Text Book for

INTERMEDIATE

Second Year

Botany

Permission and Support by

National Council of Educational Research and Training
New Delhi

Board of Intermediate Education, Andhra Pradesh

Telugu and Sanskrit Akademi, Andhra Pradesh

Intermediate

Second Year Text Book

Botany

Page: xviii + 278 + iv

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Research and Training, 2007

Reprint: **2023**

Copies: 20,000

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- Published by Telugu and Sanskrit Akademi, Andhra Pradesh under the Centrally Sponsored Scheme of Production of Books and Literature in Regional Languages at the University level of the Government of India in the Ministry of Human Resource Development, New Delhi.

Permission and Prescribed by
Board of Intermediate Education, A.P.
Vijayawada.

Published, Printed & Distributed by
Telugu and Sanskrit Akademi, A.P.

Price: Rs. **145.00**

Printed in India

Laser Typeset by P.V. Ramana Murthy, Rajamahendravaram

Published and Printed by
M/s. GBR Offset Printers & Publishers
Surampalli, NTR Dist.

on behalf of Telugu and Sanskrit Akademi

Y.S. JA GAN MOHAN REDD Y

CHIEF MINISTER
ANDHRA PRADESH

AMARA VATI

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 R eddy

Dr. Nandamuri Lakshmiparvathi
M.A., M.Phil., Ph.D.
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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 RA O, 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

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UNIT I

Chapter 1 : Transport in Plants
Chapter 2 : Mineral Nutrition
Chapter 3 : Enzymes
Chapter 4 : Photosynthesis in Higher Plants
Chapter 5 : Respiration in Plants
Chapter 6 : Plant Growth and Development

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

1.1 Means of Transport
1.2 Plant-Water Relations
1.3 Long Distance Transport of Water
1.4 Transpiration
1.5 Uptake and Transport of Mineral Nutrients
1.6 Phloem Transport: Flow from Source to Sink

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 .

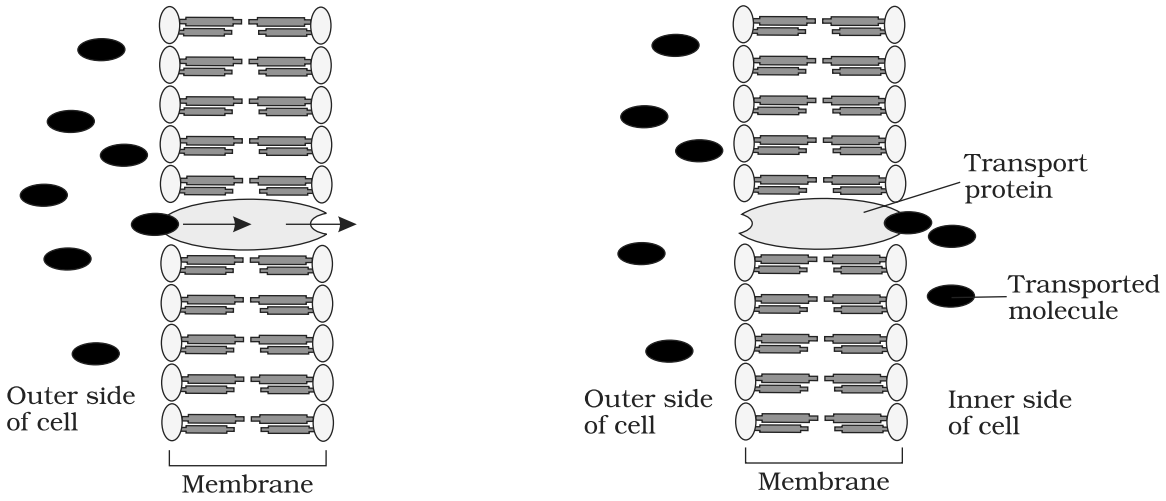


Figure 1.1 Facilitated diffusion

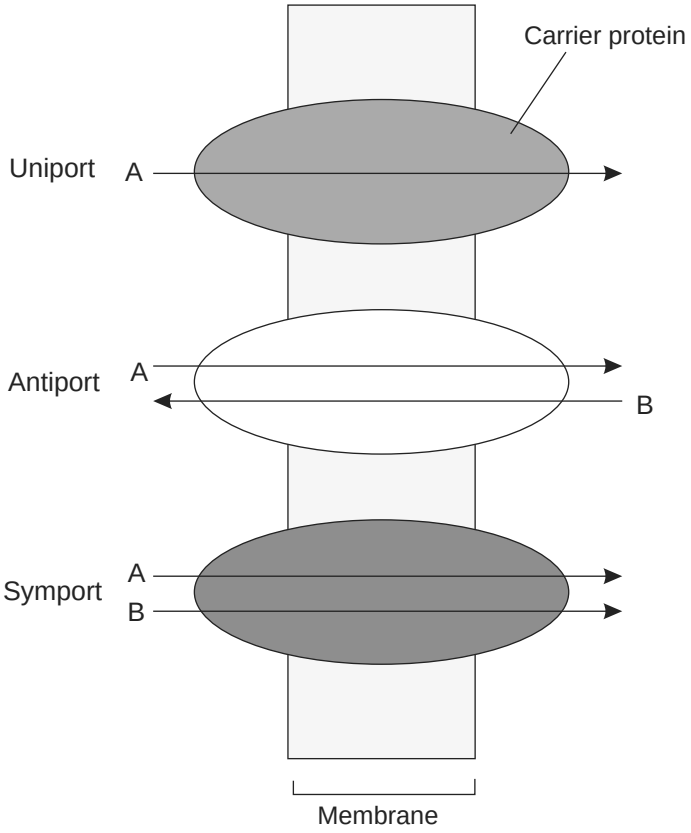


Figure 1.2 Facilitated diffusion

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 \ 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.

