a biochemist, **Jacque Monod**. These scientists were the first to elucidate a transcriptionally regulated system. In *lac* operon (here *lac* refers to lactose), a polycistronic structural gene is regulated by a common promoter and regulatory genes. Such an arrangement is very common in bacteria and is referred to as **operon**. To name a few such examples, *lac* operon, *trp* operon, *ara* operon, *his* operon, *val* operon, etc.

The *lac* operon consists of one regulatory gene (the *i* gene – here the term *i* does not refer to inducer, rather it is derived from the word inhibitor) the promoter (p) and operator (o); and three structural genes (*z*, *y*, and *a*). The *i* gene codes for the repressor of the *lac* operon. The *z* gene codes for beta-galactosidase (β-gal) enzyme which is primarily responsible for the hydrolysis of the disaccharide, lactose, into its monomeric units, galactose and glucose. The *y* gene codes for enzyme permease, which increases permeability of the cell to β-galactosides. The *a* gene encodes a transacetylase enzyme. Hence, all the three gene products in *lac* operon are required for metabolism of lactose. In most other operons as well, the genes present in the operon are needed

together to function in the same or related metabolic pathway (Figure 10.17).

Lactose is the substrate for the enzyme beta-galactosidase and it regulates switching on and off of the operon. Hence, it is termed as **inducer**. In the absence of a preferred carbon source such as glucose, if lactose is provided in the growth medium of the bacteria, it is transported into the cells through the action of permease (remember, a very low level of expression of *lac* operon has to be present in the cell all the time, otherwise lactose cannot enter the cells). The lactose then induces the operon in the following manner.

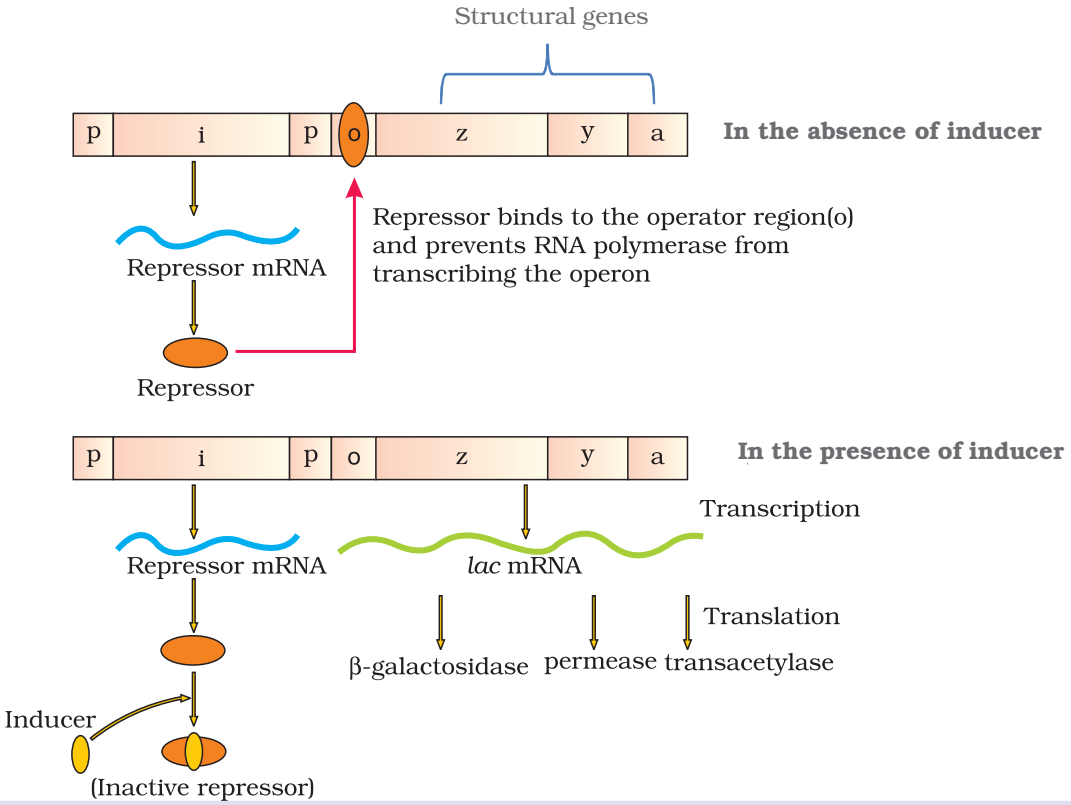


Figure 10.17 The *lac* Operon

The repressor of the operon is synthesised all the time (hence said to be constitutively) from the *i* gene. The repressor protein binds to the operator region of the operon and prevents RNA polymerase from transcribing the operon. In the presence of an inducer, such as lactose or allolactose, the repressor is inactivated by interaction with the inducer. This allows RNA polymerase to access the promoter and transcription proceeds (Figure 10.17). Essentially, regulation of *lac* operon can also be visualised as regulation of enzyme synthesis by its substrate.

Remember, glucose or galactose cannot act as inducers for lac operon. Can you think for how long the lac operon would be expressed in the presence of lactose?

Regulation of *lac* operon by repressor is referred to as **negative regulation**. *Lac* operon is under the control of positive regulation as well, but it is beyond the scope of discussion at this level.

SUMMARY

SUMMARY

Nucleic acids are long polymers of nucleotides. While DNA stores genetic information, RNA mostly helps in transfer and expression of information. Though DNA and RNA both function as genetic material, DNA is chemically and structurally more stable and is a better genetic material. However, RNA was the first to evolve and DNA was derived from RNA. The hallmark of the double stranded helical structure of DNA is the hydrogen bonding between the bases from opposite strands. The rule is that Adenine pairs with Thymine through two H-bonds, and Guanine with Cytosine through three H-bonds. This makes one strand complementary to the other. The DNA replicates semiconservatively, the process is guided by the complementary H-bonding. A segment of DNA that codes for RNA may, in simplistic term be referred to as gene. During transcription also, one of the strands of DNA acts as a template to direct the synthesis of complementary RNA. In bacteria, the transcribed mRNA is functional, hence can directly be translated. In eukaryotes, the gene is split. The coding sequences, exons, are interrupted by non-coding sequences, introns. Introns are removed and exons are joined to produce functional RNA by a process called splicing. The messenger RNA contains the base sequences that are read in a combination of three (to make triplet genetic code) to code for an amino acid. The genetic code is read again on the principle of complementarity by tRNA that acts as an adapter molecule. There are specific tRNAs for every amino acid. The tRNA binds to specific amino acid at one end and pairs through H-bonding with codes on mRNA through its anticodons. The site of translation (protein synthesis) is ribosomes, which bind to mRNA and provide a platform for joining of amino acids. One of the rRNA acts as a catalyst for peptide bond formation, which is an example of RNA as an enzyme (ribozyme). Translation is a process that has evolved around RNA, indicating that life began around RNA. Since, transcription and translation are energetically very expensive processes, these have to be tightly regulated. Regulation of transcription is the primary step for regulation of gene expression. In bacteria, more than one gene is arranged together and regulated in units called operons. *Lac* operon is the prototype operon in bacteria, which codes for genes responsible for metabolism of lactose. The operon is regulated by the amount of lactose in the medium where the bacteria are grown. Therefore, this regulation can also be viewed as regulation of enzyme synthesis by its substrate.

GLOSSARY

Anticodon: A sequence of three adjacent nucleotides in tRNA that is complementary to the codon in messenger RNA which specifies the amino acid.

Complementarity: It is the property shared by two nitrogenous bases or two polynucleotide strands which, when positioned opposite to each other, can pair by forming hydrogen bonds between A & T or U and between G & C.

Double helix: It is the second order structure formed by two complementary deoxy polynucleotide chains twisted around each other in mutually opposite orientation and held together by hydrogen bonds.

Gene regulation : It is the process of turning genes on and off so that appropriate genes are expressed at proper times and places.

Genetic Code: The set of all possible three-nucleotide combinations in DNA / mRNA that determine the specific amino acid sequence in a polypeptide chain.

Genomics: It is the field of study dealing with determination of the nucleotide sequence of the genome and its analysis using computer programmes.

Histones: Histones are the chief protein components of chromatin in eukaryotic cells, acting as spools around which DNA winds, and play a role in gene regulation.

hnRNA: Heterogenous nuclear RNA found in the nucleus which becomes mRNA after undergoing processing or maturation.

m RNA: It is the form of RNA that carries information from DNA to the ribosomes and functions as the template for protein synthesis.

Negative regulation: The method of regulation of operons or gene expression where repressor protein binds to the operator site preventing the expression of structural genes.

Nucleosome: It is the basic unit of DNA packaging into chromatin and consists of a segment of DNA (200 bp) wound around a protein core of 8 histone molecules.

Operon: It is a group of closely placed structural genes and regulatory elements (DNA sequences) such as a regulator, a promoter and an operator, which function in a co-ordinated manner

Operator: It is a segment of DNA to which the product of regulatory gene (protein) binds. In the *lac operon* it is a segment between the promoter and the structural genes of the operon.

Promoter: It is a region of DNA where RNA polymerase binds and initiates transcription.

Replication fork: It is a site on a DNA double helix where both unwinding of the helix and synthesis of daughter molecules occur.

Structural gene: It codes for mRNA that determines the amino acid sequence in a protein or polypeptide chain other than a regulatory protein.

sn RNA: Small nuclear ribonucleic acids found within the eukaryotic nucleus. They are involved in some important process such as RNA splicing and regulation of transcription factors.

t RNA: Transfer RNA is an adaptor molecule, that is used by all living organisms to bridge the genetic code in messenger RNA with the twenty amino acids in proteins. tRNAs carry amino acids to ribosomes, where they are linked into proteins.

QUESTIONS

Very Short Answer Type Questions

1. What is the function of histones in DNA packaging?
2. Distinguish between heterochromatin and euchromatin. Which of the two is transcriptionally active?
3. Who proved that DNA is genetic material? What is the organism they worked on?
4. What is the function of DNA polymerase?
5. What are the components of a nucleotide?
6. Write the structure of chromatin.
7. What is the cause of discontinuous synthesis of DNA on one of its parental strands? What happens to these short stretches of synthesised DNA?
8. Given below is the sequence of coding strand of DNA in a transcription Unit

   3 'A A T G C A G C T A T T A G G – 5'

   write the sequence of

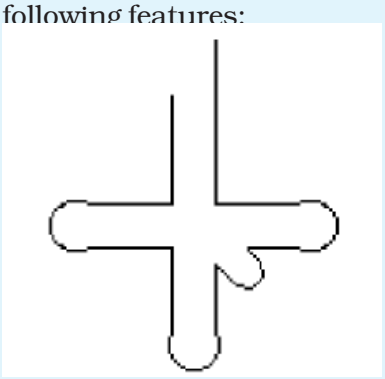
   a) its complementary strand

   b) the mRNA
9. In a nucleus, the number of ribonucleoside triphosphates is 10 times the number of deoxy ribonucleoside triphosphates, but only deoxyribonucleotides are added during the DNA replication. Suggest a mechanism.
10. Name any three viruses which have RNA as the genetic material.
11. Write the sequence of nucleotides after single base insertion and deletion in the given gene. Gene: THE CAT ATE THE FAT RAT.
12. Why was DNA chosen over RNA as genetic material in the majority of the organisms?
13. What are the components of a transcription unit?
14. What is the difference between exons and introns?
15. What is meant by capping and tailing?
16. What is meant by point mutation? Give an example.
17. Define peptide bond. Why are proteins referred to as polypeptide chains?
18. What is meant by charging of tRNA?
19. What is a regulator and a promoter?
20. During DNA replication, why is it that the entire molecule does not open in one go? Explain replication fork.
21. What is the function of the codon-AUG?
22. Define stop codon. Write the codons.
23. What is the difference between the template strand and a coding strand in a DNA molecue?
24. Write any two chemical differences between DNA and RNA.
25. In a typical DNA molecule, the proportion of Thymine is 30% of the N bases. Find out the percentages of other N bases.

26. The proportion of nucleotides in a given nucleic acid are: Adenine 18%, Guanine 30%, Cytosine 42%, and Uracil 10%. Name the nucleic acid and mention the number of strands in it.
27. If the base sequence of a codon in mRNA is 5'AUG-3'.What is the sequence of tRNA pairing with it?

Short Answer Type Questions

1. Define transformation in Griffith's experiment. Discuss how it helps in the identification of DNA as genetic material.
2. Who revealed the biochemical nature of the transforming principle? How was it done?
3. Discuss the significance of heavy isotope of nitrogen in Meselson and Stahl's experiment.
4. Define a cistron. Differentiate between monocistronic and polycistronic transcription unit with suitable examples.
5. Recall the experiments done by Frederick Griffith in which DNA was speculated to be the genetic material. If RNA, instead of DNA, was the genetic material, would the heat killed strain of Pneumococcus have transformed the R-strain into virulent strain? Explain.
6. There is only one possible sequence of amino acids when deduced from a given nucleotides. But a multiple nucleotide sequence can be deduced from a single amino acid sequence. Explain this phenomenon.
7. A single base mutation in a gene may not always result in loss or gain of function. Do you think the statement is correct? Defend your answer.
8. A low level of expression of lac operon occurs all the time. Can you explain the logic behind this phenomenon.
9. What background information did Watson and Crick have for developing a model of DNA? What was their contribution?
10. What are the functions of (i) methylated guanosine cap, (ii) poly-A "tail" in a mature on RNA?
11. How many types of RNA polymerases exist in cells? Write their names and functions.
12. Write briefly about DNA polymerase.
13. What are the contributions of George Gamow, H.G.Khorana, Marshall Nirenberg in deciphering the genetic code?
14. On the diagram of the secondary structure of tRNA shown below indicate the location of the following features:



a) Anticodon b) Acceptor stem
c) Anticodon stem
d) 5' end e) 3' end

15. If there are 2.9×10^9 complete turns in a DNA molecule estimate the length of the molecule.
(1 angstrom=10^{-8} cm).
16. Draw the schematic/ diagrammatic presentation of the lac operon.
17. What are the differences between DNA and RNA.
18. Write the important features of Genetic code?
19. Describe the sequential steps in the replication of a DNA molecule.
20. Give a diagrammatic presentation of the process of transcription in a bacterial cell.
21. Write briefly on nucleosomes.

Long Answer Type Questions

1. Give an account of the Hershey and Chase experiment. What did it conclusively prove? If both DNA and proteins contained phosphorus and sulphur do you think the result would have been the same.
2. Give an account of post transcriptional modifications of a eukaryotic mRNA.
3. Discuss the process of translation in detail.
4. Write briefly about Griffith's experiments on *S. pnuemoniae* bacteria. What was his conclusion?
5. Define an operon, giving an example. Explain what is an Inducible operon.
6. Give the salient features of the Double helix structure of DNA.
7. Replication was allowed to take place in the presence of radioactive Deoxy- nucleotide precursors in E.coli that was a mutant for DNA ligase. Explain how the newly synthesised radioactive DNA will be when purified.

Exercises

1. Group the following as nitrogenous bases and nucleosides: Adenine, Cytidine, Thymine, Guanosine, Uracil and Cytosine.
2. If a double stranded DNA has 20 per cent of cytosine, calculate the percent of adenine in the DNA.
3. If the sequence of one strand of DNA is written as follows:
Write down the sequence of complementary strand in 3'→5' direction.
5'-ATGCATGCATGCATGCATG CATGCATGC-3'
4. If the sequence of the coding strand in a transcription unit is writtenas follows:
5'-ATGCATGCATGCATGCAT GCATGCATGC-3'
Write down the sequence of mRNA.
5. Which property of DNA double helix led Watson and Crick to hypothesise semi-conservative mode of DNA replication? Explain.
6. Depending upon the chemical nature of the template (DNA or RNA) and the nature of nucleic acids synthesized from it (DNA or RNA), list the types of nucleic acid polymerases.

7. How did Hershey and Chase differentiate between DNA and protein in their experiment while proving that DNA is the genetic material?
8. Differentiate between the followings:
   (a) mRNA and tRNA
   (b) Template strand and Coding strand
9. List two essential roles of ribosome during translation.
10. In the medium where E. coli was growing, lactose was added, which induced the lac operon. Then, why does lac operon shut down some time after addition of lactose in the medium?
11. Explain (in one or two lines) the function of the followings:
    (a) Promoter (b) tRNA (c) Exons
12. Briefly describe the following:
    (a) Transcription (b) Translation
13. How the polymerization of nucleotides can be prevented in a DNA molecule.
14. In an experiment, DNA is treated with a compound which tends to place itself amongst the stacks of nitrogenous base pairs. As a result of this, the distance between two consecutive base pairs increases. from 0.34nm to 0.44 nm calculate the length of DNA double helix (which has 2×10^9 bp) in the presence of saturating amount of this compound.
15. Recall the experiments done by Frederick Griffith. Where DNA was speculated to be the genetic material. If RNA, instead of DNA was the genetic material, would the heat killed strain of Pneumococcus have transformed the R-strain into virulent strain? Explain.
16. You are repeating the Hershey-Chase experiment and are provided with two isotopes: ^{32}P and ^{15}N (in place of ^{35}S in the original experiment). How do you expect your results to be different?
17. Do you think that the alternate splicing of exons may enable a structural gene to code for several isoproteins from one and the same gene? If yes, how? If not, why so?
18. Can you recall what centrifugal force is, and think why a molecule with higher mass/density would sediment faster?
19. Do Retroviruses follow central Dogma? Give one example.

