

Biyani's Think Tank

Concept based notes

Plant Physiology & Bio Chemistry

(B.Sc. Part-II)

Dr. Anupama Goyal

Deptt. of Science
Biyani Girls College, Jaipur



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Ph : 011-2871, 28591-95 • Fax : 011-28007

E-mail : acad@biyanicolleges.org

Website : www.gurukpo.com; www.biyanicolleges.org

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the “Teach Yourself” style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

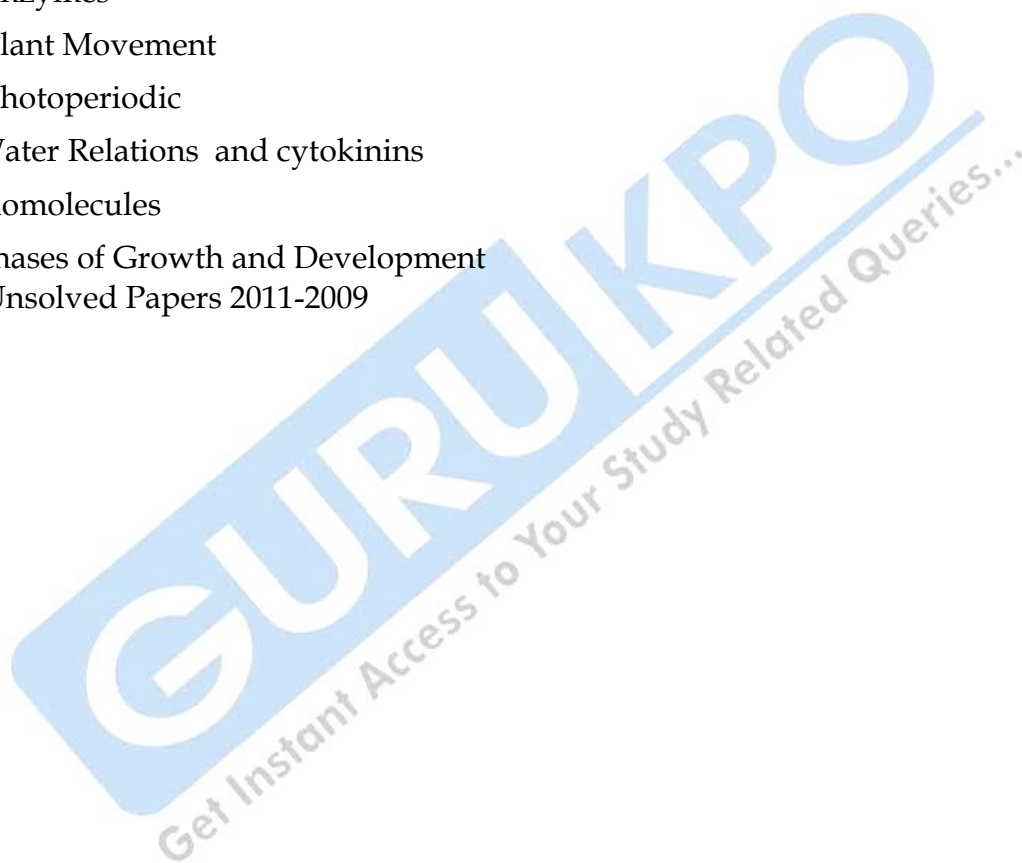
I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director (Acad.)* Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Author

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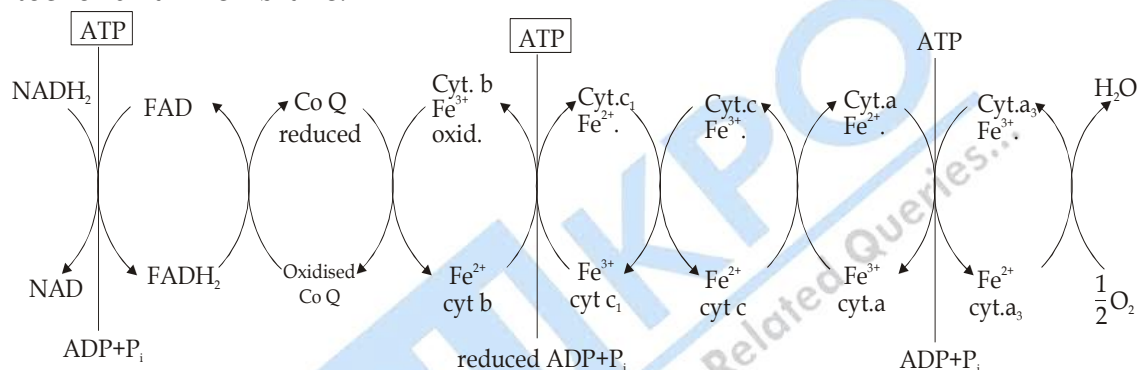


Chapter 1

Respiration & Photosynthesis

Q.1. Discuss the electron transport system in Mitochondria.