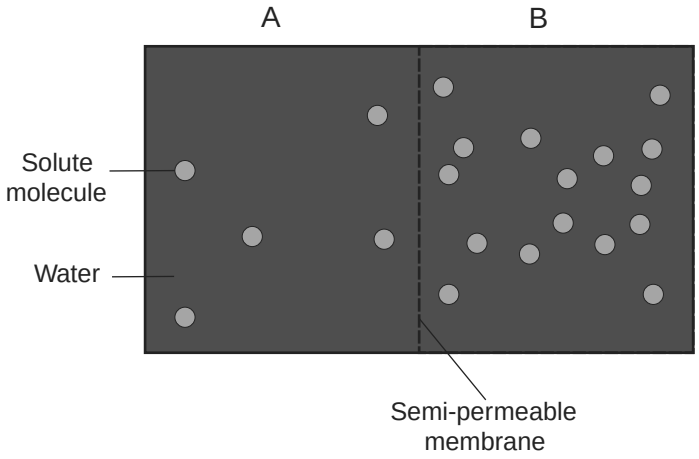


Figure 1.3

(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 \ of – 2000 kPa and the other – 1000 kPa, which is the chamber that has the higher \?

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?

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.

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

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

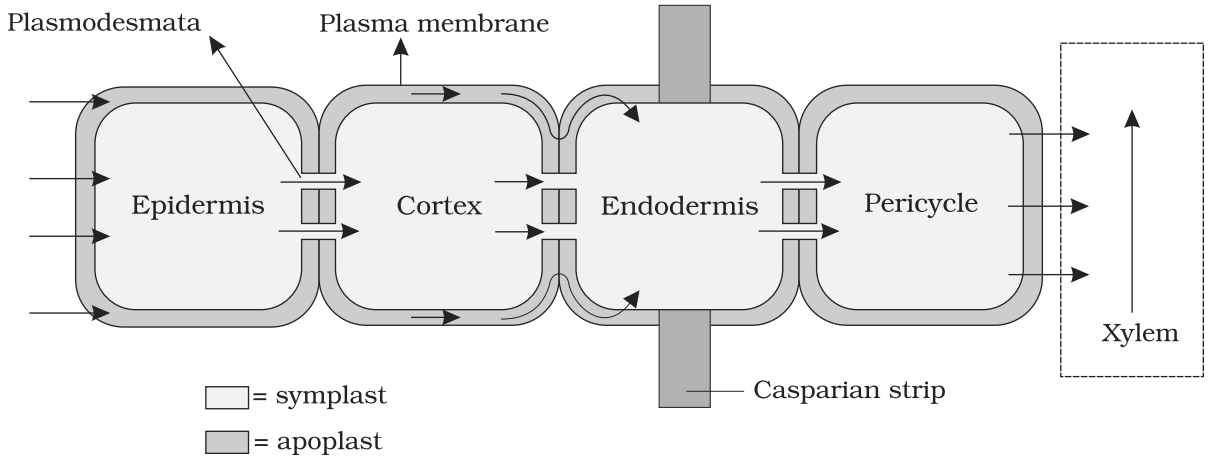


Figure 1.6 Pathway of water movement in the root

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.

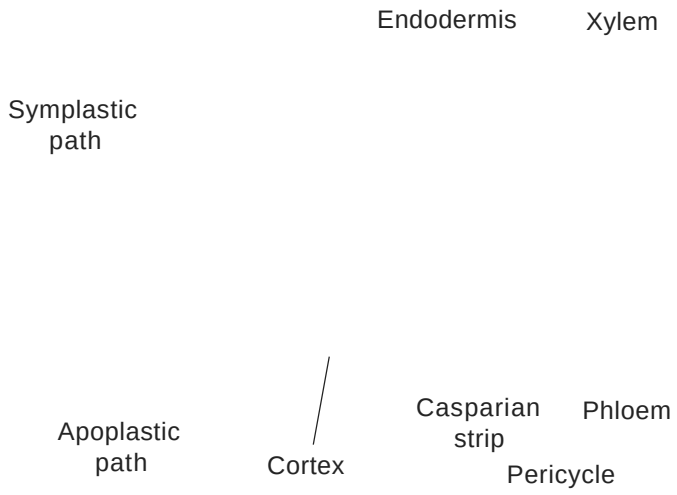


Figure 1.7 Symplastic and apoplastic pathways of water and ion absorption and movement in roots

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 T ranspiration 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).

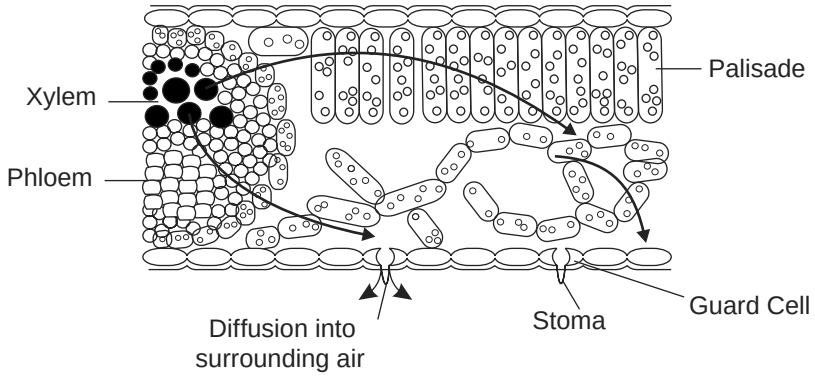


Figure1.8 Water movement in the leaf. Evaporation from the leaf sets up a pressure gradient between the outside air and the air spaces of the leaf. The gradient is transmitted into the photosynthetic cells and on the water-filled xylem in the leaf vein.

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.

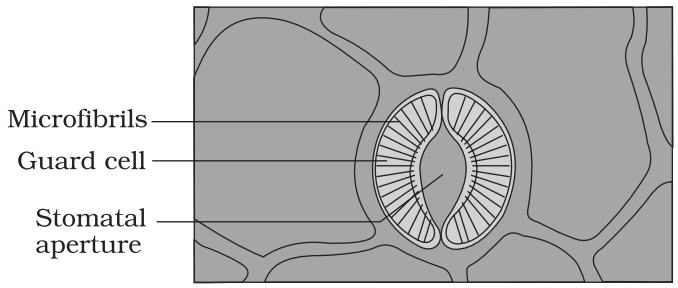


Figure1.9 A stomatal aperture with guard cells

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

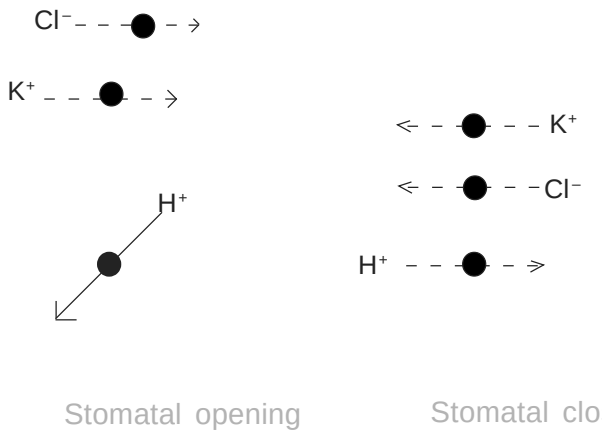


Figure1.10 Stomatal opening and closing : Broken arrows indicate passive movement and continous arrow indicates active movement.