UNIT V

BIOTECHNOLOGY

Ever since the days of **Rene Descartes**, the French philosopher, mathematician and biologist of the seventeenth century, all human knowledge, especially natural sciences, were directed to develop technologies which add to the comforts and welfare of human lives. The whole approach to understanding natural phenomena became anthropocentric. Physics and chemistry gave rise to engineering, technologies and industries which all worked for human comfort and welfare. The major utility of the biological world is as a source of food. Biotechnology, the twentieth century off-shoot of modern biology, changed our daily life as its products brought qualitative improvement in health and food production. The basic principles underlying biotechnological processes and some applications are highlighted and discussed in this unit.

Herbert Boyer

Herbert Boyer, born in 1936, grew up in a corner of western Pennsylvania where railroads and mines were the destiny of most young men. He completed graduate work at the University of Pittsburgh in 1963, followed by three years of post-graduate studies at Yale.

In 1966 Boyer took over assistant professorship at the University of California at San Francisco. By 1969, he performed studies on a couple of restriction enzymes of the *E. coli* bacterium with especially useful properties. Boyer observed that these enzymes have the capability of cutting DNA strands in a particular fashion, which left what has become known as 'sticky ends' on the strands. These clipped ends made pasting together pieces of DNA a precise exercise.

This discovery, in turn, led to a rich and rewarding conversation in Hawaii with a Stanford scientist named Stanley Cohen. Cohen had been studying small ringlets of DNA called plasmids which float about freely in the cytoplasm of certain bacterial cells and replicate independently from the coding strand of DNA. Cohen had developed a method of removing these plasmids from the cell and then reinserting them in other cells. Combining this process with that of DNA splicing enabled Boyer and Cohen to recombine segments of DNA in desired configurations and insert the DNA in bacterial cells, which could then act as manufacturing plants for specific proteins. This breakthrough was the basis upon which the discipline of biotechnology was founded.

Chapter 11

Biotechnology: Principles and Processes

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans. In this sense, making curd, bread or wine, which are all microbe-mediated processes, could also be considered as a form of biotechnology. However, the term is used in a restricted sense today, to refer to such of those processes which use genetically modified organisms to produce products useful to human beings on a larg scale. Further, many other processes/techniques are also included under biotechnology. For example, in vitro fertilisation leading to a 'test-tube' baby, synthesising a gene and using it, developing a DNA vaccine or correcting a defective gene, are all part of biotechnology.

The European Federation of Biotechnology (EFB) has given a definition of biotechnology that combines both the traditional view and the modern molecular emphasis. The definition given by EFB is as follows:

'The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'. This means biotechnology is a science which utilizes properties & uses of micro-organisms or exploits cells and the cell constituents at the industrial level for generating useful products essential to life and human welfare.

11.1 Principles of Biotechnology

The two core techniques that enabled birth of modern biotechnology are:

(i) **Genetic engineering :** This includes techniques to alter the chemistry of genetic material (DNA and RNA), to introduce this material into host organisms and thus change the phenotype of the host organism.

(ii) **Tissue culture:** This involves the growth of only the desired microbe/ eukaryotic cell in large quantities for the manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc. A sterile (microbial contamination-free) environment in chemical engineering processes is essential for this.

The conceptual development of the principles of genetic engineering

Sexual reproduction provides opportunities for variations and re-combinations of genes, some of which may be beneficial to the organism as well as the population. Asexual reproduction preserves genetic information, while sexual reproduction permits variation. Traditional hybridisation procedures used in plant and animal breeding very often lead to inclusion and multiplication of undesirable genes, along with the desired genes. The techniques of genetic engineering which include creation of recombinant DNA, use of gene cloning and gene transfer, overcome this limitation and allow us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into the target organism.

A piece of DNA, which is somehow transferred into an alien organism, would not be able to multiply itself in the progeny cells of the organism. But, when it gets integrated into the genome of the recipient, it may multiply and be inherited along with the host DNA. This is because the alien piece of DNA has become part of a chromosome, which has the ability to replicate. In a chromosome there is a specific DNA sequence called the origin of replication, which is responsible for initiating replication. Therefore, multiplication of the alien piece of DNA in an organism is not possible unless it becomes a part of a chromosome(s) which has a specific sequence known as 'origin of replication'. Thus, an alien DNA is linked with the origin of replication, so that, this alien piece of DNA can replicate and multiply itself in the host organism. This process is also called cloning or making multiple identical copies of any template DNA.

Construction of the first artificial recombinant DNA molecule

The first recombinant DNA was constructed by linking a gene encoding antibiotic resistance with a native plasmid (autonomously replicating circular extra-chromosomal DNA) of *Salmonella typhimurium*. Stanley Cohen and Herbert Boyer accomplished this in 1972 by isolating the antibiotic resistance gene by cutting out a piece of DNA from a plasmid which was responsible for conferring antibiotic resistance. The cutting of DNA at specific locations became possible with the discovery of the so-called 'molecular scissors'– restriction enzymes. The cut piece of DNA was then linked with the plasmid DNA. These plasmid DNAs act as vectors to transfer the piece of DNA attached to it. A plasmid can be used as vector to deliver an alien piece of DNA into the host organism. The linking of antibiotic resistance gene with the plasmid vector became possible with the enzyme DNA ligase, which acts on cut DNA molecules and joins their ends. This makes a new combination of circular autonomously replicating DNA created in vitro and is known as recombinant DNA. When this DNA is transferred into *Escherichia coli*, a bacterium closely related to *Salmonella*, it could replicate using the new host's DNA polymerase enzyme and make multiple copies. The ability to multiply copies of antibiotic resistance gene in *E. coli* was called cloning of antibiotic resistance gene in *E. coli*.

There are three basic steps in genetically modifying an organism —

(i) identification of DNA with desirable genes

(ii) introduction of the identified DNA into the host

(iii) maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

11.2 Tools of Recombinant DNA Technology

Genetic engineering or recombinant DNA technology can be accomplished only if we have the key tools, i.e., restriction enzymes, polymerase enzymes, ligases, vectors and the host organism.

11.2.1 Restriction Enzymes

In the year 1963, the two enzymes responsible for restricting the growth of bacteriophage in *Escherichia coli* were isolated. One of these added methyl groups to DNA, while the other cut DNA. The latter was called restriction endonuclease.

The first restriction endonuclease–Hind II, whose functioning depended on a specific DNA nucleotide sequence was isolated and characterised later. It was found that Hind II always cut DNA molecules at a particular point by recognising a specific sequence of six base pairs. This specific base sequence is known as the **recognition sequence** for Hind II. Besides Hind II, today we know more than 900 restriction enzymes that have been isolated from over 230 strains of bacteria, each of which recognises a different recognition sequence.

The convention for naming these enzymes is that the first letter of the name comes from the genus and the second two letters come from the species of the prokaryotic cell from which they were isolated, e.g., EcoRI comes from *Escherichia coli* RY 13. In EcoRI, the letter 'R' is derived from the name of strain. Roman numbers following the names indicate the order in which the enzymes were isolated from that strain of bacteria.

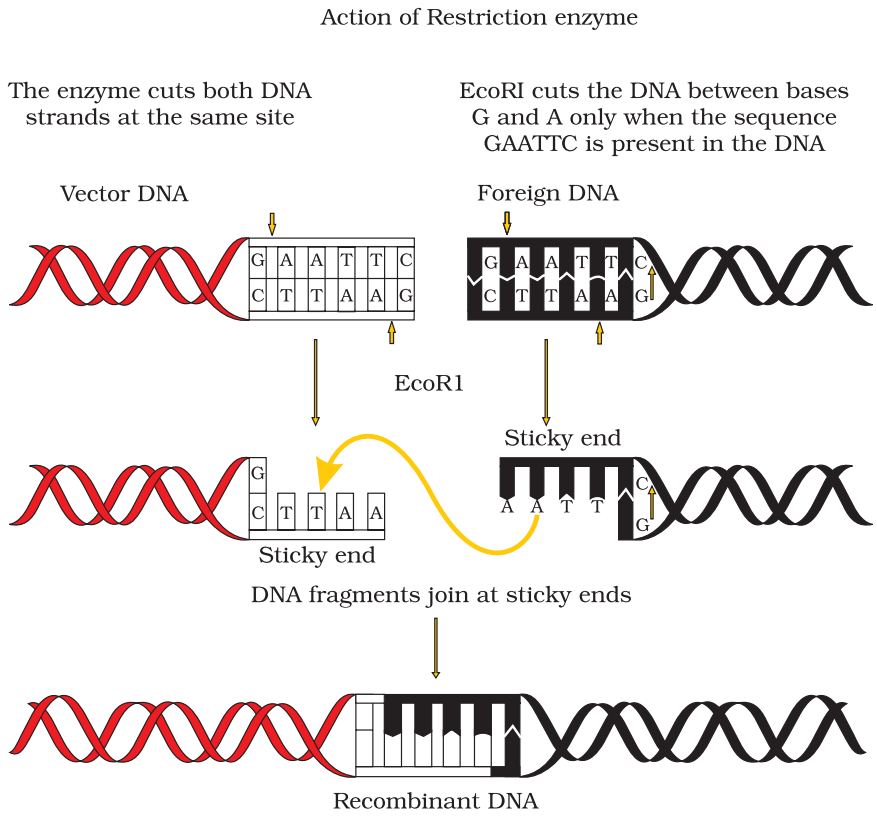


Figure 11.1 Steps in the formation of recombinant DNA by the action of restriction endonuclease enzyme - EcoRI

Restriction enzymes belong to a larger class of enzymes called nucleases. These are of two kinds; exonucleases and endonucleases. Exonucleases remove nucleotides from the ends of the DNA whereas, endonucleases make cuts at specific positions within the DNA.

Each restriction endonuclease functions by 'inspecting' the length of a DNA sequence. Once it finds its specific recognition sequence, it binds to the DNA and cuts each of the two strands of the double helix at specific points in their sugar-phosphate backbones (Figure 11.1). Each restriction endonuclease recognises a specific **palindromic nucleotide sequence** in the DNA.

Palindromes are groups of letters that form the same words when read in both forward and backward directions. For example, 'MALAYALAM'. The palindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same. For example, the following sequences read the same on the two strands in 5' → 3' direction. This is also true if read in the 3' → 5' direction.

5' —— GAATTC —— 3'
3' —— CTTAAG —— 5'

Commonly most restriction enzymes cut the two stands of DNA double helix at different locations. Such a cleavage is generally termed as **staggered cut**.

Restriction enzymes cut the strand of DNA a little away from the centre of the palindrome sites, but between the same two bases on the opposite strands. EcoRI recognizes 5' GAATTC 3' sites on the DNA and cuts it between G and A. This leaves single stranded portions at the ends. These overhanging stretches are called sticky ends or cohesive ends (Figure 11.1). These are named so because they form hydrogen bonds with their complementary cut counterparts. This stickiness of the ends facilitates the action of the enzyme DNA ligase.

Can you calculate theoritically how frequent is the EcoRI recognition sequence in a DNA?

There are four types of nucleotides in DNA. Hence, the probability that any one of these occurs at a place is 1/4. Assuming that the occurence of bases in

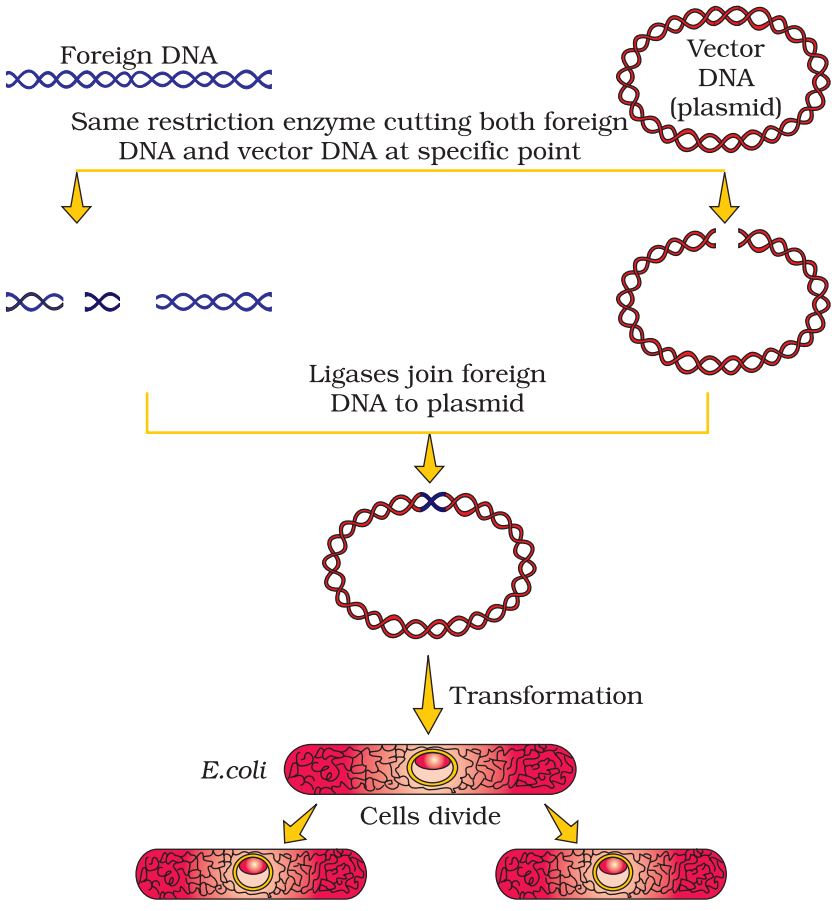


Figure 11.2 Diagrammatic representation of recombinant DNA technology

adjacent positions is random, the probability that GAATTC occurs is $(1/4)^6$ or once in a stretch of 4096 nucleotides. However, in reality, the location and frequency of such sequences in a given genome is not random but species - specific.

Restriction endonucleases are used in genetic engineering to form 'recombinant' molecules of DNA, which are composed of DNA from different sources/genomes.

When cut by the same restriction enzyme, the resultant DNA fragments have the same kind of 'sticky-ends' and, these can be joined together (end-to-end) using DNA ligases (Figure 11.2).

Which chemical bonds of DNA are cut by restriction endonucleases? Which are formed by DNA ligases?

11.2.2 Cloning Vectors

The DNA used as a carrier for transferring a fragment of foreign DNA into a suitable host is called **vector**. Vectors used for multiplying the foreign DNA sequences are called **cloning vectors**. Commonly used cloning vectors are plasmids, bacteriophages, cosmids (*cos* site of phage incorporated into a plasmid) and artificial chromosomes etc.

Plasmids are extra chromosomal circular DNA molecules found in almost all bacterial species. They are inheritable and carry few genes which determine a variety of biological functions. The advantage of a plasmid is that it is very easy to isolate and reintroduce into the bacterium (host).

Apart from natural vectors, artificially restructured plasmids like pBR322 (after Boliver and Rodriguez) pUC 19,101 (after University of California) are popularly used.

Plasmids and bacteriophages have the ability to replicate within bacterial cells, independent of the control of chromosomal DNA. Bacteriophages, because of their high number per cell, have very high copy numbers of their genome within the bacterial cells. Some plasmids may have only one or two copies per cell whereas others may have 15-100 copies per cell. Their numbers can go even higher. If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage. Vectors used at present are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants.

The following are the features that are required to facilitate cloning into a vector.

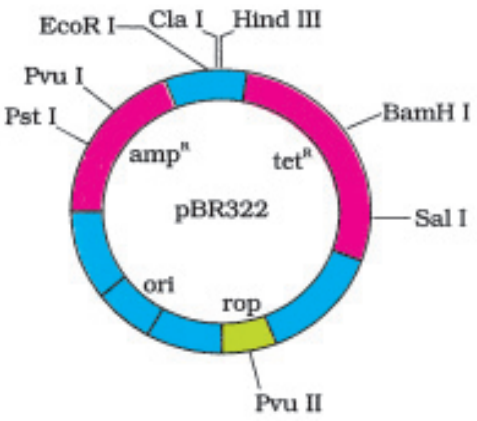


Figure 11.3 *E. coli* cloning vector pBR322 showing restriction sites (*Hind* III, *Eco*R I, *Bam*H I, *Sal* I, *Pvu* II, *Pst* I, *Cla* I), ori and antibiotic resistance genes (amp^R and tet^R). rop codes for the proteins involved in the replication of the plasmid.