Ans. In eukaryotic cells, the electron transport chain is located within the inner mitochondrial membrane.



Electron transport system : During oxidative phosphorylation, electrons from the reduced coenzymes NADH and FADH_2 are transferred through a series of membrane-bound carriers that form an electron transport chain. The transfer of electrons during the electron transport chain involves a series of oxidation-reduction reactions of the membrane bound carrier molecules and the eventual reduction of a terminal electron acceptor.

Flavoproteins (containing FMN) and iron-sulfur proteins (non-heme proteins) transfer hydrogen from NADH to coenzyme Q (Quinone). Electrons from coenzyme Q reduce a series of cytochromes, usually beginning with cytochrome C or cytochrome b. Cytochromes contain a central iron ion, which can be cycled between the oxidized ferric state (Fe^{+3}) and the reduced ferrous (Fe^{2+}) state. Ultimately electrons are passed to a cytochrome a/cytochrome a_3 complex and then to O_2 in aerobic respiration.

The electrons from CoQ move to cytochromes. The cytochromes are Fe containing proteins. The Fe ion undergoes valency changes (ferrous - ferric) during oxidation-reduction of cytochromes. The electrons from CoQ are accepted by cytochrome b, cytochrome b then donates electrons to cytochrome C_1 . The free energy change during this step of electron transfer (cytochrome b to c_1) is large enough to generate a molecule of ATP. This step is inhibited by the antibiotic

antimycin A. The electrons from cytochrome C_1 then move to cytochrome C and then to cytochrome $a_1 + a_3$ (a_1 and a_3 are two types of cytochromes a). There is a production of another molecule of ATP during electron transfer from cytochrome C to cytochrome $a_1 + a_3$. The cytochrome a complex ($a_1 + a_3$) is known as terminal oxidase or sometimes as cytochrome oxidase. This complex itself catalysed the transfer of electrons ultimately to O_2 which is reduced to form H_2O . Thus, when both the electrons from NADH are transported to O_2 , 3ATP molecules are generated.

Q.2. Describe briefly glycolysis.

Ans. Glycolysis is the sequence of ten enzyme catalyzed reactions that converts glucose into pyruvate with the simultaneous production of ATP. Glycolysis takes place in the extramitochondrial part of the cell or the soluble cytoplasm.

Reaction Steps of Glycolysis:

(1) Phosphorylation of Glucose: Glucose is activated by phosphorylation to yield glucose-6-phosphate using ATP as phosphate donor. This reaction is catalyzed by an inducible enzyme hexokinase.

(2) Isomerization of glucose - 6- phosphate: Glucose -6-P is reversibly isomerized to fructose-6- phosphate by phosphoglucose isomerase.

(3) Phosphorylation of Fructose 6- phosphate: This is the 2nd activation/primary reaction of glycolysis. F-6-P is phosphorylated by ATP to produce fructose-1,6 diphosphate by another inducible allosteric enzyme phospho-fructokinase (PFK).

Step 4: Cleavage of Fructose 1, 6 diphosphate:

Fructose-1,6 diphosphate cleaves into, two 3C triose phosphate – glyceraldehyde-3-phosphate and dihydroxy acetone phosphate, catalyzed via fructose diphosphate aldolase.

Step 5: Isomerization of Dihydroxyacetone phosphate:-

DHAP is reversibly converted into glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase.

Step 6: Oxidative phosphorylation of Glyceraldehyde 3-phosphate:

This is the first of the two energy conserving reactions of glycolysis in which a high energy phosphate compound 1,3 diphosphoglycerate is formed by the enzyme glyceraldehyde-3-P dehydrogenase, which is NAD^+ dependent.