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.

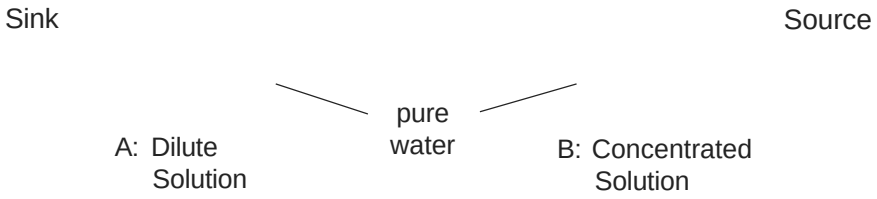


Figure 1.11 Illustration of Mass flow by Munch

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.

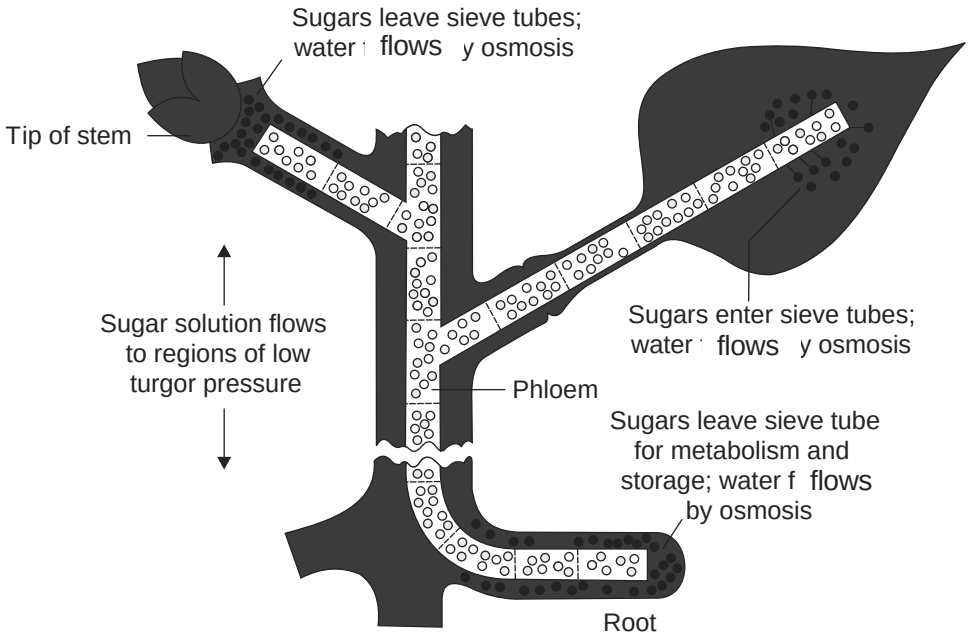


Figure1.12 Diagrammatic presentation of mechanism of translocation

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

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?

C

Chapter 2

Mineral Nutrition

2.1 Methods to Study the Mineral Requirements of Plants
2.2 Essential Mineral Elements
2.3 Mechanism of Absorption of Elements
2.4 Translocation of Solutes
2.5 Soil as Reservoir of Essential Elements
2.6 Metabolism of Nitrogen

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

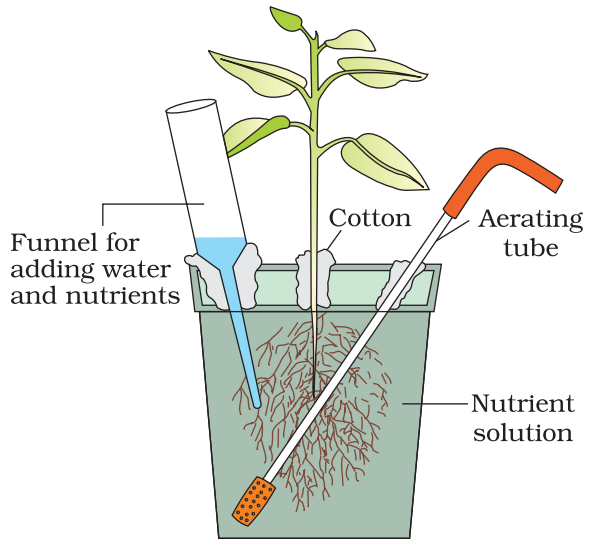


Figure 2.1 Diagram of a typical set-up for nutrient solution culture

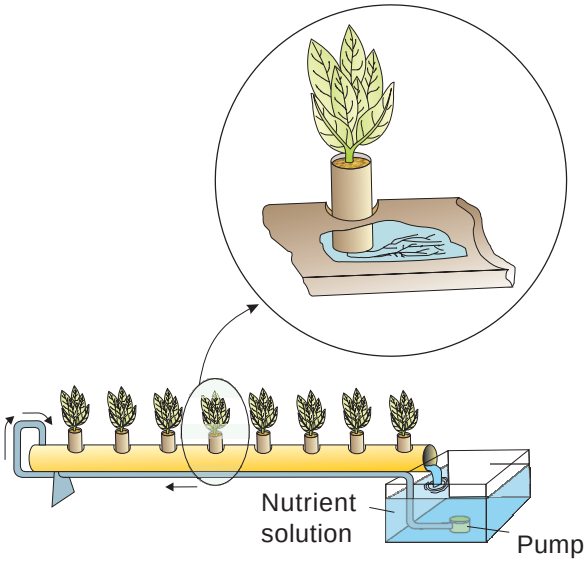


Figure 2.2 Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots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