(i) Origin of replication (*ori*): This is a sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells. This sequence is also responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose 'ori' supports high copy number.

(ii) Selectable marker : In addition to '*ori*', the vector requires a selectable marker, which helps in identifying and eliminating non-transformants and selectively permitting the growth of transformants. Transformation is a procedure through which a piece of DNA is introduced in a host bacterium. Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for *E. coli*. The normal *E. coli* cells do not carry resistance against any of these antibiotics.

(iii) Cloning sites: In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes. The presence of more than one recognition site for the same enzyme within the vector will generate several fragments, which will complicate the gene cloning (Figure 11.3).

(iv) Molecular weight: The cloning vector should have low molecular weight.

(v) Vectors for cloning genes in plants and animals: We have learnt how bacteria and viruses transfer genes into plants and animals and, thereby, transform eukaryotic cells and force them to do what they (bacteria or viruses) want. For example, *Agrobacterium tumifaciens*, a pathogen of several dicot plants is able to deliver a piece of DNA known as 'T-DNA' to transform normal plant cells into a tumor and direct these tumor cells to produce the chemicals required by the pathogen. Similarly, retroviruses in animals have the ability to transform normal cells into cancerous cells.

The tumor inducing (Ti) plasmid of *Agrobacterium tumifaciens* has now been modified into a cloning vector such that it is no more pathogenic to plants but is still able to use the mechanisms to deliver genes of our interest into a variety of plants. Similarly, retroviruses have also been disarmed and are now used to deliver desirable genes into animal cells.

11.2.3 Competent Host (For Transformation with Recombinant DNA)

Since DNA is a hydrophilic molecule, it cannot pass through cell membranes. In order to force bacteria to take up the plasmid, the bacterial cells must first be made 'competent' to take up DNA. This is done by treating them with a specific concentration of a divalent cation, such as calcium, which increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

11.3 Processes of Recombinant DNA Technology

Recombinant DNA technology involves several steps in specific sequence such as isolation of DNA, fragmentation of DNA by restriction endonucleases, isolation of a desired DNA fragment, ligation of the DNA fragment into a vector, transferring the recombinant DNA into the host, culturing the host cells in a medium at large scale and extraction of the desired product. Let us examine each of these steps in some detail.

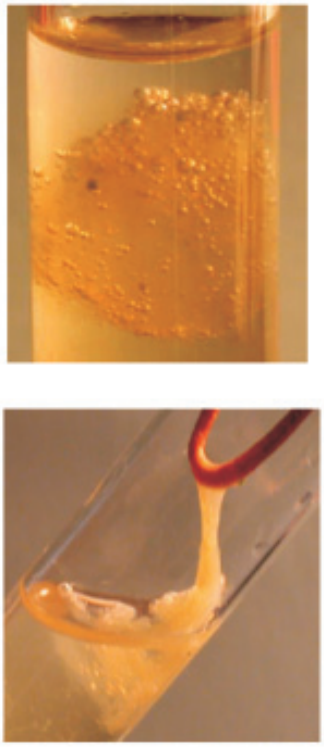


Figure 11.4 DNA that separates out can be removed by spooling

11.3.1 Isolation of the Genetic Material (DNA)

Nucleic acid is the genetic material of all organisms without exception. In the majority of organisms this is in the form of deoxyribonucleic acid or DNA. In order to cut the DNA with restriction enzymes, DNA needs to be in a pure form, free from other macro-molecules. Since the DNA is enclosed within the membranes, we have to break the cell open to release DNA along with other macromolecules such as RNA, proteins, polysaccharides and also lipids. The cell wall is digested by treating the bacterial cells/plant tissue with enzymes such as lysozyme (bacteria), cellulase (plant cells), chitinase (fungus) etc. This is followed by the dissolution of all the biological membranes within a cell by detergent lysis (using high powered detergents). We know that genes are located on long molecules of DNA interwined with proteins such as histones. The RNA can be removed by

treatment with ribonuclease whereas proteins can be removed by treatment with protease. Other molecules can be removed by appropriate treatments. Purified DNA ultimately precipitates out after the addition of chilled ethanol. This can be seen as a collection of fine threads in the suspension. DNA that seperates out can be removed by spooling (Figure 11.4).

11.3.2 Cutting of DNA at Specific Locations

The purified DNA is cut into a number of fragments by restriction endo nucleases. The process of cutting DNA with restriction enzymes is called restriction enzyme digestion. Restriction enzyme digestions are performed by incubating purified DNA molecules with the restriction enzyme at the optimal conditions for that specific enzyme. Agarose gel electrophoresis is employed to check the progression of restriction enzyme digestion. The process is repeated with the vector DNA also.

Separation and isolation of DNA fragments

The cutting of DNA by restriction endonucleases results in the fragments of DNA. These fragments can be separated by a technique known as **gel electrophoresis**. Since DNA fragments are negatively charged molecules they can be separated by forcing them to move towards the anode under an electric field through a medium/matrix. Nowadays the most commonly used matrix is agarose which is a natural polymer extracted from sea weeds. The DNA fragments separate (resolve) according to their size through the sieving effect provided by the agarose gel. Hence, the smaller the fragment size, the farther away it moves (Figure 11.5).

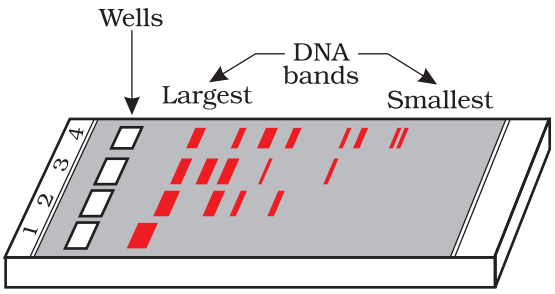


Figure 11.5 A typical agarose gel electrophoresis showing migration of undigested (lane 1) and digested set of DNA fragments (lane 2 to 4)

The separated DNA fragments can be visualised only after staining the DNA with a compound known as ethidium bromide followed by exposure to UV radiation (we cannot see pure DNA fragments in the visible light and without staining). We can see bright orange coloured bands of DNA in an ethidium bromide stained gel exposed to UV light (Figure 11.5). The separated bands of DNA are cut out from the agarose gel and extracted from the gel piece. This step is known as **elution**. The DNA fragments purified in this way are used in constructing recombinant DNA by joining them with cloning vectors.

Insertion of isolated gene into a suitable vector

To isolate a plasmid the bacterial cell is treated with lysozyme to digest the cell wall. Then the bacterial cell is subjected to centrifugation to separate the plasmid.

The joining of DNA involves several processes. After having cut the source DNA as well as the vector DNA with the same restriction enzyme (sticky end ligation technique), the cut 'gene of interest' from the source DNA and the cut vector are mixed and ligase is added. This results in the formation of **recombinant DNA** (rDNA) or **chimaeric DNA**.

11.3.3 Amplification of Gene of Interest using PCR

PCR stands for Polymerase Chain Reaction. In this reaction, multiple copies of the gene (or DNA) of interest are synthesised *in vitro* using two sets of

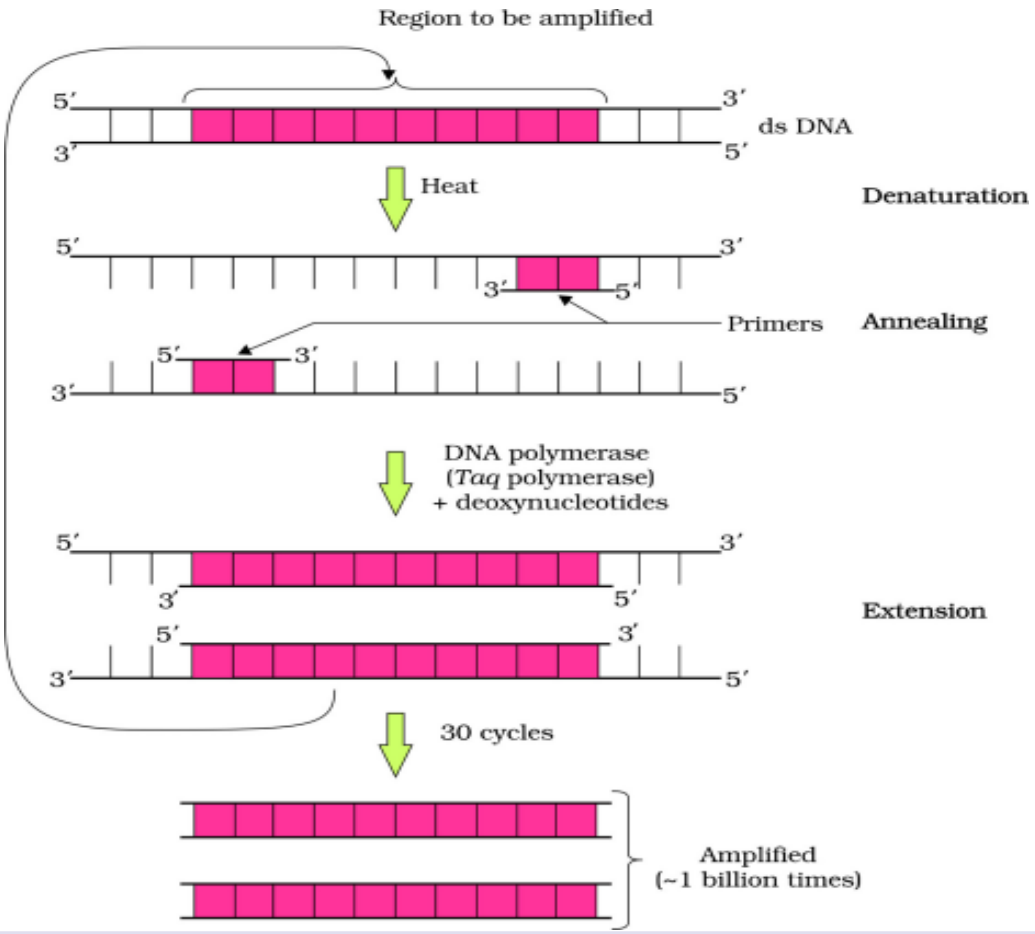


Figure 11.6 Polymerase chain reaction (PCR) : Each cycle has three steps: (i) Denaturation; (ii) Primer annealing; and (iii) Extension of primers

primers (small chemically synthesised oligonucleotides that are complementary to the regions of DNA) and the enzyme DNA polymerase. The enzyme extends the primers, using the nucleotides provided in the reaction and the genomic DNA as template. If the process of replication of DNA is repeated many times, the segment of DNA can be amplified to approximately billion times, i.e., 1 billion copies are made. Such repeated amplification is achieved by the use of a thermostable DNA polymerase such as Taq polymerase (isolated from a bacterium, *Thermus aquaticus*), which remain active even during the high temperature induced denaturation of double stranded DNA. The amplified fragment, if desired, can now be used to ligate with a vector for further cloning (Figure11.6).

DNA fingerprint is the pattern of DNA fragments on the gel. Gene amplification is one technique for DNA fingerprinting.

11.3.4 Insertion of Recombinant DNA into the Host Cell/Organism

There are several methods of introducing the ligated DNA into recipient cells. Recipient cells after making them 'competent' to receive, take up the DNA present in its surrounding. Recombinant DNA can be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 42^{0}C (heat shock), and then putting them back on ice. This enables the bacteria to take up the recombinant DNA.

This is not the only way to introduce alien DNA into host cells. In a method known as micro-injection, recombinant DNA is directly injected into the nucleus of an animal cell. In another method, suitable for plants, cells are bombarded with high velocity micro-particles of gold or tungsten coated with DNA in a method known as biolistic or gene gun method. And the last method uses 'disarmed pathogen' vectors, which when allowed to infect the cell, transfer the recombinant DNA into the host.

Selection of Transformed host cells:

So, if a recombinant DNA bearing gene for resistance to an antibiotic (e.g., Ampicillin) is transferred into *E. coli* cells, the host cells become transformed into ampicillin-resistant cells. If we spread the transformed cells on agar plates containing ampicillin, only transformants will grow untransformed recipient cells will die. Due to the ampicillin resistance gene, one is able to select a transformed cell in the presence of ampicillin. The ampicillin resistance gene in this case is called a **selectable marker**.

Sometimes the ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. For example, you can ligate a foreign DNA at the BamH I site of tetracycline resistance gene in the vector pBR322. It may be noted that when an experiment is performed to insert a DNA fragment into a vector like pBR322, two types of vector molecules are formed namely (1) Some vector molecules will contain the DNA insert (recombinant or chimaeric vector) but (2) Some others will contain only the vector sequences (unaltered vector). This mixture of vector molecules is used for transformation of host cells. Some host cells will receive the recombinant vector. Some others will contain normal unaltered vector. Some others will contain no vector i.e will not be transformed. The recombinant plasmids will lose tetracycline resistance due to insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformants on ampicillin containing medium. The transformants growing on ampicillin containing medium are then transferred on to a medium containing tetracycline. The recombinants will grow in ampicillin containing medium but not on that containing tetracycline. But, non- recombinants will grow on the medium containing both the antibiotics. In this case, one antibiotic resistance gene helps in selecting the transformants, whereas the other antibiotic resistance gene gets inactivated due to insertion of alien DNA, and helps in selection of recombinants.

Selection of recombinants by inactivation of antibiotics is a cumbersome procedure because it requires simultaneous plating on two plates having different antibiotics. Therefore, alternative selectable markers have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of a chromogenic substrate. In this, a recombinant DNA is inserted within the coding sequence of an enzyme, β-galactosidase. This results in the inactivation of the enzyme. This phenomenon is referred to as **insertional inactivation**. The presence of a chromogenic substrate gives blue coloured colonies if the plasmid in the bacteria does not have an insert. The presence of insert results in insertional inactivation of the β-galactosidase and the colonies do not produce any colour. They are called recombinant colonies.

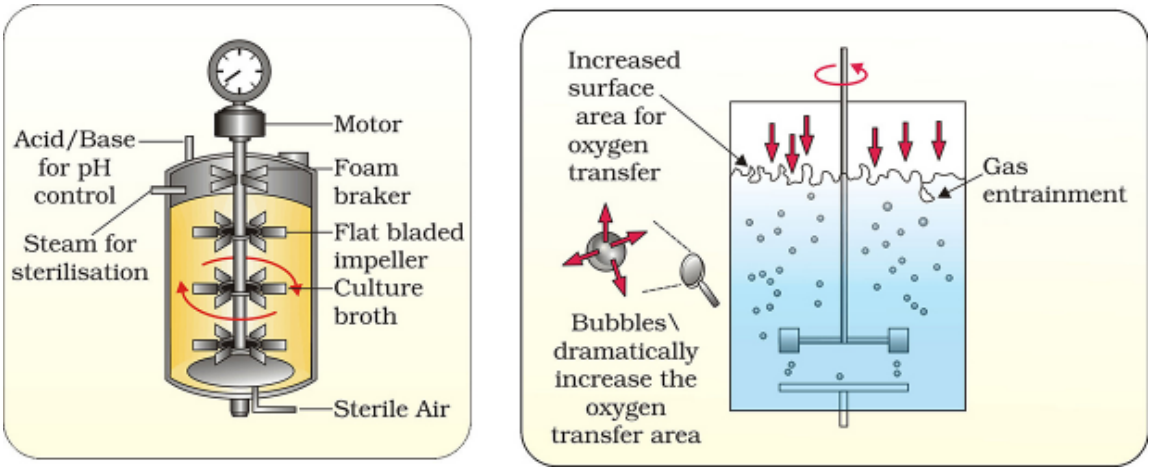
In order to select the cells with the desired gene, the method of colony hybridization is also used. In this method gene specific probes are used. A probe is a small fragment of single stranded DNA or RNA which is tagged with a radio active molecule and is complementary to atleast one part of the desired DNA.

11.3.5 Obtaining the Foreign Gene Product

When we insert a piece of alien DNA into a cloning vector and transfer it into a bacterial, plant or animal cell, the alien DNA gets multiplied. In almost all recombinant DNA technologies, the ultimate aim is to produce a desirable protein. Hence, there is a need for the recombinant DNA to be expressed. The foreign gene gets expressed under appropriate conditions. The expression of foreign genes in host cells involves many technical steps.

After having cloned the gene of interest and having optimised the conditions to induce the expression of the target protein, one has to consider producing it on a large scale. If any protein encoding gene is expressed in a heterologous (new) host, it is called a recombinant protein. The cells harbouring cloned genes of interest may be grown on a small scale in the laboratory. The cultures may be used for extracting the desired protein and then purifying it by using different separation techniques.

The cells can also be multiplied in a continuous culture system wherein the used medium is drained out from one side while fresh medium is added from the other to maintain the cells in their physiologically most active log/ exponential phase. This type of culturing method produces a larger biomass, leading to higher yields of desired protein.