Step 7: Transfer of phosphate from 1,3 DPG to ADP:

It involves the transfer of high energy phosphate group from carboxylic group of 1,3-DPG to ADP by the enzyme phosphoglycerate kinase, thus producing ATP and leaving 3-phosphoglycerate. Since 2 moles of triose phosphate are produced per mole of glucose, 2 moles of ATP are generated at this stage per mole of glucose oxidized.

Step 8: Isomerization of 3-phosphoglycerate:-

The 3- phosphoglycerate is converted into 2- phosphoglycerate in a reversible reaction catalyzed by phosphoglycerate mutase enzyme.

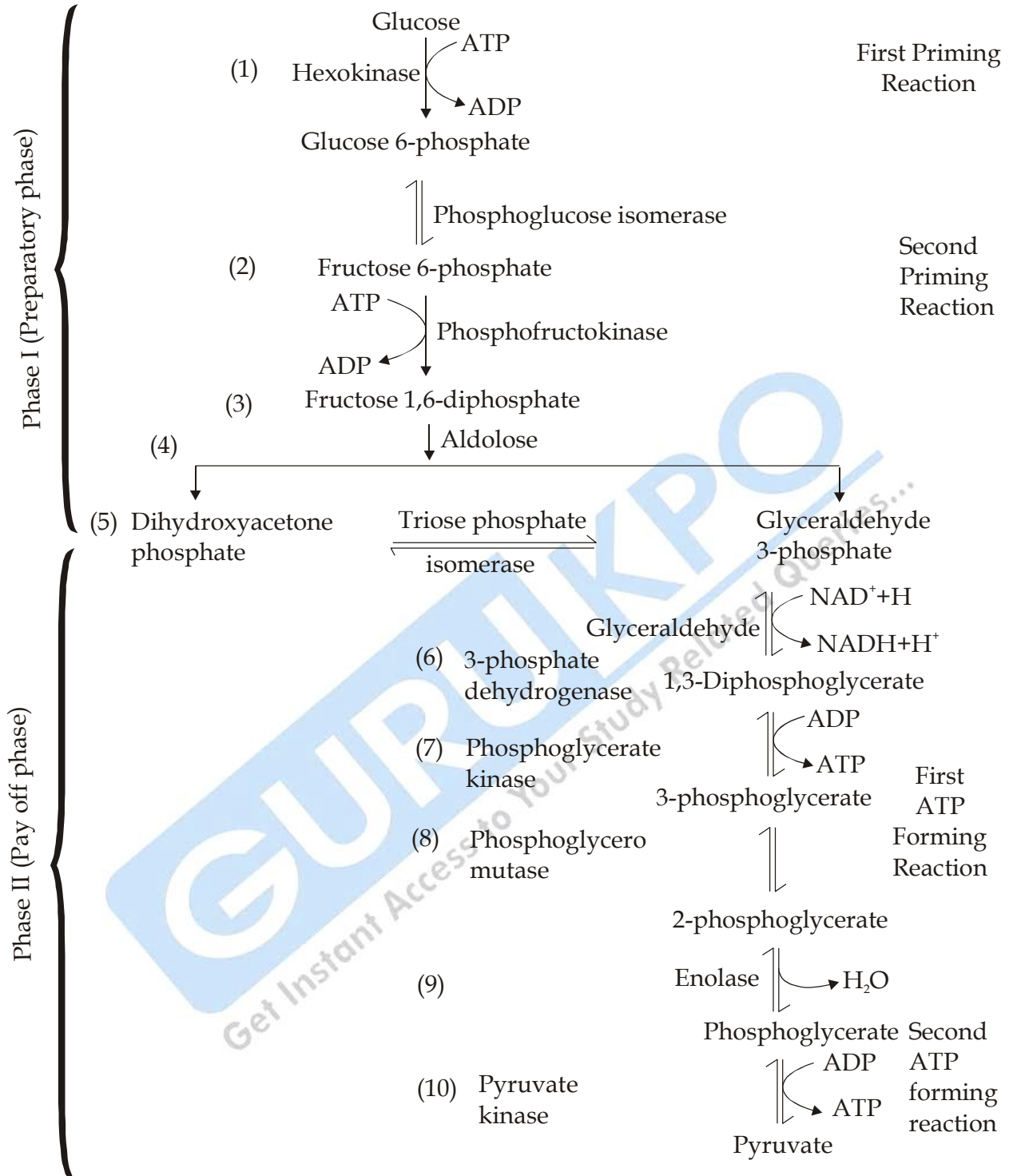
Step 9: Dehydration of 2-phosphoglycerate:-

This is the second reaction of glycolysis in which a high energy phosphate compound phosphoenol pyruvate (PEP) is formed by enolase enzyme in a reversible reaction.