$2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O$ .... (i)

$2NO_2^- + O_2 \rightarrow 2NO_3^-$ ..... (ii)

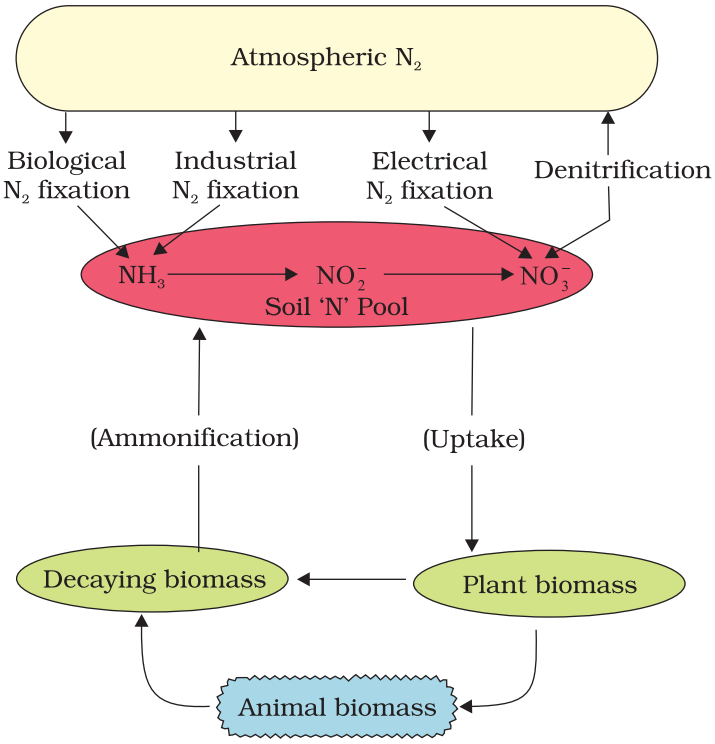


Figure 2.3 The nitrogen cycle showing the relationship between the three main nitrogen pools – atmosphere, soil, and biomass

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,

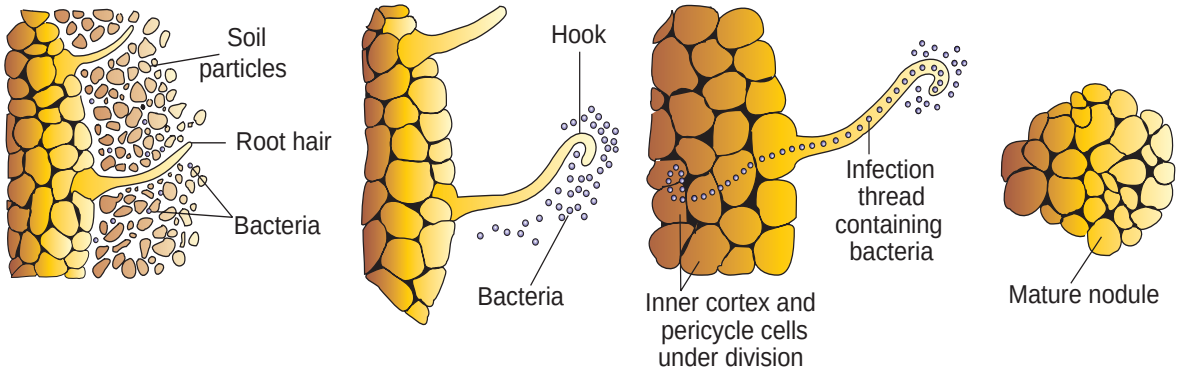


Figure 2.4 Development of root nodules in soyabean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it. (b) Successful infection of the root hair causes it to curl. (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids 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:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \xrightarrow{\quad} 2NH_3 + H_2 + 16ADP + 16Pi$$

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

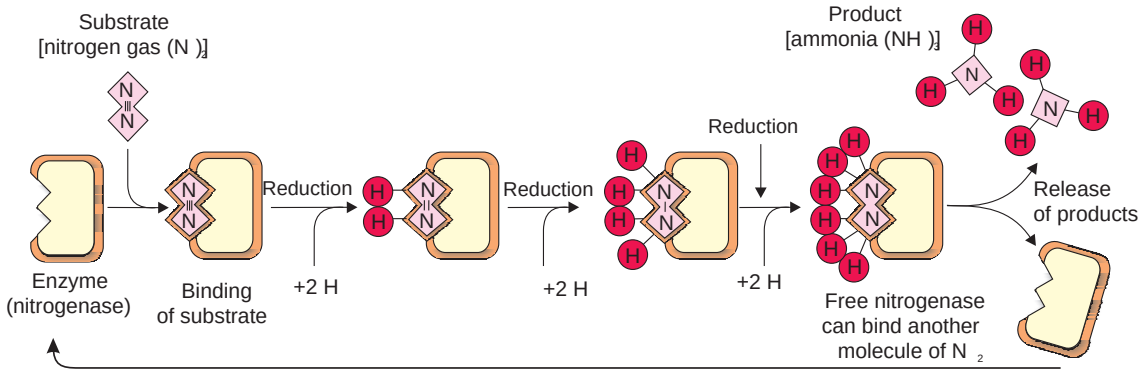


Figure 2.5 Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

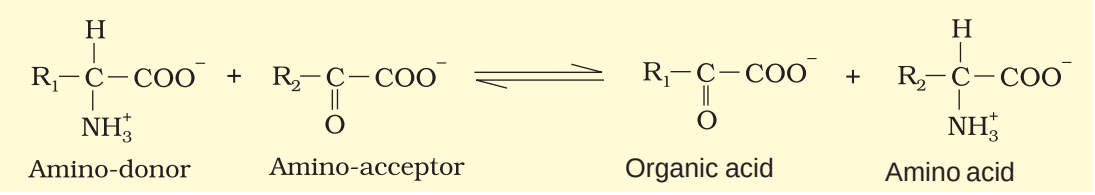
these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they adapt to anaerobic conditions, thus protecting the nitrogenase enzyme. You must have noticed in the above reaction that the ammonia synthesis by nitrogenease requires 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 :

$$\alpha\text{-ketoglutaric acid} + NH_3 + NADPH \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{glutamate} + H_2O + NADP^+$$