(a) (b)

Figure 11.7 (a) Simple stirred-tank bioreactor; (b) Sparged stirred-tank bioreactor through which sterile air bubbles are sparged

Small volume cultures cannot yield appreciable quantities of products. Production of large quantities of proteins and enzymes requires the use of bioreactors. Large volumes (100 -1000 litres) of culture can be processed in bioreactors. Thus, bioreactors can be thought of as vessels in which raw materials are biologically converted into specific products, individual enzymes, etc., using microbial plant, animal or human cells. A bioreactor provides the optimal conditions for achieving the desired product by providing optimum growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).

The most commonly used bioreactors are of the stirring type, which are shown in Figure 11.7.

A stirred-tank reactor is usually cylindrical or with a curved base to facilitate the mixing of the reactor contents. The stirrer facilitates even mixing and oxygen availability throughout the bioreactor. Alternatively air can be bubbled through the reactor. If we look at the figure closely we will see that the bioreactor has an agitator system, an oxygen delivery system, a foam control system, a temperature control system, pH control system and sampling ports so that small volumes of the culture can be withdrawn periodically.

11.3.6 Downstream Processing

After completion of the biosynthetic stage, the product has to be subjected through a series of processes before it is ready for marketing as a finished product. The processes include separation and purification, which are collectively referred to as downstream processing. The product has to be formulated with suitable preservatives. Such formulation has to undergo thorough clinical trials as in the case of drugs. Strict quality control testing for each product is also required. The downstream processing and quality control testing vary from product to product.

SUMMARY

Biotechnology deals with large scale production and marketing of products and processes using live organisms, cells or enzymes. Modern biotechnology using genetically modified organisms was made possible only when man learnt to alter the DNA sequences and construct novel DNA. This key process is called recombinant DNA technology or genetic engineering. This process involves the use of restriction endonucleases, DNA ligase, appropriate plasmid or viral vectors to isolate and ferry the foreign DNA into host organisms, expression of the foreign gene, purification of the gene product, i.e., the functional protein and finally making a suitable formulation for marketing. Large scale production involves use of bioreactors.

SUMMARY

GLOSSARY

Annealing: It is a step in amplification of gene of interest in which two oligonucleotide primers hybridize to each of single stranded template DNA.

Artificial chromosome vectors: These are linear vectors constructed by adding centromere, telomere and origin of replication sites to a DNA fragment.

Bio-reactors: Are large vessels which are used for biological conversion of raw material into specific products.

Competent hosts: Microbial cells which are capable of taking in DNA molecules from outside and thereby undergo transformation.

Culture medium: Consist of nutrients prepared for microbial growth in a laboratory.

Denaturation: The complete unfolding of a protein or sepration of two complementary strands of a DNA molecule by break down of bonds.

DNA Ligases: A class of enzymes that join foreign DNA to plasmids by phosphodiester bonds to form recombinant DNAs.

Elution: It is a technique of extracting seperated bands of DNA from agarose gel.

Origin of replication: It is the specific DNA sequence which is responsible for initiating replication.

Selectable marker: It helps in identifying and eliminating non-transformants.

Sticky ends or cohesive ends: Single stranded strings of nucleotides that extend from the end of a fragment of double-stranded DNA.

QUESTIONS

Very Short Answer Type Questions

1. Define biotechnology.
2. What are molecular scissors? Where are they obtained from?
3. Name any two artificially restructured plasmids.
4. What is EcoR1? How does it function?
5. What are cloning vectors? Give an example.
6. What is recombinant DNA?
7. What is palindromic sequence?
8. What is the full form of PCR? How is it useful in biotechnology?
9. What is down-stream processing?
10. How does one visualize DNA on an agar gel.
11. How can you differentiate between exonucleases and endonucleases?

Short Answer Type Questions

1. Write short notes on restriction enzymes.
2. Give an account of amplification of gene of interest using PCR.
3. What is a bio-reactor? Describe briefly the stirring type of bio-reactor.
4. What are the different methods of insertion of reombinant DNA into the host cell?

Long Answer Type Questions

1. Explain briefly the various processes of recombinant DNA technology.
2. Give a brief account of the tools of recombinant DNA technology.

Exercises

1. Do eukaryotic cells have restriction endonucleases? Justify your answer.
2. Besides better aeration and mixing properties, what other advantages do stirred tank bioreactors have over shake flasks?
3. Can you recall meiosis and indicate at what stage a recombinant DNA is made?
4. Describe briefly the following:
   (a) Origin of replication
   (b) Bioreactors
   (c) Downstream processing
5. Explain briefly
   (a) PCR
   (b) Restriction enzymes and DNA
   (c) Chitinase
6. Discuss with your teacher and find out how to distinguish between
   (a) Plasmid DNA and Chromosomal DNA
   (b) RNA and DNA
   (c) Exonuclease and Endonuclease

7. What does 'H' 'in' 'd' and 'III' refer to in the enzyme Hind III?
8. Restriction enzymes should not have more than one site of action in the cloning site of a vector. Comment.
9. What does 'competent' refer to in 'competent cells' used in transformation experiments?
10. What is the significance of adding proteases at the time of isolation of genetic material (DNA).
11. While doing a PCR, 'denaturation' step is missed. What will be its effect on the process?
12. What modification is done on the *Ti* plasmid of *Agrobacterium tumefaciens* to convert it into a cloning vector?
13. What is meant by gene cloning?
14. Decide the ratio between ester bonds and hydrogen bonds that are broken in each palindromic sequence of a DNA when treated with EcoRI during the formation of sticky ends.

Chapter 12

Biotechnology and its applications

Biotechnology essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals. The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production. Three critical research areas of biotechnology are:

(i) Providing the best catalyst in the form of improved organism, usually a microbe or pure enzyme.

(ii) Creating optimal conditions through engineering for a catalyst to act, and

(iii) Downstream processing technologies to purify the protein/organic compound.

Let us now learn how human beings have used biotechnology to improve the quality of human life, especially in the field of food production and health.

12.1 Biotechnological Applications In Agriculture

There are three options that can be used for increasing food production

(i) agro-chemical based agriculture

(ii) organic agriculture

(iii) genetically engineered crop-based agriculture

Around 1960s, several countries including India experienced substantial and dramatic increase in agricultural production which was termed as **green revolution** by William Gaud (1968), the then director of United States Agency for International Development (USAID). **Norman Borlaug** to whom the credit of initiating efforts that led to green revolution, is regarded as **"Father of Green Revolution"**. **Dr. M.S. Swaminathan** and his team were instrumental for the success of green revolution in our country. Green revolution was possible due to a number of factors both economical and scientific, and include use of improved varieties, chemical fertilizers and pesticides, improved irrigational facilities, adoption of better agricultural management strategies, land reforms etc.

Even though Green Revolution succeeded in tripling the food supply, it was not enough to feed the growing human population. Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilisers and pesticides). However, for farmers in the developing world, agrochemicals are often too expensive, and they show harmful effects on the environment. Further increases in yield with existing varieties are not possible using conventional breeding. Use of genetically modified crops is a possible solution.

With the advent of biotechnology, especially the genetic engineering, a new ray of hope to increase food production and to reduce the use of chemical fertilizers and pesticides has been envisaged which might lead to other type of revolution, **the Gene revolution**. With the help of genetic engineering, it is possible to break through natural species barriers and systematically transfer genes from one species to another that may not be possible in nature. The gene revolution is likely to provide new plants that would lead to a more environmentally sound agricultural production.

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants are useful in many ways. In addition to the production of high yielding and disease resistant varieties, genetic modification has:

(i) made crops more tolerant to abiotic stresses (cold, drought, salt, heat)

(ii) reduced reliance on chemical pesticides (pest-resistant crops), e.g Bt cotton

(iii) helped to reduce post harvest losses

(iv) increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil)

(v) enhanced nutritional value of food, e.g., Vitamin A enriched rice.

In addition to these uses, genetic engineering has been used to create tailor – made plants to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Some of the applications of biotechnology in agriculture are the production of pest resistant plants, which could decrease the amount of pesticide used. Bt toxin is produced by a bacterium called *Bacillus thuringiensis* (Bt for short). Bt toxin gene has been cloned from this bacterium and has been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt Cotton: Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes). *B. thuringiensis* forms protein crystals during a particular phase of growth. These crystals contain a toxic insecticidal protein.

Why does this toxin not kill the Bacillus?

Actually, the Bt toxin proteins exists as inactive *protoxins* but once an insect ingests the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilises the crystals. The activated toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis and eventually cause the death of the insect.

Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into several crop plants such as cotton (Figure 12.1). The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific. The toxin is coded by a gene named 'cry'. There are a number of them, for example, the proteins encoded by the genes *cryIAc* and *cryIIAb* control the cotton bollworms, while that of *cryIAb* controls corn borer.

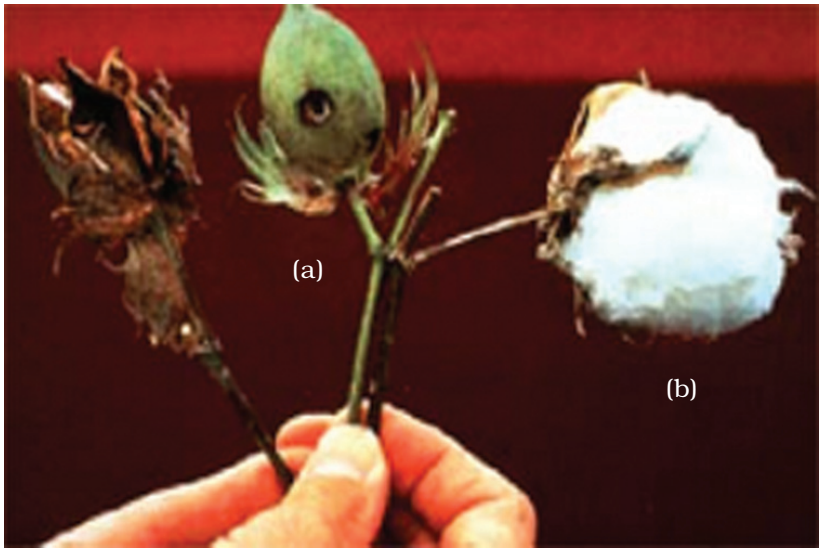


Figure 12.1 Cotton boll: (a) destroyed by bollworms; (b) a fully mature cotton boll

Pest Resistant Plants: Several nematodes parasitize a wide variety of plants and animals including human beings. A nematode *Meloidegyne incognitia* infects the roots of tobacco plants and causes a great reduction in yield. A novel strategy was adopted to prevent this infestation which was based on the process of RNA interference (RNAi). RNAi takes place in all eukaryotic organisms as a method of cellular defence. This method involves silencing of a specific mRNA due to a complementary RNA molecule that binds to and prevents translation of the mRNA (silencing). The source of this complementary RNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons) that replicate via an RNA intermediate.

Using *Agrobacterium* vectors, nematode-specific genes were introduced into the host plant (Figure 12.2). The introduction of DNA was such that it produced both sense and anti-sense RNAs in the host cells. These two RNAs being complementary to each other formed a double stranded RNA (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode. The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite (Figure 12.2).

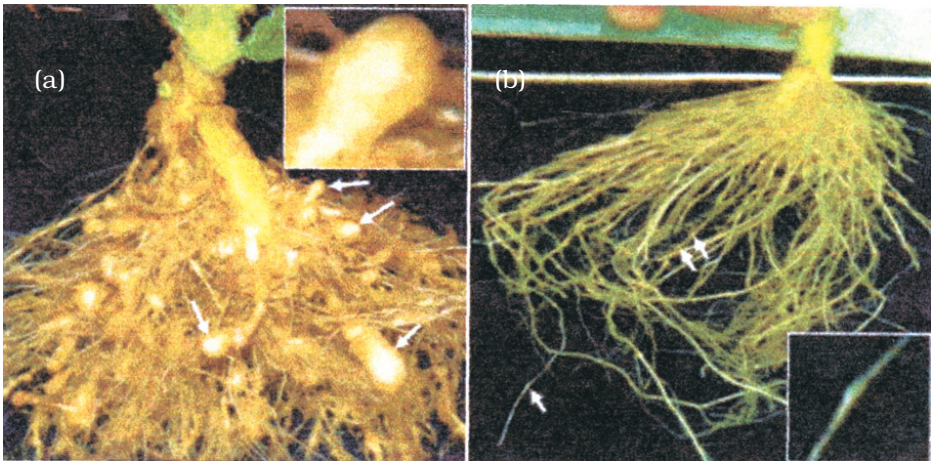


Figure 12.2 Host plant-generated dsRNA triggers protection against nematode infestation: (a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

12.2 Other applications of Biotechnology

The recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. Cloned DNAs are utilized in the commercial synthesis of hormones like insulin, interferons and vaccines. Micro-organisms are commercially exploited for production of vitamins, antibiotics and other commercial chemicals at lower costs. Further, recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources. At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs. Human insulin is now made in bacteria, yet its structure is absolutely identical to that of the natural molecule. In 1983, Eli Lilly, an American company prepared two DNA sequences corresponding to A and B chains of human insulin and introduced them into plasmids of *E. coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin. The possibility of production through novel method helped to have continuous supply of insulin and stabilization of its market price etc.

Gene therapy is a corrective therapy to be taken for hereditary diseases. Here genes are inserted into a person's cells and tissues to treat a disease. It

involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the non-functional gene.

Molecular Diagnosis: For effective treatment of a disease, early diagnosis and understanding its pathophysiology is very important. Using conventional methods of diagnosis (serum and urine analysis, etc.) early detection is not possible. Recombinant DNA technology, Polymerase Chain Reaction (PCR) and Enzyme Linked Immuno-sorbent Assay (ELISA) are some of the techniques that serve the purpose of early diagnosis.

The presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom. By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid through PCR.

Can you explain how PCR can detect very low amounts of DNA?

PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful techqnique to identify many other genetic disorders.

ELISA is based on the principle of antigen-antibody interaction. Infection by a pathogen can be detected by the presence of antigens or by detecting the antibodies synthesised against the pathogen.

DNA fingerprinting has succesfully helped forensic science in the search of criminals and also solving parentage disputes etc.

12.3 Transgenic plants

Plants with desirable characters created through gene transfer methods are called transgenic plants. Though a number of methods have been developed to introduce the cloned genes into plant cells, Ti plasmid of *Agrobacterium tumefaciens* has been widely used as an effective vector for obtaining transgenic plants. Several transgenic plants have been produced to meet specific needs of agriculture and human life. Some of these are given below.

A. Transgenic crop plants having resistance to pathogens and pests:

i. Transgenic papaya is resistant to papaya ring spot virus
ii. Bt.cotton is resistant to insects
iii. Transgenic tomato plants are resistant to the bacterial pathogen *Pseudomonas.*
iv. Transgenic potato plants are resistant to the fungus *Phytophthora.*

B. Transgenic plants suitable for food processing technology:

i. Transgenic tomato 'Flavr Savr' is bruise resistant i.e., suitable for storage and transport due to delayed ripening and offers longer shelf-life.

C. Transgenic plants with improved nutritional value:

i. Transgenic golden rice obtained from 'Taipei' is rich in vitamin A and prevents blindness.

D. Transgenic plants useful for hybrid seed production:

i. Male sterile plants of *Brassica napus* are produced. This will eliminate the problem of manual emasculation and reduce the cost of hybrid seed production.

E. Transgenic plants tolerant to abiotic stresses caused by chemicals, cold, drought, salt, heat etc.

i. Basmati variety of rice was made resistant against biotic and abiotic stresses.

ii. Round up ready soyabean is herbicide tolerant.

Besides these, genetically modified crops have evolved as alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

Transgenic plants have been shown to experess the genes of insulin, interferon, human growth hormones, antibiotics, antibodies etc. These biochemicals produced by plants are as good as or sometimes better than those produced in bacteria.

Utilization of plants as biofactories or bioreactors for obtaining commercially useful products, specialized medicines, chemicals and antibodies on a large scale is described as molecular farming. In the near future this field is expected to revolutionise both the farming as well as biochemical industry.

12.4 Bio-safety and Ethical issues

Despite several advantages, genetic modifications of organisms can have unpredictable results when they are introduced into the natural ecosystem. Some of the apprehensions towards bio-safety issues of genetically engineered crops are :

i. There is fear of transferring allergins or toxins to humans and animals as side effects.
ii. There is a risk of changing the fundamental nature of vegetables.
iii. They may pose a harmful effect on biodiversity and have an adverse impact on environment.
iv. There is a risk of gene pollution due to transfer of the new genes into related wild species through natural out-crossing. This may result in the development of super-weeds which may be fast-growing than the crops and may be resistant to weedicides.
v. They may bring about changes in natural evolutionary pattern.

Going beyond the morality of such issues, their biological significance is also important. The manipulation of living organisms by the human race can not go on any further without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms. Therefore, the Indian Government has set up organisations such as GEAC (Genetic Engineering Approval Committee), which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.

The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.

There is growing public anger that certain companies are being granted patents for products and technologies that make use of genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.