Step 10: Transfer of phosphate from PEP to ADP:

This is the second ATP generating reaction in glycolysis. PEP gets converted into pyruvate by an inducible allosteric enzyme pyruvate kinase (PK). The enzyme PK catalyzes the transfer of a phosphoryl group from PEP to ADP, thus forming ATP.

The conversion of PEP into pyruvate is the second-example of substrate level phosphorylation in glycolysis.



Two phases of glycolysis

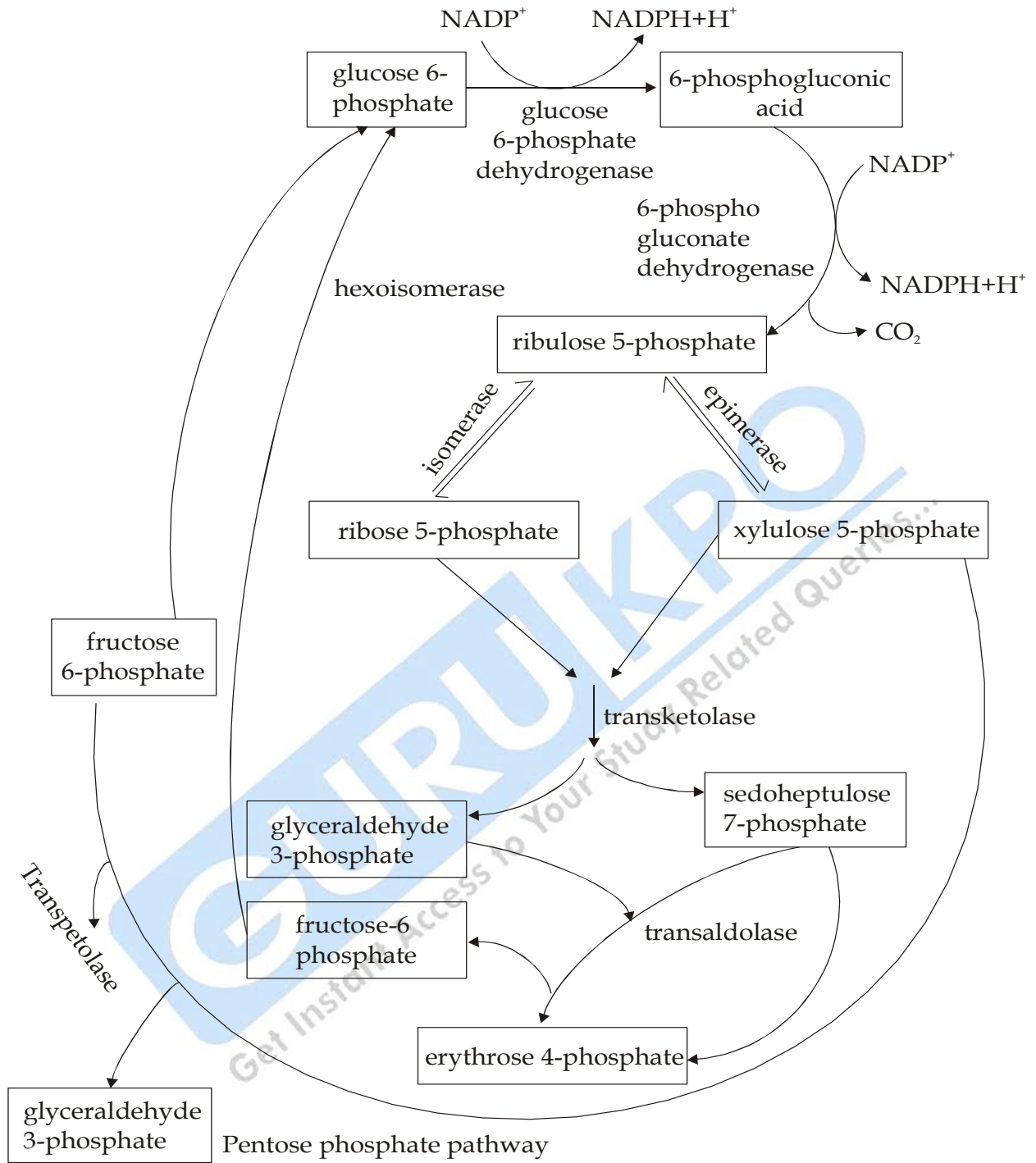
Q.3. Describe Pentose phosphate pathway.