(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

3.1 Chemical reactions
3.2 Enzymatic conversions
3.3 Nature of enzyme action
3.4 Factors affecting enzyme activity
3.5 Classification and nomenclature of Enzymes
3.6 Cofactors

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:

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

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:

$$\text{rate} = \frac{\text{Change in product}}{\text{Time taken}}$$

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,

Glucose 2 Pyruvic acid

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

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 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

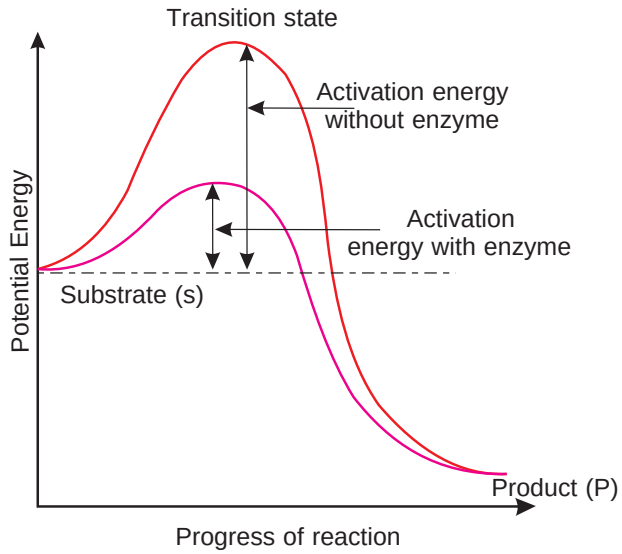


Figure 3.1 Concept of activation energy

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.

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

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

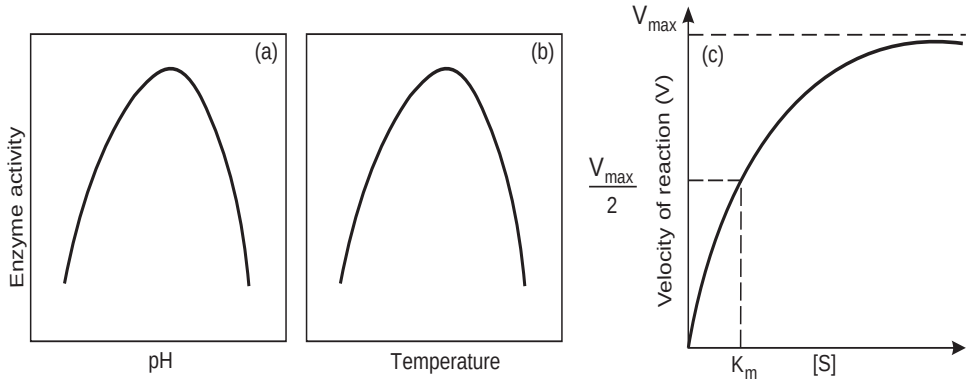


Figure 3.2 Effect of change in: (a) pH (b) Temperature and (c) Concentration of substrate on enzyme activity

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 reduced + S' oxidised S oxidised + S' 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.,

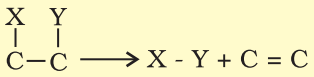
S - G + S' S + S' - G

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?

C

Chapter 4

Photosynthesis in Higher Plants

4.1 What do we Know?
4.2 Early Experiments
4.3 What is the site of Photosynthesis ?
4.4 How many Pigments are involved in Photosynthesis?
4.5 What is Light Reaction?
4.6 The Electron Transport
4.7 Where are the ATP and NADPH Used?
4.8 The C_4 Pathway
4.9 Photorespiration
4.10 Factors affecting Photosynthesis

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?*

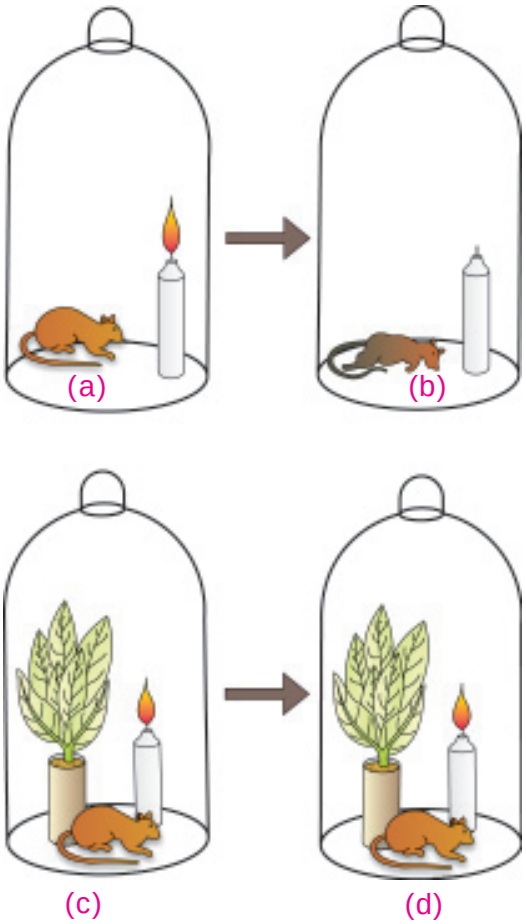


Figure 4.1 Priestley's experiment

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:

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

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 $C_6H_{12}O_6$ 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,