For example, rice is an important food grain, the presence of which goes back to thousands of years in Asia's agricultural history. There are an estimated

200,000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries. In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new' variety of Basmati, in the US and abroad. This 'new' variety of Basmati had actually been derived from Indian farmers' varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an

invention or a novelty. The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent. Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, such as turmeric and neem. If we are not vigilant and do immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

Biopiracy is the term coined to refer to the use of bio-resources by multinational companies and other organizations without proper authorization from the countries and people concerned or without compensatory payment.

Most of the industrialized nations are rich financially but poor in biodiversity and traditional knowledge. In contrast, the developing and underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialization.

There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.

The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiatives.

SUMMARY

Biotechnology has given humans several useful products by using microbes, plant, animals and their metabolic machinery. Recombinant DNA technology has made it possible to engineer microbes, plants and animals such that they have novel capabilities. Genetically Modified Organisms have been created by transferring one or more genes from one organism to another using techniques such as recombinant DNA technology.

Such genetically modified plants have been useful in increasing crop yields, reduce post-harvest losses and make crops more tolerant to stresses. There are crop plants with improved nutritional value of foods and those which reduced the reliance on chemical pesticides.

Recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutics.

Gene therapy is the insertion of genes into an individual's cells and tissues to treat hereditary diseases. It does so by replacing a defective mutant allele with a functional one.

The current interest in the manipulation of microbes, plants, and animals has raised serious ethical questions about usage and protection of biodiversity.

SUMMARY

GLOSSARY

Bio-remediation: The process of using microbes and plants to break down or recycle environmental pollutants.

'Cry' protein: It is a protein toxin produced by *Bacillus thuringiensis* that kills certain insects.

DNA finger printing: A technique for the identification of individuals based on small differences in DNA fragment patterns detected by electrophoresis.

Enzyme linked immuno-sorbent assay (ELISA): A method to detect a very small amounts of specific proteins utilising antibodies linked to enzymes that catalyse the formation of coloured products.

RNA Interfernece (RNAi): It Involves the use of a complementary RNA molecule to prevent another mRNA molecule from taking part in translation there by preventing the expression of a gene.

Transgenic plant: A plant that has been altered to contain a gene from a different species.

QUESTIONS

Very Short Answer Type Questions

1. Expand GMO. How is it different from a hybrid?
2. Give different types of *cry genes* and pests which are controlled by the proteins encoded by these genes.
3. Can a disease be detected before its symptoms appear? Explain the principle involved.
4. Many toxic proteins are produced in their inactive form by micro-organisms.Explain how the mechanism is useful for the organism producing the toxin.
5. Why has the Indian parliament cleared the second amendment of the country's patents bill?
6. Give any two reasons why the patent on Basmati should not have gone to an American company.
7. PCR is a useful tool for early diagnosis of an infectious disease. Elaborate.
8. What is GEAC and what are its objectives?
9. Name the nematode that infects the roots of tobacco plants. Name the strategy adopted to prevent this infestation.
10. For which variety of Indian rice, has a patent been filed by a USA company.
11. Give one example for each of transgenic plants which are suitable for food processing and those with improved nutritional quality.

Short Answer Type Questions

1. List out the beneficial aspects of transgenic plants.
2. What are some bio-safety issues concerned with genetically modified crops?
3. Give a brief account of
   A) Bt. cotton
   B) Pest resistant plants
4. Write notes on green revolution and gene revolution.

Long Answer Type Questions

1. Give an account of bio-technological applications in agriculture and other fields.

Exercises

1. Crystals of Bt toxin produced by some bacteria do not kill the bacteria themselves because –
   (a) bacteria are resistant to the toxin
   (b) toxin is immature;
   (c) toxin is inactive;
   (d) bacteria encloses toxin in a special sac.
2. What are transgenic bacteria? Illustrate using any one example.
3. Compare and contrast the advantages and disadvantages of production of genetically modified crops.
4. What are Cry proteins? Name an organism that produces it. How has man exploited this protein to his benefit?
5. List the advantages of recombinant insulin.
6. What is meant by the term bio-pesticide? Name and explain the mode of action of a popular bio-pesticide.

UNIT VI

PLANTS, MICROBES AND HUMAN WELFARE

Biology is the youngest of the formalised disciplines of natural science. Progress in physics and chemistry proceeded much faster than in Biology. Applications of physics and chemistry in our daily life also have a higher visibility than those of biology. However, advances made in the twentieth century and twenty-first century have demonstrated the utility of biological knowledge in furthering human welfare, be it in the health sector or in agriculture. The discovery of antibiotics, synthetic plant-derived drugs and anaesthetics have changed medical practice on one hand and human health on the other hand. Life expectancy of human beings has dramatically changed over the years. Agricultural practices, food processing and diagnostics have brought socio-cultural changes in human communities. These are briefly described in the following two chapters of this unit.

M.S Swaminathan

Born in August 1925 in Kumbakonam in Tamil Nadu, ***Monkambu Sambasivan Swaminathan*** *did his graduation and post-graduation in Botany from Madras University. He worked in different capacities in large a number of institutions in India and abroad and developed expertise in genetics and plant breeding.*

The School of Cytogenetics and Radiation Research established at the ***Indian Agricultural Research Institute*** *(IARI) enabled Swaminathan and his team to develop short-duration high-yielding varieties of rice including scented Basmati. Swaminathan is also known for the development of the concept of crop cafeteria, crop scheduling and genetic improvement of the yield and quality of crops.*

Swaminathan's collaboration with Norman Borlaug culminated in the 'Green Revolution' through introduction of Mexican varieties of wheat in India. This was highly recognised and appreciated. He is also the initiator of 'Lab-to-Land', food security and several other environmental programmes. He has been honoured with the Padma Bhushan and several other prestigious awards, medals and fellowships by institutions of excellence.

Chapter 13

Strategies for Enhancement in Food Production

With the ever-increasing population of the world, enhancement of food production is a major necessity. You are already aware of the economic importance of plants (Unit-IV of First Year) as sources of food, medicine and other useful products. Biological principles as applied to animal husbandry and plant breeding have a major role in our efforts to increase food production. Several new techniques like mutation breeding, tissue culture and r-DNA techniques are going to play a pivotal role in further enhancing food production.

13.1 Plant Breeding

Traditional farming can only yield a limited biomass as food for humans and animals. Better management practices and increase in acreage can increase yield, but only to a limited extent. Plant breeding as a technology has helped increase yields to a very large extent. Who in India has not heard of the **Green Revolution**? The Green Revolution made it possible for our country to not merely meet the national requirements in food production but also helped us even to export it. The Green Revolution was dependent to a large extent on plant breeding techniques for development of high-yielding and disease resistant varieties in wheat, rice, maize, etc.

13.1.1 What is Plant Breeding?

Plant breeding is the purposeful manipulation of plant species in order to create desired plant types that are better suited for cultivation, give better yields and are disease resistant. Conventional plant breeding has been practised for thousands of years, since the beginning of human civilisation; recorded evidence of plant breeding dates back 9000-11,000 years. Many present-day crops are the result of domestication in ancient times. Today all our major food crops are derived from domesticated varieties. Classical plant breeding involves crossing or hybridisation of pure lines, followed by artificial selection to produce plants with desirable traits of higher yield, nutrition and resistance to diseases. With advancements in genetics, molecular biology and tissue culture, plant breeding is now increasingly being carried out by using molecular genetic tools.

If we were to list the traits or characters that breeders have tried to incorporate into crop plants, the first we list would be increased crop yield and improved quality. Increased tolerance to environmental stresses (salinity, extreme temperatures, drought), resistance to pathogens (viruses, fungi and bacteria) and increased tolerance to insect pests would be on our list too.

Plant breeding programmes are carried out in a systematic way worldwide in government institutions and commercial companies. The main steps in breeding a new genetic variety of a crop are–

(i) ***Collection of variability:*** Genetic variability is the root of any breeding programme. Generally pre-existing genetic variability is available from wild relatives of crop plants. Collection and preservation of all the different wild varieties, species and relatives of the cultivated species (followed by their evaluation for their characteristics) is a pre-requisite for effective exploitation of natural genes available in the populations. The entire collection (of plants/seeds) having all the diverse alleles for all genes in a given crop is called **germplasm collection**.

(ii) ***Evaluation and selection of parents:*** The germplasm is evaluated so as to identify plants with desirable combination of characters. The selected plants are multiplied and used in the process of hybridisation. Purelines are created wherever desirable and possible.

(iii) ***Cross hybridisation among the selected parents:*** The desired characters have often to be combined from two different plants (parents). For example high protein quality of one parent may need to be combined with disease resistance from another parent. This is possible by cross hybridising the two parents to produce hybrids that genetically combine the desired characters in one plant. This is a very time-consuming and tedious process since the pollen grains from the desirable plant chosen as male parent have to be collected and placed on the stigma of the flowers selected as female parent (In Chapter 7 of First Year details on how to make crosses have been described). Also, it is not necessary that the hybrids do combine the desirable characters; usually only one in a few hundred to a thousand crosses shows the desirable combination.

(iv) ***Selection and testing of superior recombinants:*** This step consists of selecting, among the progeny of the hybrids, those plants that have the desired character combination. The selection process is crucial to the success of the breeding objective and requires careful scientific evaluation of the progeny. This step yields plants that are superior to both the parents (very often more than one superior progeny plant may become available). These are self-pollinated for several generations till they reach a state of uniformity (homozygosity), so that the characters will not segregate in the progeny.

(v) ***Testing, release and commercialisation of new cultivars:*** The newly selected lines are evaluated for their yield and other agronomic traits of quality, disease resistance, etc. This evaluation is done by growing these in research fields and recording their performance under ideal fertiliser application, irrigation, and other crop management practices. The evaluation in research fields is followed by testing the materials in farmers' fields, for at least three growing seasons at several locations in the country, representing all the agroclimatic zones where the crop is usually grown. The material is evaluated in comparison to the best available local crop cultivar – a check or reference cultivar.

India is mainly an agricultural country. Agriculture accounts for approximately 33 per cent of India's Gross Domestic Product (GDP) and employs nearly 62 per cent of the population. After independence, one of the main challenges facing the country was that of producing enough food for the increasing population. As only limited land is fit for cultivation, India has to strive to increase yields per unit area in existing farm land. The development of several high yielding varieties of wheat and rice in the mid-1960s as a result of various plant breeding techniques led to a dramatic increase in food production in our country. This phase is often referred to as the Green Revolution. Figure 13.1 represents some Indian hybrid crops of high yeilding varieties.

(a)

(b)

(c)

Figure 13.1 Some Indian hybrid crops: (a) Maize; (b) Wheat; (c) Garden peas

Wheat and Rice: During the period 1960 to 2000, wheat production increased from 11 million tonnes to 75 million tonnes while rice production went up from 35 million tonnes to 889.5 million tonnes. This was due to the development of semi-dwarf varieties of wheat and rice. Nobel laureate Norman E. Borlaug, at International Centre for Wheat and Maize Improvement in Mexico, developed semi-dwarf wheat. In 1963, several varieties such as *Sonalika* and *Kalyan Sona*, which were high yielding and disease resistant, were introduced all over the wheat-growing belt of India. Semi-dwarf rice varieties were derived from IR-8, (developed at International Rice Research Institute (IRRI), Philippines) and Taichung Native-1 (from Taiwan). The derivatives were introduced in 1966. Later better-yielding semi-dwarf varieties *Jaya* and *Ratna* were developed in India.

Sugarcane: *Saccharum barberi* was originally grown in north India, but had poor sugar content and yield. Tropical canes grown in south India *Saccharum officinarum* had thicker stems and higher sugar content but did not grow well in north India. These two species were successfully crossed to get sugarcane varieties combining the desirable qualities of high yield, thick stems, high sugar and ability to grow in the sugarcane areas of north India.

Millets: Hybrid maize, jowar and bajra have been successfully developed in India. Hybrid breeding have led to the development of several high yielding varieties resistant to water stress.

13.1.2 Plant Breeding for Disease Resistance

A wide range of fungal, bacterial and viral pathogens affect the yield of cultivated crop species, especially in tropical climates. Crop losses can often be significant, up to 20-30 per cent, or sometimes even total. In this situation, breeding and development of cultivars resistant to disease enhances food production. This also helps reduce the dependence on the use of fungicides and bacteriocides. Resistance of the host plant is the ability to prevent the pathogen from causing disease and is determined by the genetic constitution of the host plant. Before breeding is undertaken, it is important to know about the causative organism and the mode of transmission. Some of the diseases caused by fungi are rusts, e.g., brown rust of wheat, red rot of sugarcane and late blight of potato; by bacteria – black rot of crucifers; and by viruses – tobacco mosaic, turnip mosaic, etc.

Methods of breeding for disease resistance: Breeding is carried out by conventional breeding techniques (described earlier) or by mutation breeding. The conventional method of breeding for disease resistance is that of hybridisation and selection. The steps are essentially identical to

those for breeding for any other agronomic characters such as high yield. The various sequential steps are : screening germplasm for resistance sources, hybridisation of selected parents, selection and evaluation of the hybrids and testing and release of new varieties.

Some crop varieties bred by hybridisation and selection for disease resistance to fungi, bacteria and viral diseases are given below (Table 13.1).

Table 13.1

Crop	Variety	Resistance to diseases
Wheat	*Himgiri*	*Leaf and stripe rust, hill bunt*
Brassica	*Pusa swarnim (Karan rai)*	*White rust*
Cauliflower	*Pusa Shubhra, Pusa Snowball K-1*	*Black rot and Curl blight black rot*
Cowpea	*Pusa Komal*	*Bacterial blight*
Chilli	*Pusa Sadabahar*	*Chilly mosaic virus, Tobacco mosaic virus and Leaf curl*

Conventional breeding is often constrained by the availability of a limited number of disease resistance genes that are present and identified in various crop varieties or wild relatives. Inducing mutations in plants through diverse means and then screening the plant materials for resistance sometimes leads to desirable genes being identified. Plants having these desirable characters can then be either multiplied directly or used in breeding. Other breeding methods that are used are selection amongst somaclonal variants and genetic engineering.

Mutation is the process by which genetic variations are created through changes in the base sequence within genes **(see Chapter 9 & 10)** resulting in the creation of a new character or trait not found in the parental type. It is possible to induce mutations artificially through the use of chemicals or radiations (like gamma radiations), and selecting and using the plants that have the desirable character as a source in breeding. This process is called **mutation breeding**. In mung bean, resistance to yellow mosaic virus and powdery mildew were induced by mutations.

Several wild relatives of different cultivated species of plants have been shown to have certain resistant characters but have very low yield. Hence, there is a need to introduce resistant genes into high-yielding cultivated varieties.

Resistance to yellow mosaic virus in *bhindi* (*Abelmoschus esculentus*) was transferred from a wild species, which resulted in a new variety called **Parbhani Kranti**.

All the above examples involve sources of resistance genes that are in the same crop species or in a related wild species. Transfer of resistance genes is achieved by sexual hybridisation between the target and the source plant followed by selection.

13.1.3 Plant Breeding for Developing Resistance to Insect Pests

Another major cause for large scale destruction of crop plant and crop produce is insect and pest infestation. Insect resistance in host crop plants may be due to morphological, biochemical or physiological characteristics. Hairy leaves in several plants are associated with resistance to insect pests, e.g, resistance to jassids in cotton and cereal leaf beetle in wheat. In wheat, solid stems lead to non-preference by the stem sawfly. Smooth leaved and nectar-less cotton varieties do not attract bollworms. High aspartic acid, low nitrogen and sugar content in maize leads to resistance to maize stem borers.

Breeding methods for insect pest resistance involve the same steps as those for any other agronomic trait such as yield or quality and are as discussed earlier. Sources of resistance genes may be cultivated varieties, germplasm collections of the crop or wild relatives.

Some released crop varieties bred by hybridisation and selection, for insect pest resistance are given in Table 13.2.

Table 13.2

Crop	Variety	Insect Pests
Brassica (rapeseed, mustard)	*Pusa Gaurav*	*Aphids*
Flat bean	*Pusa Sem 2, Pusa Sem 3*	*Jassids, aphids and fruit borer*
Okra (Bhindi) (Lady's Finger)	*Pusa Sawani*	*Shoot and Fruit borer Pusa A-4*

13.1.4 Plant Breeding for Improved Food Quality

More than 840 million people in the world do not have adequate food to meet their daily food and nutritional requirements. A far greater number–three billion people – suffer from micronutrient, protein and vitamin deficiencies or 'hidden hunger' because they cannot afford to buy enough fruits, vegetables, legumes, fish and meat. Diets lacking essential micronutrients – particularly iron, vitamin A, iodine and zinc – increase the risk for disease, reduce life span and effect mental abilities. **Biofortification**, which aims at breeding crops with higher levels of vitamins and minerals, or higher protein and healthier fats, is the most practical means to improve public health.