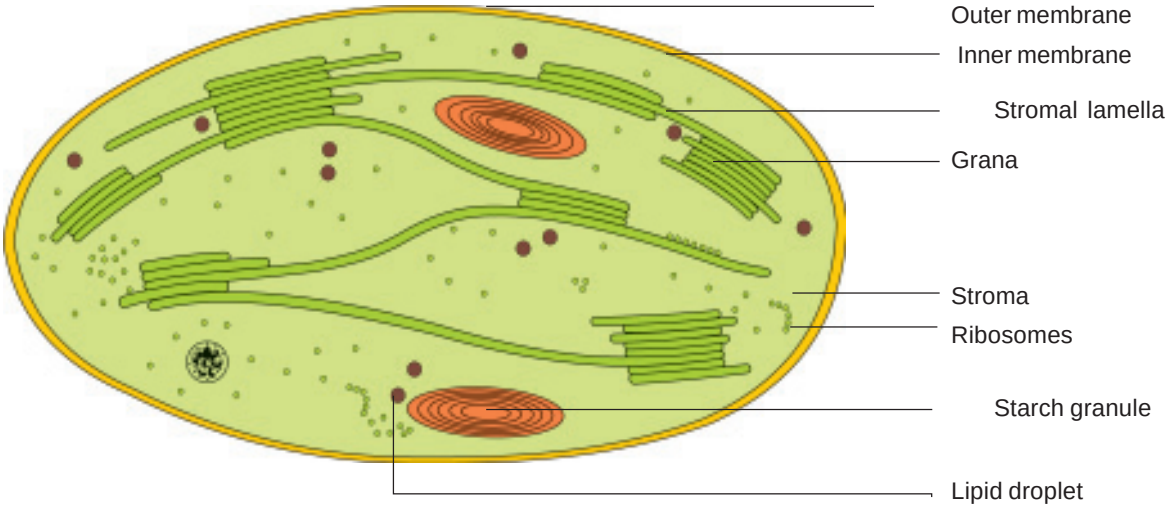


Figure 4.2 Diagrammatic representation of an electron micrograph of a section of chloroplast

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.

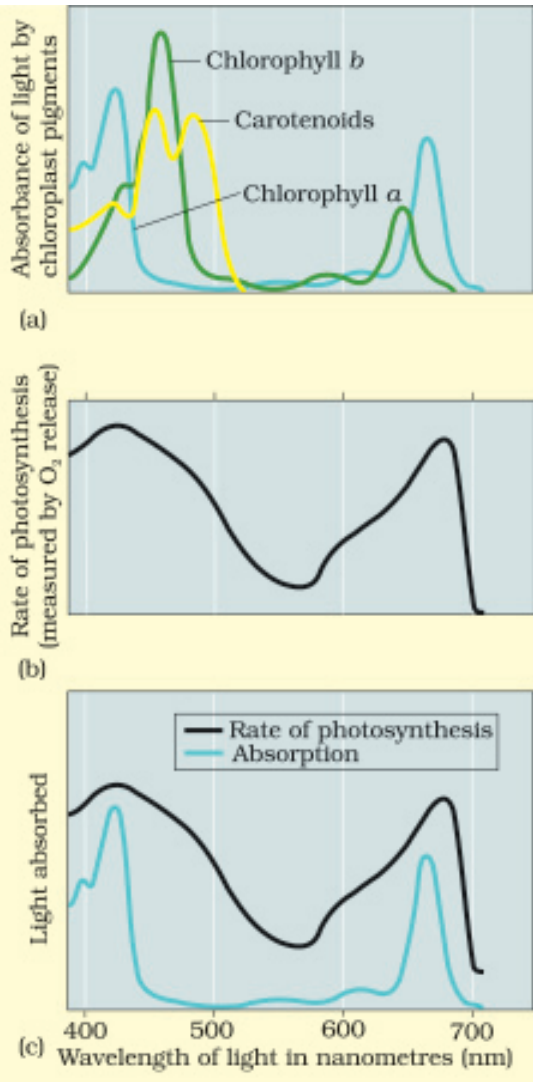


Figure 4.3a Graph showing the absorption spectrum of chlorophyll *a, b* and the carotenoids

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.

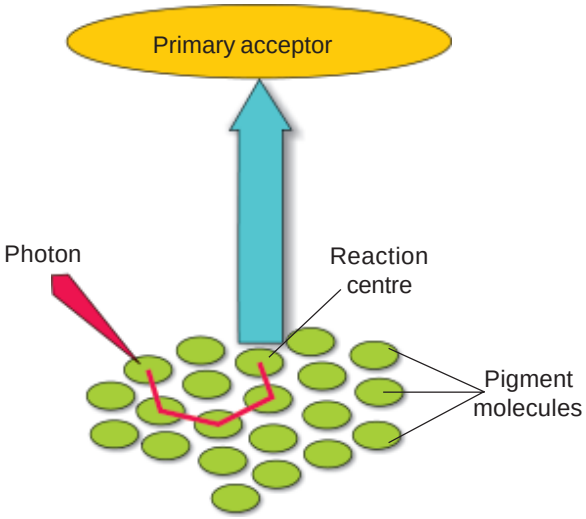


Figure 4.4 The light harvesting complex

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

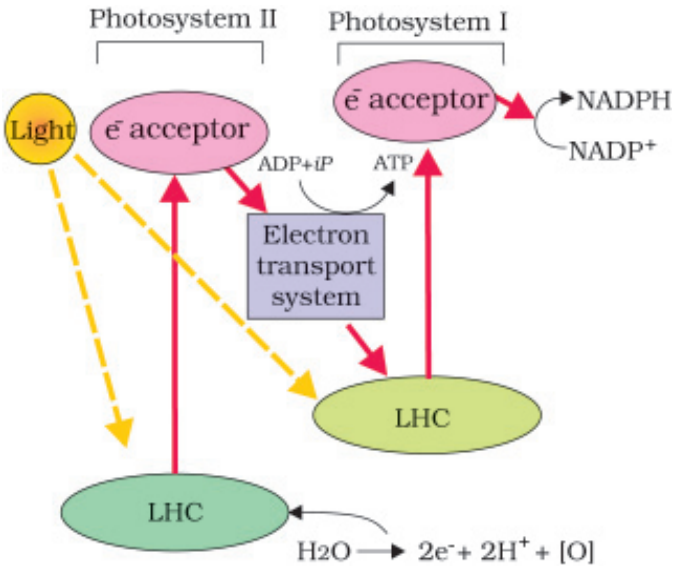


Figure 4.5 Z scheme of light reaction

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 W ater

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.

$$2H_2O \rightarrow 4H^{+} + O_2 + 4e^{-}$$

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).

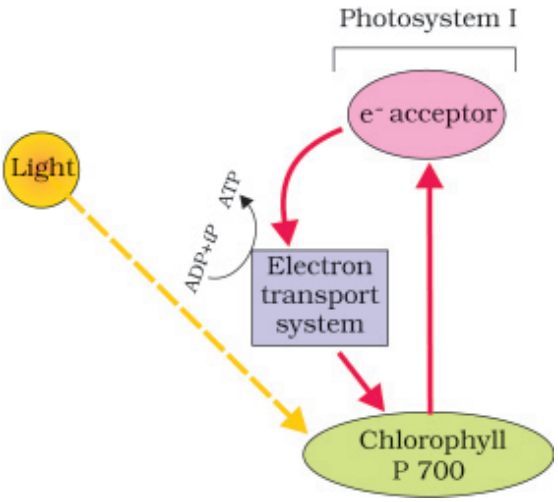


Figure 4.6 Cyclic photophosphorylation

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

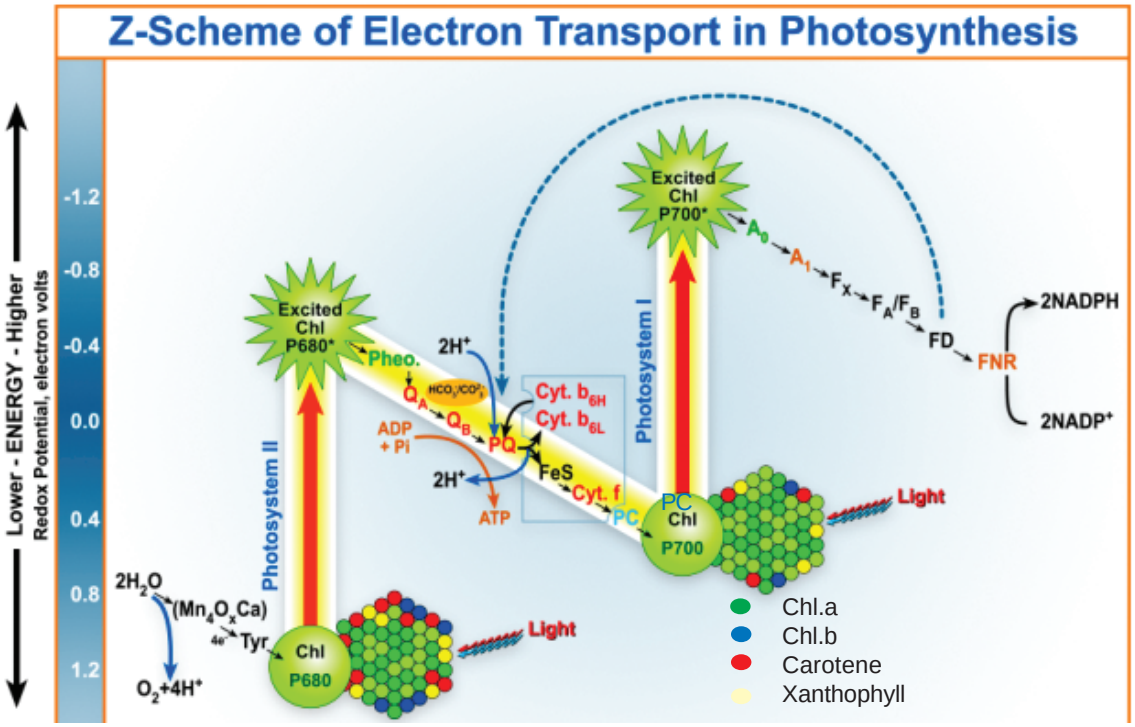


Figure 4.6a Modern scheme of photosynthetic electron transport

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.