Breeding for improved nutritional quality is undertaken with the objectives of improving –

(i) Protein content and quality
(ii) Oil content and quality
(iii) Vitamin content
(iv) Micronutrient and mineral content.

In the year 2000, maize hybrids that had twice the amount of amino acids, lysine and tryptophan compared to existing maize hybrids were developed. Wheat variety, *Atlas 66*, having a high protein content, has been used as a donor for improving cultivated wheat. It has been possible to develop an iron-fortified rice variety containing over five times as much iron as in commonly consumed varieties and β-carotene-containing rice variety named **Golden rice**.

The Indian Agricultural Research Institute, New Delhi has also released several vegetable crops that are rich in vitamins and minerals, e.g., vitamin A enriched carrots, spinach, pumpkin; vitamin C enriched bitter gourd, *bathua* (*Chenopodium*, king of green vegetables), mustard, tomato; iron and calcium enriched spinach and *bathua*; protein enriched beans – broad, lablab, French and garden peas.

13.2 Single Cell Protein (SCP)

Conventional agricultural production of cereals, pulses, vegetables, fruits, etc., may not be able to meet the demand of food at the rate at which human and animal population is increasing. The shift from grain to meat diets also creates more demand for cereals as it takes 3-10 Kg of grain to produce 1 Kg of meat by animal farming.

Can you explain the above statement in the light of your knowledge of food chains?

Table 13.3 Some examples of SCP producing organisms

Algae	Fungi	Bacteria
1. *Spirulina maxima*	1. *Candida utilis* (Torula yeast)	1. *Brevibacterium ketoglutamicum*
2. *Chlorella pyrenoidosa*	2. *Saccharomyces cerevisiae* (Bakers yeast)	2. *Methylophilus methylotrophus*
3. *Scenedesmus acutus*	3. *Chaetomium cellulolyticum*	

More than 25 per cent of human population is suffering from hunger and malnutrition. One of the alternate sources of proteins for animal and human nutrition is **Single Cell Protein (SCP)**.


Spirulina

Microbes are being grown on an industrial scale as a source of good protein. Algae, Fungi and Bacteria are used in SCP production. Microbes like *Spirulina* can be grown easily on materials like waste water from potato processing plants (containing starch), straw, molasses, animal manure and even sewage, to produce large quantities and can serve as food which is rich in protein, minerals, fats, carbohydrate and vitamins. Incidentally such utilization also reduces environmental pollution.

It has been calculated that a 250 Kg cow produces 200 g of protein per day. In the same period, 250g of a micro-organism like *Methylophilus methylotrophus*, because of its high rate of biomass production and growth, can be expected to produce 25 tonnes of protein. The fact that mushrooms are eaten by many people and large scale mushroom culture is a growing industry makes it believable that microbes too will soon become acceptable as food.

13.3 Tissue Culture

As traditional breeding techniques are inadequate to keep pace with demand and to provide sufficiently fast and efficient systems for crop improvement, another technology called **tissue culture** was developed. What does tissue culture mean? It was learnt by scientists, during 1950s, that whole plants could be regenerated from **explants**, i.e., any part of a plant taken out and grown in a test tube under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell is called **totipotency**.

It is important to stress here that the nutrient medium must provide a carbon source such as sucrose and also inorganic salts, vitamins, amino acids and growth regulators like auxins, cytokinins etc. The culture medium is rich in nutrients and therefore attracts the growth of microorganisms, thereby resulting in the contamination and spoiling of the medium. Therefore, the culture medium is sterilized to kill the microorganisms. Serilization is carried out in a steam sterilizer called the **autoclave**. **The culture medium is autoclaved for 15 min, at 121° C & 15 pounds of pressure.** The transfer of explants onto the sterilized nutrient culture medium is called **inoculation** and is carried out in an aseptic environment.

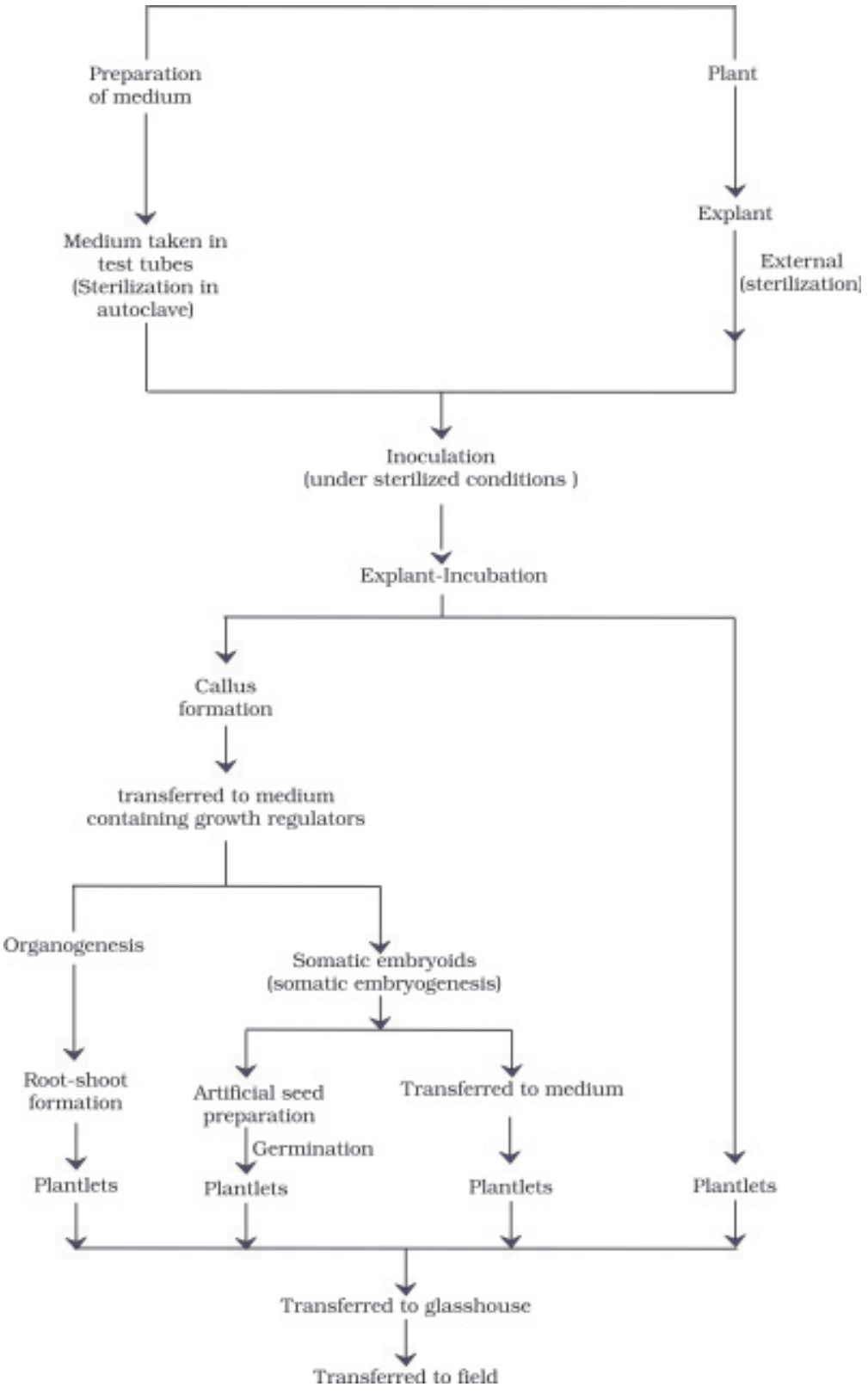
The cultures are incubated for 3 to 4 weeks during which period the cells of the explants absorb the nutrients, grow and undergo repeated divisions to produce a proliferating undifferentiated mass of cells known as '**callus**' (Fig. 13.2a). Sometims shoots or roots may be produced directly. The explants or callus cultured on different combinations of auxins and cytokinins will produce shoots or roots and this process is called '**organogenesis**' (Fig. 13.2 b & c). Alternately, embryo like structures develop from the callus and this phenomenon is known as **somatic embryogenesis**, the embryo- like structures which develop from the callus are called **embryoids**. Since these embryoids develop from somatic tissue they are also referred to as '**somatic embryos**'. Sometimes, the explants produces the embryoids directly without callus formation.

By applying this technique, it is possible to produce a large number of plants in a very short time and in limited space. Hence the technique is called **micro-propagation**. Plants thus produced are genetically identical to the original or source plant and hence they are called **somaclones**. Many economically important plants like tomato, banana, apple, teak, eucalyptus, bamboo etc., have been produced on a commercial scale by the use of this method.

Try to visit a tissue culture laboratory with your teacher to better understand and appreciate the process of micro-propagation.

Another important application of the method is the recovery of healthy plants from diseased plants. Although the plant is infected with a virus, the **meristem** (apical and axillary) is free of virus. Hence, one can remove the meristem and grow it *in vitro* to obtain virus-free plants. Scientists have succeeded in culturing meristems of banana, sugarcane, potato, etc.

Flow Chart showing plant Tissue Culture Technique



Scientists have even isolated single cells or protoplasts (naked cells) from plants after digesting their cell walls which act as physical barriers, using cell wall hydrolyzing enzymes like cellulase and pectinase. Isolated protoplasts from two different varieties of plants – each having a desirable character – can be fused to get hybrid protoplasts, which can be further cultured to form a novel plant. These hybrids are called **somatic hybrids** while the process is called **somatic hybridisation**. Some plants show physical or chemical incompatibility in normal sexual crosses. Somatic hybridizastion technique provides the opportunity for bypassing the conventional breeding barriers through direct transfer of cytoplasmic and nuclear genomes to plant cells and this facilitates the introduction of desirable traits. Imagine a situation when a protoplast of tomato is fused with that of potato, and then they are grown to form new hybrid plants combining tomato and potato characteristics. Well, this has been achieved resulting in the formation of **pomato**; unfortunately this plant did not have all the desired combination of characteristics for its commercial utilisation.

a) Callus b) Regeneration c) Test tube Plants

Figure 13.2 Tissue culture

Summary

Plant breeding may be used to create varieties which are resistant to pathogens and insect pests. This increases the yield of the food. This method has also been used to increase the protein content of plant foods and thereby enhance the quality of food. In India, several varieties of different crop plants have been produced. All these measures enhance the production of food. Techniques of tissue culture and somatic hybridisation offer vast potential for manipulation of plants *in vitro* to produce new varieties.

SUMMARY

GLOSSARY

Germplasm (Collection): The entire collection of plants/seeds, having all the diverse alleles for all genes in a given crop is called germplasm collection.

Green Revolution: It is the dramatic increase in food production due to plant breeding techniques.

Hybridisation: The process of producing new crop varieties by crossing two genetically different parents.

Mutation breeding: Induction of desirable mutations in plants and their utilization for the production of new superior varieties is called mutation breeding.

Protoplasts: Cells whose cellwalls have been removed by enzymatic digestion.

Somaclones: Plants grown through tissue culture which are genetically identical with the original (source) plant are called somaclones.

Somatic hybridisation: The process of developing hybrid plants by the fusion of isolated protoplasts.

Totipotency: The ability of a cell (or) an explant to regenerate into a complete plant is called totipotency.

QUESTIONS

Very Short Answer Type Questions

1. What is meant by 'hidden hunger'?
2. Name two semi-dwarf varieties of rice developed in India.
3. Give two examples of wheat varieties introduced in India, which are high yielding and disease resistant.
4. Give two examples of fungi used in SCP production.
5. Why are plants obtained by protoplast fusion called somatic hybrids?
6. What is protoplast fusion?
7. Why is it easier to culture meristems compared to permanent tissues?
8. Why are proteins synthesized from *Spirulina* called single cell proteins?
9. A person who is allergic to pulses was advised to take a capsule of *Spirulina* daily. Give reasons for the advice.
10. Would it be wrong to refer to plants obtained through micro-propagation as 'clones'? Explain.
11. How is a somatic hybrid different from a hybrid?
12. What is emasculation? Why and when is it done?
13. Discuss the two main limitations of plant hybridization programme.
14. Give two important contributions of Dr. M.S. Swaminathan.
15. Which two species of sugarcane were crossed for better yield?

Short Answer Type Questions

1. What is meant by germplasm collection? What are its benefits?
2. Name the improved characteristics of wheat that helped India to achieve green revolution.
3. Suggest some of the features of plants that prevent insect and pest infestation.
4. The culture medium (nutrient medium) can be referred to as a 'highly enriched laboratory soil'. Justify the statement.
5. Plants raised through tissue cultures are clones of the 'parent' plant. Discuss the utility of these plants.
6. Discuss the importance of testing of new plant varieties in a geographically vast country like India.
7. Give few examples of biofortified crops. What benefits do they offer to the society?
8. Mutations are beneficial for plant breeding. Taking an example, justify the statement.
9. Discuss briefly the technology that made us self-sufficient in food production.

Long Answer Type Questions

1. You are a Botanist working in the area of plant breeding. Describe the various steps that you will undertake to release a new variety.

2. Describe the tissue culture technique and what are the advantages of tissue culture over conventional method of plant breeding in crop improvement programmes?
3. Modern methods of breeding plants can alleviate the global food 'shortage'. Comment on the statement and give suitable examples.
4. Discuss how the property of plant cell totipotency has been utilized for plant propagation and improvement.
5. What are three options to increase food production? Discuss each giving the salient features, merits and demerits?

Exercises

1. Describe in brief various steps involved in plant breeding.
2. What is meant by biofortification.
3. Which part of the plant is best suited for making virus-free plants and why?
4. What is the major advantage of producing plants by micropropagation?
5. Find out what are the various components of the medium used for propagation of an explant *in vitro* ?
6. Name any five hybrid varieties of crop plants which have been developed in India.
7. The term 'desirable trait' can mean different things for different plants. Justify the statement with suitable examples.
8. Is there any relationship between dedifferentiation and the higher degree of success achieved in plant tissue culture experiments?
9. "Give me a living cell of any plant and I will give you a thousand plants of the same type" Is this only a slogan or is it scientifically possible? Write your comments and justify them
10. What are the physical barriers of a cell in the protoplast fusion experiment ? How are the barriers overcome?

Chapter 14

Microbes in Human Welfare

Besides macroscopic plants and animals, microbes are the major components of biological systems on this earth. You have studied about the diversity of living organisms in First Year. *Do you remember which Kingdoms among the living organisms contain micro-organisms? Which are the ones that are only microscopic?* Microbes are present everywhere – in soil, water, air, inside our bodies and that of other animals and plants. They are present even at sites where no other life-form could possibly exist–sites such as deep inside the geysers (thermal vents) where the temperature may be as high as 140^{0}C, deep in the soil, under the layers of snow several metres thick, and in highly acidic environments. Microbes are diverse–protozoa, bacteria, fungi and microscopic plant viruses, viroids and also prions that are proteinacious infectious agents.

Microbes like bacteria and many fungi can be grown on nutritive media to form colonies (Figure 14.1), that can be seen with the naked eye. Such cultures are useful in studies on micro-organisms.

In unit II, you have read that microbes cause a large number of diseases in human beings, animals and plants. But this should not make you think that all microbes are harmful. Several microbes are useful to man in diverse ways. Some of the most important contributions of microbes to human welfare are discussed in this chapter.

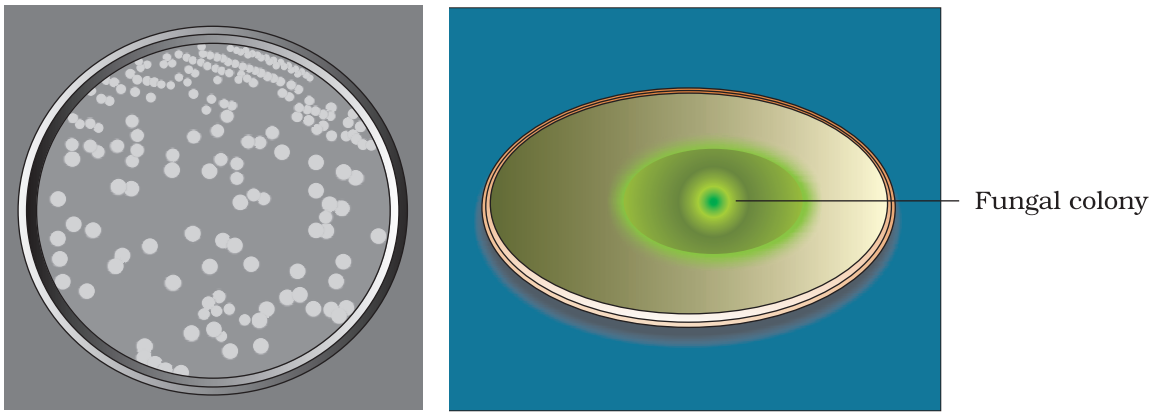


Figure 14.1 (a) Colonies of bacteria growing in a petri dish; (b) Fungal colony growing in a petri dish

14.1 Microbes in Household Products

You would be surprised to know that we use microbes or products derived from them everyday. A common example is the production of curd from milk. Micro-organisms such as *Lactobacillus* and others, commonly called **lactic acid bacteria (LAB)**, grow in milk and convert it to curd. During growth, the LAB produce acids that coagulate and partially digest the milk proteins. A small amount of curd added to fresh milk as inoculum or starter contain millions of LAB, which at suitable temperatures multiply, thus converting milk to curd, which also improves its nutritional quality by increasing vitamin B_{12}. In our stomach too, LAB play a very beneficial role in checking disease-causing microbes. The use of such friendly bacteria for therapeutic purposes and for the betterment of human health has led to the concept of **Probiotics**.

The dough which is used for making foods such as *dosa* and *idli* is also fermented by bacteria. The puffed-up appearance of dough is due to the production of CO_2 gas. *Can you tell which metabolic pathway is taking place resulting in the formation of CO_2? Where do you think the bacteria for these fermentations came from?* Similarly the dough which is used for making bread is fermented using baker's yeast (*Saccharomyces cerevisiae*). A number of traditional drinks and foods are also made by fermentation by the microbes. 'Toddy', a traditional drink of some parts of southern India, is made by fermenting sap from palms. Microbes are also used to ferment fish, soyabean and bamboo-shoots to make foods. Cheese is one of the oldest food items in which microbes were used. Different varieties of cheese are known by their

characteristic texture, flavour and taste, the specificity coming from the microbes used. For example, the large holes in 'Swiss cheese' are due to the production of a large amount of CO_2 by a bacterium named *Propionibacterium sharmanii*. The 'Roquefort cheese' is ripened by growing a specific fungi on it, which gives it a particular flavour.

14.2 Microbes in Industrial Products

Even in industry, microbes are used to synthesise a number of products valuable to human beings. Beverages and antibiotics are some examples. Production on an industrial scale requires growing microbes in very large vessels called **fermentors** (Figure 14.2).

14.2.1 Fermented Beverages

Microbes, especially yeasts, have been used from time immemorial for the production of beverages like wine, beer, whisky, brandy and rum. The same yeast *Saccharomyces cerevisiae,* used for bread-making and commonly called brewer's yeast, is used for fermenting malted cereals and fruit juices, to produce ethanol. *Do you recollect the metabolic reactions which result in the production of ethanol by yeast?* Depending on the type of the raw material used for fermentation and the type of processing (with or without distillation) different types of alcoholic drinks are obtained. Wine and beer are produced without distillation whereas whisky, brandy and rum are produced by the distillation of the fermented broth. The photograph of a fermentation plant is shown in Figure 14.3.

Figure 14.2 Fermentors

Figure 14.3 Fermentation Plant

14.2.2 Antibiotics

Antibiotics produced by microbes are regarded as one of the most significant discoveries of the twentieth century and have greatly contributed towards the welfare of human society. *Anti* is a Greek word that means 'against', and *bio* means 'life'; together they mean 'against life' (in the context of disease causing

organisms); whereas with reference to human beings, they are 'pro life' and not against. Antibiotics are chemical substances which are produced by some microbes and can kill or retard the growth of other (disease-causing) microbes.

You are familiar with the commonly used antibiotic, **Penicillin**. Do you know that Penicillin was the first antibiotic to be discovered, and it was a chance discovery? **Alexander Fleming** while working on ***Staphylococci*** bacteria, once observed a mould growing in one of his unwashed culture plates around which *Staphylococci* could not grow. He found out that it was due to a chemical produced by the mould and he named it Penicillin after the mould *Penicillium notatum.* However, its full potential as an effective antibiotic was established only much later by Ernest Chain and Howard Florey. This antibiotic was extensively used to treat American soldiers wounded in World War II. Fleming, Chain and Florey were awarded the Nobel Prize in 1945 for this discovery.

After Penicillin, other antibiotics were also purified from other microbes. *Can you name some other antibiotics and find out their sources?* Antibiotics have greatly improved our capacity to treat deadly diseases such as the plague, whooping cough (*kali khansi*), diphtheria (*gal ghotu*) and leprosy (*kusht rog*), which used to kill millions all over the globe. Today we cannot imagine a world without antibiotics.

14.2.3 Chemicals, Enzymes and other Bioactive Molecules

Microbes are also used for commercial and industrial production of certain chemicals like organic acids, alcohols and enzymes. Examples of acid producers are *Aspergillus niger* (a fungus) of citric acid, *Acetobacter aceti* (a bacterium) of acetic acid; *Clostridium butylicum* (a bacterium) of butyric acid and *Lactobacillus* (a bacterium) of lactic acid.

Yeast (*Saccharomyces cerevisiae)* is used for commercial production of ethanol. Microbes are also used for production of enzymes. Lipases are used in detergent formulations and are helpful in removing oily stains from laundry. You must have noticed that bottled fruit juices bought from the market are clearer than those made at home. This is because bottled juices are clarified by the use of pectinases and proteases. Streptokinase, produced by the bacterium *Streptococcus* and modified by genetic engineering is used as a 'clot buster' for removing clots from the blood vessels of patients who have undergone myocardial infection leading to heart attack.

Another bioactive molecule, cyclosporin A, that is used as an immunosuppressive agent in organ-transplant patients, is produced by the fungus *Trichoderma polysporum*. Statins produced by the yeast *Monascus purpureus* have been commercialised as blood-cholesterol lowering agents. The act by competitively inhibiting the enzyme responsible for the synthesis of cholesterol.

14.3 Microbes in Sewage Treatment

We know that large quantities of waste water are generated everyday in cities and towns. A major component of this waste water is human excreta. Municipal waste-water is also called sewage. It contains large amounts of organic matter and microbes, many of which are pathogenic. *Have you ever wondered where this huge quantity of sewage or urban waste water is disposed off daily?* This cannot be discharged into natural water bodies like rivers and streams directly – you can understand why. Before disposal, hence, sewage is treated in sewage treatment plants (STPs) to make it less polluting. Treatment of waste water is done by heterotrophic microbes naturally present in the sewage. This treatment is carried out in two stages:

Primary treatment : The treatment involves physical removal of particles – large and small – from the sewage through filtration and sedimentation. These particles are removed in stages; initially, floating debris is removed by sequential filtration. Then the grit (soil and small pebbles) is removed by sedimentation. All solids that settle form the **primary sludge**, and the supernatant forms, the effluent. The effluent from the primary settling tank is taken for secondary treatment.

Secondary treatment or Biological treatment : The primary effluent is passed into large aeration tanks (Figure 14.4) where it is constantly agitated mechanically and air is pumped into it. This allows vigorous growth of useful aerobic microbes into **flocs** (masses of bacteria associated with fungal filaments to form mesh like structures). While growing, these microbes consume the major part of the organic matter in the effluent. This significantly reduces the **BOD**

Figure 14.4 Secondary treatment

(biochemical oxygen demand) of the effluent. BOD refers to the amount of the oxygen that would be consumed if all the organic matter in one liter of water were oxidised by bacteria. The sewage water is treated till the BOD is reduced. The BOD test measures the rate of uptake of oxygen by micro-organisms in a sample of water and thus, indirectly, BOD is a measure of the organic matter present in the water. The greater the BOD of waste water, the more is its polluting potential.

Once the BOD of sewage or waste water is reduced significantly, the effluent is then passed into a settling tank where the bacterial 'flocs' are allowed to sediment. This sediment is called **activated sludge**. A small part of the activated sludge is pumped back into the aeration tank to serve as the inoculum. The remaining major part of the sludge is pumped into large tanks called **anaerobic sludge digesters**. Here other kinds of bacteria, which grow anaerobically, digest the bacteria and the fungi in the sludge. During this digestion, bacteria produce a mixture of gases such as methane, hydrogen sulphide and carbon dioxide. These gases form **biogas** which can be used as a source of energy as it is inflammable.

Figure 14.5 An aerial view of a sewage plant

The effluent from the secondary treatment plant is generally released into natural water bodies like rivers and streams. An aerial view of such a plant is shown in Figure 14.5.

You can appreciate how microbes play a major role in treating millions of gallons of waste water everyday across the globe. This methodology has been practiced for more than a century now, in almost all parts of the world. Till date, no man-made technology has been able to rival the microbial treatment of sewage.

You are aware that due to increasing urbanisation, sewage is being produced in much larger quantities than ever before. However the number of sewage treatment plants has not increased enough to treat such large quantities. So the untreated sewage is often discharged directly into rivers, leading to their pollution and an increase in water-borne diseases.

The Ministry of Environment and Forests has initiated **The Ganga Action Plan** and **The Yamuna Action Plan** to save the major rivers of our country

from pollution. Under these plans, it is proposed to build a large number of sewage treatment plants so that only treated sewage may be discharged in the rivers. *A visit to a sewage treatment plant situated in any place near you would be a very interesting and educating experience.*

Besides the above, microbes can be used to remove toxic substances that are accidently released into the environment such as oil or chemical spills and lands contaminated with toxic wastes. This process is known as **bioremediation**.

14.4 Microbes in Production of Biogas

Biogas is a mixture of gases (containing predominantly methane) produced by microbial activity and which may be used as fuel. You have learnt that microbes produce different types of gaseous end-products during growth and metabolism. The type of the gas produced depends upon the microbes and the organic substrates they utilise. In the examples cited in relation to fermentation of dough, cheese making and production of beverages, the main gas produced is $CO_{2.}$. However, certain bacteria which grow anaerobically on cellulosic material produce large amount of methane along with CO_2 and H_2. These bacteria are collectively called **methanogens**, and one such common bacterium is *Methanobacterium*. These bacteria are commonly found in the anaerobic sludge during sewage treatment. These bacteria are also present in the rumen (a part of stomach) of cattle. A lot of cellulosic material present in the food of cattle is also present in the rumen. In rumen, these bacteria help in the breakdown of cellulose and play an important role in the nutrition of cattle. Thus, the excreta (dung) of

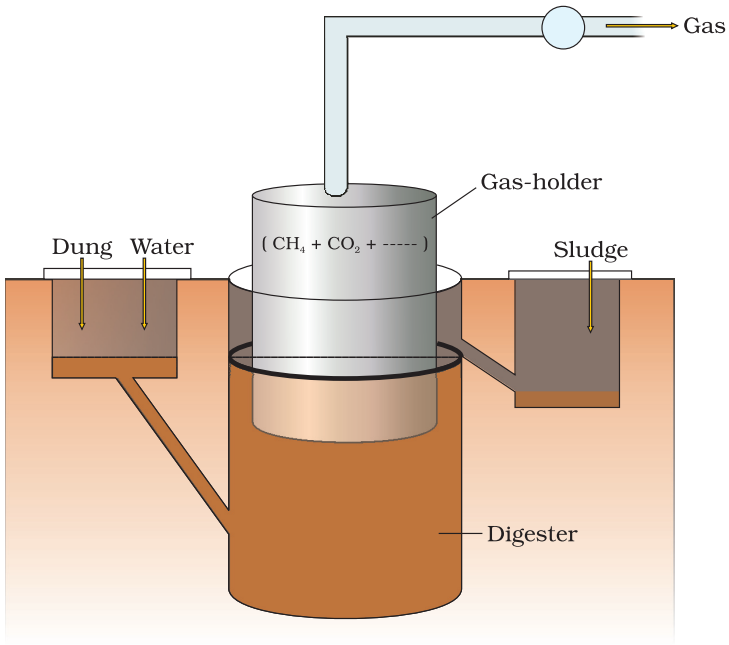


Figure 14.6 A typical biogas plant

cattle, commonly called *gobar*, is rich in these bacteria. Dung can be used for generation of biogas, commonly called *gobar gas*. *Do you think we, human beings are able to digest the celluose present in our foods?*

The biogas plant consists of a concrete tank (14-15 feet deep) in which bio-wastes are collected and a slurry of dung is fed. A floating cover is placed over the slurry, which keeps on rising as the gas is produced in the tank due to the microbial activity. The biogas plant has an outlet which is connected to a pipe to supply biogas to nearby houses. The spent slurry is removed through another outlet and may be used as fertiliser. Cattle dung is available in large quantities in rural areas where cattle are used for a variety of purposes. So biogas plants are often built in rural areas. The biogas thus produced is used for cooking and lighting. The picture of a biogas plant is shown in Figure 14.6. The technology of biogas production was developed in India mainly due to the efforts of Indian Agricultural Research Institute (IARI) and Khadi and Village Industries Commission (KVIC). If your school/ college is situated in a village or near a village, it would be very interesting to enquire if there are any biogas plants nearby. Visit the biogas plant and learn more about it from the people who are actually managing it.

14.5 Microbes as Biocontrol Agents

Biocontrol refers to the use of biological methods for controlling plant diseases and pests. In modern society these problems have been tackled increasingly by the use of chemicals – insecticides and pesticides. These chemicals are toxic and extremely harmful to human beings and animals alike, and have been polluting our environment (soil, ground water), fruits, vegetables and crop plants. Our soil is also polluted through the use of weedicides to remove weeds.

Biological control of pests and diseases: In agriculture there is a method of controlling pests that relies on natural predation rather than introduced chemicals. A key belief of the organic farmer is that biodiversity furthers health. The more variety a landscape has, the more sustainable it is. The organic farmer, therefore, works to create a system where the insects that are sometimes called pests are not eradicated, but instead are kept at manageable levels by a complex system of checks and balances within a living and vibrant ecosystem. Contrary to the 'conventional' farming practices which often use chemical methods to kill both useful and harmful life forms indiscriminately, this is a holistic approach that seeks to develop an understanding of the webs of interaction between the myriad of organisms that constitute the field fauna

and flora. The organic farmer holds the view that the eradication of the creatures that are often described as pests is not only impossible, but also undesirable, for without them the beneficial predatory and parasitic insects which depend upon them as food or hosts would not be able to survive. Thus, the use of biocontrol measures will greatly reduce our dependence on toxic chemicals and pesticides. An important part of the biological farming approach is to become familiar with the various life forms that inhabit the field, predators as well as pests, and also their life cycles, patterns of feeding and the habitats that they prefer. This will help develop appropriate means of biocontrol.

The very familiar beetle with red and black markings – the Ladybird, and Dragonflies are useful to get rid of aphids and mosquitoes, respectively. An example of a microbial biocontrol agent that can be introduced in order to control butterfly caterpillars is the bacteria *Bacillus thuringiensis* (often written as *Bt*). These are available in sachets as dried spores which are mixed with water and sprayed onto vulnerable plants such as brassicas and fruit trees, where these are eaten by the insect larvae. In the gut of the larvae, the toxin is released and the larvae get killed. The bacterial disease will kill the caterpillars, but leave other insects unharmed. Because of the development of methods of genetic engineering in the last decade or so, the scientists have introduced *B. thuringiensis* toxin genes into plants. Such plants are resistant to attack by insect pests. **Bt-cotton** and **Bt-brinjal** are two such examples. It is being cultivated in some states of our country. You have already learnt about this in chapter 12.

A biological control being developed for use in the treatment of plant disease is the fungus *Trichoderma. Trichoderma* species are free-living fungi that are very common in the root ecosystems. They are effective biocontrol agents of several plant pathogens.

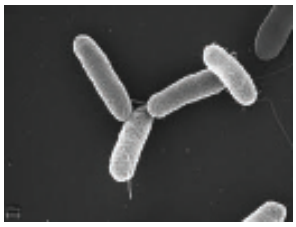
Baculoviruses are pathogens that attack insects and other arthropods. The majority of baculoviruses used as biological control agents are in the genus *Nucleopolyhedrovirus.* These viruses are excellent candidates for species-specific, narrow spectrum insecticidal applications. They have been shown to have no negative impacts on plants, mammals, birds, fish or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall integrated pest management (IPM) programme, or when an ecologically sensitive area is being treated.

14.6 Microbes as Biofertilisers

With our present day life styles, environmental pollution is a major cause of concern. The use of the chemical fertilisers to meet the ever - increasing demand of agricultural produce is one of the important causes of this pollution. Of course, we have now realised that there are problems associated with the overuse of chemical fertilisers and there is immense pressure to switch to **organic farming** – the use of **biofertilisers**. Biofertilisers are organisms that enrich the nutrient quality of the soil. The main sources of biofertilisers are bacteria, fungi and cyanobacteria. You have studied about the nodules on the roots of leguminous plants formed by the symbiotic association with *Rhizobium*. These bacteria fix atmospheric nitrogen into organic forms, which is used by the plant as a nutrient. Other bacteria can fix atmospheric nitrogen while free-living in the soil (examples *Azospirillum* and *Azotobacter*), thus enriching the nitrogen content of the soil.