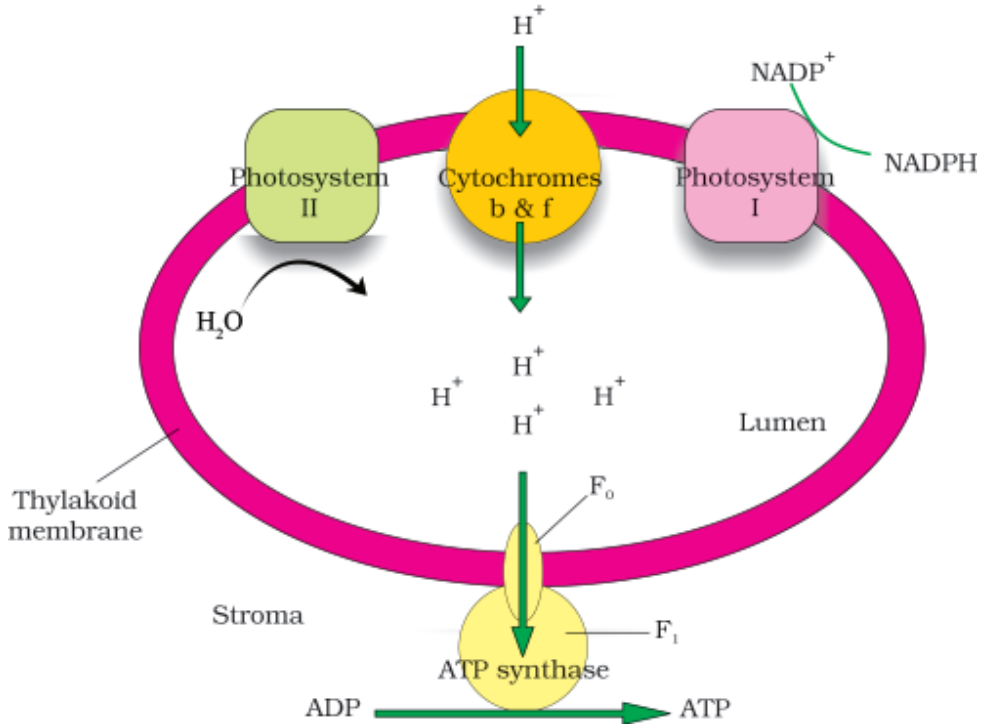


Figure 4.7 ATP synthesis through chemiosmosis

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)?*

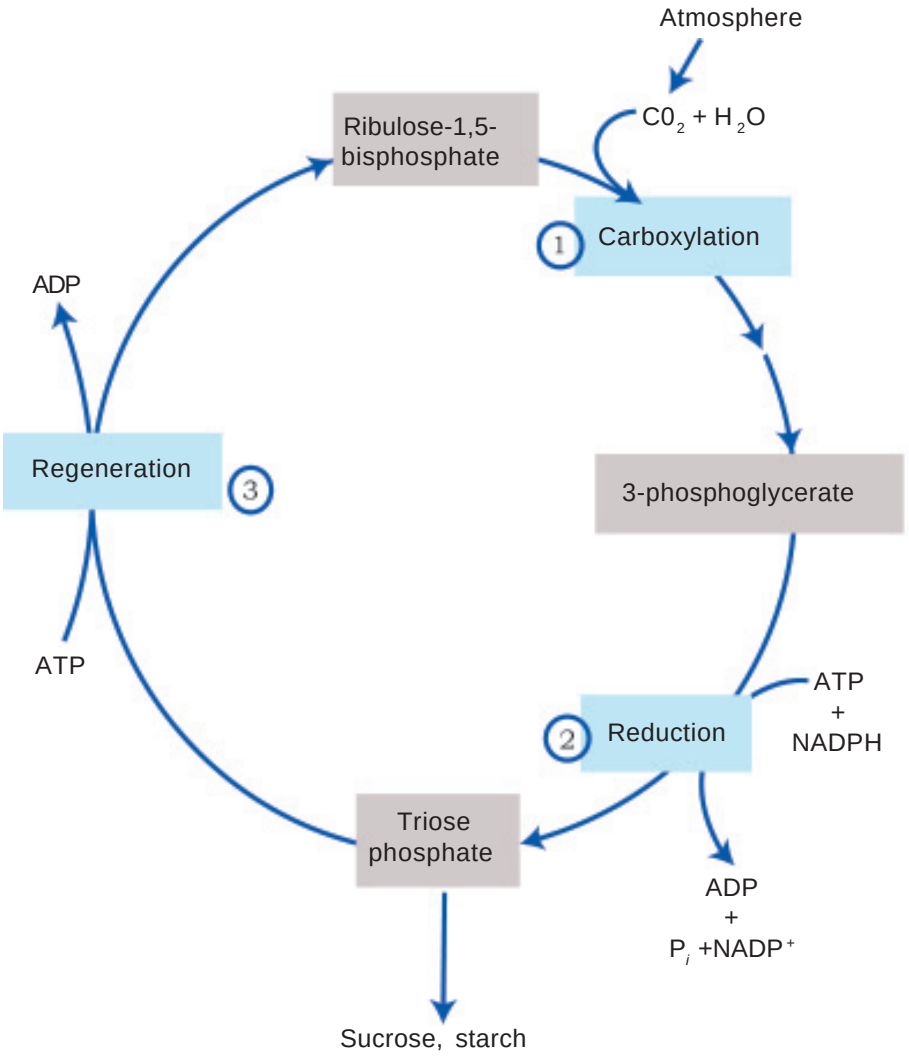


Figure 4.8 The Calvin cycle proceeds in three stages : (1) carboxylation, during which CO_2 combines with ribulose-1,5-bisphosphate; (2) reduction, during which carbohydrate is formed at the expense of the photochemically made ATP and NADPH; and (3) regeneration during which the CO_2 acceptor ribulose-1,5-bisphosphate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

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

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

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

(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 18 ATP 12 NADPH	Two trioses (One glucose) 18 ADP 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.

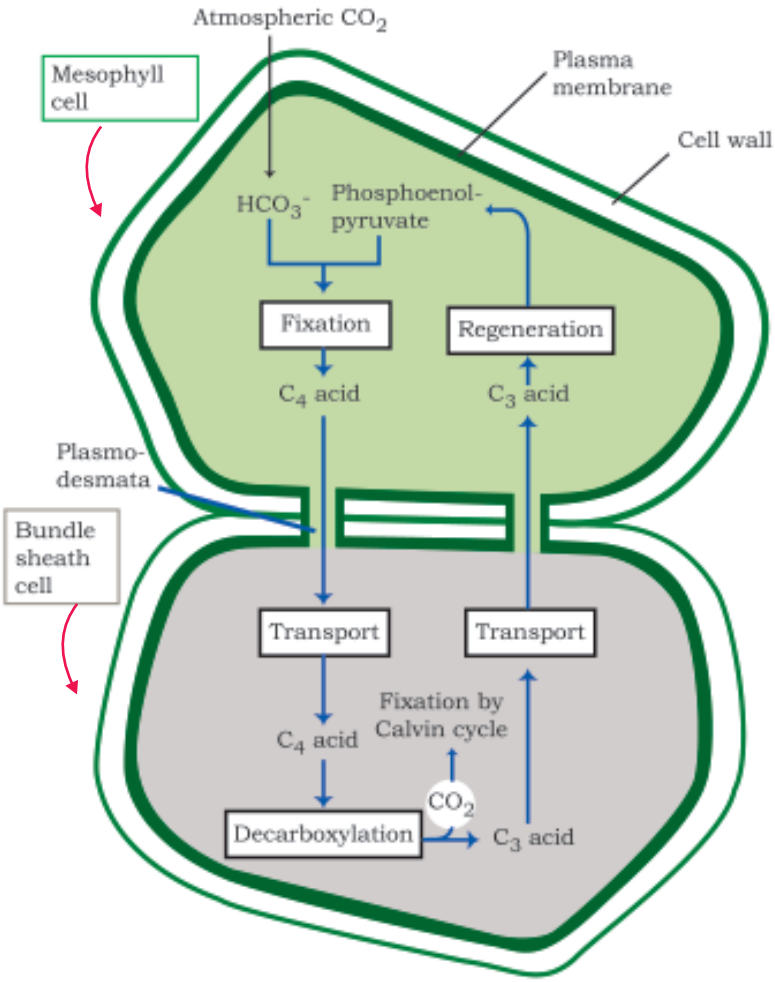


Figure 4.9 Diagrammatic representation of the Hatch and Slack Pathway

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.

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

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.

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

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

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 μL^{-1} while C_3 plants respond to increased CO_2 concentration and saturation is seen only beyond 450 μL^{-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

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 plants superior to C_3? 4
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 plants? 4

Exercises

1. By looking at a plant externally, can you tell whether a plant is C or C_4? Why and how? 3
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?