Anabaena

Azospirillum



Azotobacter

mycorrhiza

Fungi are also known to form symbiotic associations with plants (**mycorrhiza**). Many members of the genus *Glomus* form mycorrhiza. The fungal symbiont in these associations facilitates absorption of phosphorus by the plant from the soil. Plants having such associations show other benefits also, such as resistance to root-borne pathogens, tolerance to salinity and drought, and an overall increase in plant growth and development. *Can you tell what advantage the fungus derives from this association?*

Cyanobacteria are autotrophic microbes widely distributed in aquatic and terrestrial environments. Many of them can fix atmospheric nitrogen, e.g. *Anabaena*, *Nostoc*, *Oscillatoria,* etc. In paddy fields cyanobacteria serve as an important biofertiliser. Blue green algae also add organic matter to the soil and increase its fertility. Currently, in our country, a number of biofertilisers are available commercially in the market and farmers use these regularly in their fields to replenish soil nutrients and to reduce dependence on chemical fertilisers.

14.7 Challenges posed by Microbes

Inspite of the several uses of microbes described in the preceeding pages, there are certain challenges that microbes may pose to human society in future. These are to be kept in mind for the welfare of our future generations.

One major challenge is the difficulty to fight infectious diseases due to microbial pathogens evolving resistance to drugs, particularly antibiotics. Microbiologists realized that pathogens could mutate to '**supermicrobes**' which are resistant to many drugs. So there is a need to find new and effective therapeutics. Microbiologists and epidemiologists are now especially concerned with emerging and re-emerging infectious diseases. Some of the recently emerged diseases are **Acquired Immune Deficiency Syndrome (AIDS)**, **mad cow disease** and **Severe Acute Respiratory Syndrome (SARS)**. Re-emerging infectious diseases are the ones that have existed in the past but are now showing resurgence in incidence and in new geographical areas. Some of the re-emerging diseases are **Cholera**, **Tuberculosis** and **Dengue fever**.

A newly emerged pandemic Corona virus disease (COVID-19) is an infectious disease caused by SARS-CoV-2 virus posed a challenge to the global health in 2020-21 and is still persisting as a mild disease. The virus can spread from an infected person's mouth or nose in small liquid particles when they cough, sneeze, speak, sing or breath. Any one can sick with Covid-19 and become seriously ill (or) die irrespective of age. The only way to protect ourselves from such pandemic disease is by slowing down the transmission and protect yourselves and others from infection by staying at least one meter apart from others, wearing a properly fitted mask and washing your hands or using an alcohol-based sanitizer frequently. The best way to prevent such viral infections is to improve your innate immunity by staying fit with balanced diet and exercise and timely vaccination.

One of the major threats to society is **Bioterrorism.** Bioterrorism involves the actual use of or the threat to use biological agents in order to spread fear or inflict disease and death upon large populations. Advances in **genomics** and **bioinformatics** can produce rapid detection methods of these bio-terror agents and might pave way for the development of new vaccines and therapies.

SUMMARY

SUMMARY

Microbes are a very important component of life on earth. Not all microbes are pathogenic. Many microbes are very useful to human beings. We use microbes and microbially derived products almost every day. Bacteria called lactic acid bacteria (LAB) grow in milk and convert it into curd. The dough which is used to make bread is fermented by yeast called *Saccharomyces cerevisiae*. Certain dishes such as *idli* and *dosa*, are made from dough fermented by microbes. Bacteria and fungi are used to impart a particular texture, taste and flavor to cheese. Microbes are used to produce industrial products like lactic acid, acetic acid and alcohol, which are used in a variety of processes in the industry. Antibiotics like penicillin, produced by useful microbes, are used to kill disease-causing harmful microbes. Antibiotics have played a major role in controlling infectious diseases like diphtheria, whooping cough and pneumonia. For more than a hundred years, microbes have been used to treat sewage (waste water) by the process of activated sludge formation. This helps in recycling of water in nature. Methanogens produce methane (biogas) while degrading plant waste. Biogas produced by microbes is used as a source of energy in rural areas. Microbes can also be used to kill harmful pests. This process is referred to as biocontrol. Biocontrol measures help us to avoid heavy use of toxic pesticides for controlling pests. There is a need today for the aggressive use of biofertilisers in place of chemical fertilisers. Microbes, thus play an important role in the welfare of human society and have been put to diverse uses.

GLOSSARY

BOD (Bio Chemical Oxygen demand): It is the amount of oxygen that would be consumed if all the organic matter in one litre of water is oxidized by bacteria.

Biofertilizers: Organisms that enrich the nutrient quality of the soil.

Bio gas (Gobar gas): It is the gas generated by the decomposition of excreta or dung of cattle (commonly called as gobar), domestic waste material, industrial and agriculture sewage due to the activity of anaerobic bacteria present in them. It comprises methane (CH_4), CO_2, traces of H_2S and moisture.

Biopesticides: The commercial production of micro organism by use of which insects, termites, or nematode pests are killed or their level is minimized, thereby increasing the crop yield.

Fermentors: These are large vessels in which microbes are grown in large numbers on an industrial scale.

Mycorrhiza: These are the fungi living in symbiotic association with plants.

Methanogens: Certain anaerobic bacteria growing on cellulosic material produce a large amount of methane along with CO_2 and H_2 which are called methanogens.

Organic farming: The use of biofertilizers in enriching the nitrogen content of the soil.

Primary sludge: The solids that settle down during the treatment of waste water forms primary sludge.

Vermicompost: The process of compost formation by earthworms.

QUESTIONS

Very Short Answer Type Questions

1. Why does 'Swiss cheese' have big holes. Name the bacteria responsible for it.
2. What are fermentors?
3. Name a microbe used for statin production. How do statins lower blood cholesterol level?
4. Why do we prefer to call secondary waste water treatment as biological treatment?
5. What is *Nucleopolyhedrovirus* is being used for now a days?
6. How has the discovery of antibiotics helped mankind in the field of medicine?
7. Why is distillation required for producing certain alcoholic drinks?
8. Write the most important characteristic that *Aspergillus niger*, *Clostridium butylicum* and *Lactobacillus* share.
9. Give any two microbes that are useful in biotechnology.
10. Name any two genetically modified crops.
11. Why are blue green algae not popular as biofertilisers?
12. Which species of *Penicillium* produces Roquefort cheese?
13. Name any two industrially important enzymes.
14. Name an immunosuppressive agent.
15. Give an example of a rod shaped virus.
16. What is the group of bacteria found in both the rumen of cattle and sludge of sewage treatment?
17. Why are cyanobacteria considered useful in paddy fields?
18. In which food would you find lactic acid bacteria? Name the bacterium.
19. Name any two fungi which are used in the production of antibiotics.
20. Name the scientists who were credited for showing the role of penicillin as an antibiotic.

Short Answer Type Questions

1. Why are flocs important in the biological treatment of waste water?
2. How is *Bacillus thuringiensis* helpful in controlling insect pests?
3. How do mycorrhizal fungi help the plants harbouring them?
4. How was penicillin discovered?
5. How do bioactive molecules of fungal origin help in restoring good health of humans?
6. What is the chemical nature of biogas? Explain the process of biogas production.
7. Which bacterium has been used as a clot buster? What is its mode of action?
8. What are biofertilisers? Give two examples and discuss their role as biofertilisers.
9. What role do enzymes play in detergents that we use for washing clothes? Give examples.

Long Answer Type Questions

1. (a) What would happen if a large volume of untreated sewage is discharged into a river?
   (b) In what way is anaerobic sludge digestion important in sewage treatments?
2. Which type of food would have lactic acid bacteria? Discuss their useful application.
3. Write a brief essay on Microbes as biocontrol agents.
4. What is organic farming? Discuss the role of plant microbes in organic farming with examples.

Exercises

1. Bacteria cannot be seen with the naked eye, but these can be seen with the help of a microscope. If you have to carry a sample from your home to your biology laboratory to demonstrate the presence of microbes under a microscope, which sample would you carry and why?
2. Give examples to prove that microbes release gases during metabolism.
3. Name the states involved in Ganga action plan.
4. Name some traditional Indian foods made of wheat, rice and Bengal gram (or their products). Which of these foods involve the use of microbes?
5. In which way have microbes played a major role in controlling diseases caused by harmful bacteria?
6. Do you think microbes can also be used as a source of energy? If yes, how?
7. Microbes can be used to decrease the use of chemical fertilisers and pesticides. Explain how this can be accomplished.
8. Three water samples namely river water, untreated sewage water and secondary effluent discharged from a sewage treatment plant were subjected to BOD test. The samples were labelled A, B and C; but the laboratory attendant did not note which was which. The BOD values of the three samples A, B and C were recorded as 20mg/L, 8mg/L and 400mg/L, respectively. Which sample of the water is most polluted? Can you assign the correct label to each assuming the river water is relatively clean?
9. Name of the microbes from which Cyclosporin A (an immunosuppressive drug) and Statins (blood cholesterol lowering agents) are obtained.
10. Find out the role of microbes in the following and discuss it with your teacher. (a) Single cell protein (SCP) (b) Soil
11. Arrange the following in the decreasing order (most important first) of their importance, for the welfare of human society. Give reasons for your answer. Biogas, Citric acid, Penicillin and Curd.
12. What is sewage? In which way can sewage be harmful to us?
13. What is the key difference between primary and secondary sewage treatment?

BOARD OF INTERMEDIATE EDUCATION, A.P, HYDERABAD

Intermediate II Year Syllabus

Subject: BOTANY-II (W.E.F 2013-14)

UNIT I: Plant Physiology

60 Periods

Chapter 1: Transport in Plants

Means of Transport- Diffusion, Facilitated Diffusion, Passive symports and antiports, Active Transport, Comparison of Different Transport Processes**, Plant-Water Relations-** Water Potential, Osmosis, Plasmolysis, Imbibition, **Long Distance Transport of Water-** Water Movement up a Plant, Root Pressure, Transpiration pull, **Transpiration-** Opening and Closing of Stomata, Transpiration and Photosynthesis, **Uptake and Transport of Mineral Nutrients-** Uptake of Mineral Ions, Translocation of Mineral Ions, **Phloem Transport: Flow from Source to Sink-**The Pressure Flow or Mass Flow Hypothesis

Chapter 2: Mineral Nutrition

Methods to Study the Mineral Requirements of Plants, Essential Mineral Elements-Criteria for Essentiality, Macronutrients, Micronutrients, Role of Macro- and Micro-nutrients, Deficiency Symptoms of Essential Elements, Toxicity of Micronutrients, **Mechanism of Absorption of Elements, Translocation of Solutes, Soil as Reservoir of Essential Elements, Metabolism of Nitrogen-**Nitrogen Cycle, Biological Nitrogen Fixation, Symbiotic nitrogen fixation, Nodule Formation

Chapter 3: Enzymes

Chemical Reactions, Enzymatic Conversions, Nature of Enzyme Action, Factors Affecting Enzyme Activity, Temperature and pH, Concentration of Substrate, Classification and Nomenclature of Enzymes, Co-factors

Chapter 4: Photosynthesis in Higher Plants

Early Experiments, Site of Photosynthesis, Pigments Involved in Photosynthesis, Light Reaction, The Electron Transport-Splitting of Water, Cyclic and Non-cyclic Photo-phosphorylation, Chemiosmotic Hypothesis, **Biosynthetic phase-** The Primary Acceptor of CO_2, The Calvin Cycle, **The C_4 Pathway, Photorespiration, Factors affecting Photosynthesis**

Chapter 5: Respiration of Plants

Cellular respiration, Glycolysis, Fermentation, Aerobic Respiration- Tricarboxylic Acid Cycle, Electron Transport System (ETS) and Oxidative Phosphorylation, The Respiratory Balance Sheet, **Amphibolic Pathway, Respiratory Quotient**

Chapter 6: Plant Growth and Development

Growth- Plant Growth, Phases of Growth, Growth Rates, Conditions for Growth, **Differentiation, Dedifferentiation and Redifferentiation, Development, Plant Growth Regulators-** Physiological Effects of Plant Growth Regulators, *Auxins, Gibberellins, Cytokinins, Ethylene, Abscisic acid,* **Seed Dormancy, Photoperiodism, Vernalisation**

UNIT II: Microbiology

10 Periods

Chapter 7: Bacteria

Morphology of Bacteria, Bacterial cell structure-Nutrition, **Reproduction-** Sexual Reproduction, Conjugation, Transformation, Transduction, **The importance of Bacteria to Humans**

Chapter 8: Viruses

Discovery, Classification of Viruses, structure of Viruses, Multiplication of Bacteriophages- The Lysogenic Cycle, **Viral diseases in Plants, Viral diseases in Humans**

UNIT III: Genetics

10 Periods

Chapter 9: Principles of Inheritance and Variation

Mendel's Experiments, Inheritance of one gene (Monohybrid Cross)-Back cross and Test cross, Law of Dominance, Law of Segregation or Law of purity of gametes, **Deviations from Mendelian concept of dominance-**Incomplete Dominance, Co-dominance, Explanation of the concept of dominance, **Inheritance of two genes-** Law of Independent Assortment, **Chromosomal Theory of Inheritance, Linkage and Recombination, Mutations-**Significance of mutations

UNIT IV: Molecular Biology

15 Periods

Chapter 10: Molecular Basis of inheritance

The DNA- Structure of Polynucleotide Chain, Packaging of DNA Helix, **The Search for Genetic Material,** Transforming Principle, Biochemical Characterisation of Transforming Principle, The Genetic Material is DNA, Properties of Genetic Material (DNA versus RNA), **RNA World, Replication-** The Experimental Proof, The Machinery and the Enzymes, **Transcription-** Transcription Unit, Transcription Unit and the Gene, Types of RNA and the process of Transcription, **Genetic Code-**Mutations and Genetic Code, tRNA–the Adapter Molecule, **Translation, Regulation of Gene Expression-**The *Lac* operon.

UNIT V: Biotechnology

22 Periods

Chapter 11: Principles and processes of Biotechnology

Principles of Biotechnology-Construction of the first artificial recombinant DNA molecule, **Tools of Recombinant DNA Technology-**Restriction Enzymes, Cloning Vectors, Competent Host (For Transformation with Recombinant DNA), **Processes of Recombinant DNA Technology-** Isolation of the Genetic Material (DNA), Cutting of DNA at Specific Locations, Separation and isolation of DNA fragments, Insertion of isolated gene into a suitable vector, Amplification of Gene of Interest using PCR, Insertion of Recombinant DNA into the Host, Cell/Organism, Selection of Transformed host cells, Obtaining the Foreign Gene Product, Downstream Processing

Chapter 12: Biotechnology and its applications

Biotechnological Applications In Agriculture-Bt Cotton, Pest Resistant Plants, **Other applications of Biotechnology** Insulin, Gene therapy, Molecular Diagnosis, ELISA**,** DNA fingerprinting, **Transgenic plants, Bio-safety and Ethical issues-** Biopiracy

UNIT VI: Plants, Microbes and Human welfare 18 Periods

Chapter 13: Strategies for enhancement in food production

Plant Breeding- What is Plant Breeding?, Wheat and Rice, Sugarcane, Millets, Plant Breeding for Disease Resistance, Methods of breeding for disease resistance, Mutation, Plant Breeding for Developing Resistance to Insect Pests, Plant Breeding for Improved Food Quality, **Single Cell Protein (SCP), Tissue Culture**

Chapter 14: Microbes in Human Welfare

Microbes in Household Products, Microbes in Industrial Products-Fermented Beverages, Antibiotics, Chemicals, Enzymes and other Bioactive Molecules, Microbes in Sewage Treatment, Primary treatment, Secondary treatment or Biological treatment, Microbes in Production of Biogas, Microbes as Biocontrol Agents, Biological control of pests and diseases, Microbes as Biofertilisers, Challenges posed by Microbes

BOARD OF INTERMEDIATE EDUCATION

A.P., HYDERABAD

MODEL QUESTION PAPER BOTANY-II (W.E.F 2013-14)

SECTION-A **10 X 2 =20**

Answer all the questions (Very short answer type)

1. What is transpiration? How does it differs from evaporation?
2. What is Richmond Lang effect?
3. What are pleomorphic bacteria? Give one example.
4. What is linkage? Who discovered it?
5. What is translation? Where does it occur?
6. Differentiate between Purines and Pyrimidines?
7. Define Gene Revolution?
8. What is molecular farming?
9. What are synthetic seeds?
10. How do Biofertilizers enrich the fertility of the soil?

SECTION-B **6 X 4 =24**

Answer any Six Questions (Short Answer Type)

11. Explain cohesion tension theory?
12. What is active transport? Explain the mechanism involved in it?
13. Write any 8 differences between C3 and C4 plants?
14. What are growth curves? Explain the three phases of the growth curves/
15. What are viruses? Explain the chemical structure of Viruses.
16. Explain the monohybrid cross of Mendel and mention the first law of Mendel.
17. What is meant by genetic code? Write the properties of genetic code?
18. Explain genetically modified crops with 2 suitable examples.

SECTION-C **2 X 8 =16**

Answer any Two Questions (Long Answer Type)

19. Describe TCA cycle.
20. Explain various steps involved in r-DNA technology.
21. What is hybridization? Describe the procedure of hybridization.