Ans.

In this pathway, hexose sugars underwent catabolism through 5-carbon sugar intermediates and hence the pathway was called Pentose Phosphate Pathway (PPP). It is also called Warburg-Dicken Pathway, the Hexose Monophosphate shunt and the Phosphogluconate Pathway.

Steps:

- (1) With the use of one ATP molecule, glucose is phosphorylated in the 6th carbon via hexokinase converting into glucose-6-phosphate.
- (2) In the presence of enzyme Glucose 6-phosphate dehydrogenase glucose 6-P is converted into 6-phosphogluconic acid. NADP gets reduced to NADPH₂.
- (3) 6- Phosphogluconic acid is oxidatively decarboxylated to ribulose 5-phosphate via 6-phosphogluconate dehydrogenase. The oxidizing agent is NADP which itself gets reduced to NADPH₂.
- (4) Some of the ribulose 5-P is isomerised to ribose 5-phosphate in the presence of enzyme phosphoriboisomerase.
- (5) Some of the ribulose 5-phosphate is catalyzed by the enzyme epimerase and produces xylulose 5-phosphate.
- (6) Under the influence of enzyme transketolase in presence of TPP, two pentose sugars xylulose 5-phosphate and ribose 5-phosphate react to produce a heptose sugar sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate.
- (7) In the presence of enzyme transaldolase and TPP, sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate reduce into 4-C sugar erythrose 4-phosphate and a 6-C sugar fructose 6-phosphate.
- (8) Under the influence of the enzyme transketolase, xylulose 5-phosphate and erythrose 4-phosphate combine to produce another molecule of hexose sugar fructose 6-phosphate and glyceraldehyde 3-phosphate. The 3-phosphoglyceraldehyde also gets converted into Pyruvic acid.
- (9) Fructose 6-phosphate is then converted to glucose 6-phosphate by the enzyme phosphohexoisomerase. Glucose 6-phosphate can go to PPP again and the cycle is completed.



TCA Cycle

Q.4. Describe Tricarboxylic Acid Cycle.

Ans. The most nearly universal pathway for aerobic metabolism is the cyclic series of reactions, termed citric acid cycle (CAC) or krebs cycle. It is also called as Tricarboxylic acid cycle.

Steps of the Citric Acid Cycle:

Step 1: Condensation of acetyl CoA with Oxaloacetate: The cycle starts with the joining of a 4-carbon unit, oxaloacetate (OAA) and a 2-carbon acetyl CoA to produce a tricarboxylic acid, citric acid.

Step 2: Citric acid is then isomerised to isocitric acid by the enzyme aconitase. In this isomerization, an intermediate compound cis-aconitic acid is formed which remains tightly bound to the enzyme. Cis-aconitic acid is then hydrolysed to isocitric acid.

Step 3: Isocitric acid is decarboxylated to yield 2-oxoglutaric acid in a two step reaction. The intermediate compound is oxalo succinic acid. The reaction generates NADH also and the enzyme for the reaction is isocitrate dehydrogenase.

Step 4: 2-Oxoglutaric acid is decarboxylated by an enzyme complex, 2-oxoglutarate dehydrogenase complex and produces succinyl CoA, CO_2 and NADH. Cofactors required for this reaction are coenzyme A, TPP and NAD^+ .

At this stage of TCA cycle two molecules of CO_2 have been evolved which are equivalent to a two carbon molecule e.g. acetyl CoA.

Step 5: Succinyl CoA, splits into succinic acid and coenzyme A by the catalytic activity of succinyl CoA synthetase or succinate thio kinase. The reaction also generates ATP from ADP and Pi. The enzyme can also phosphorylate GDP and IDP.

Step 6: Succinic acid is oxidized to fumaric acid by the enzyme succinate dehydrogenase. The enzyme reduces FAD to FADH_2 .

Step 7: The enzyme fumarase catalyzes the hydration of fumaric acid, which is converted into malic acid.

Step 8: Malic acid is oxidised to oxaloacetic acid by the enzyme Malate dehydrogenase. NAD^+ is reduced to NADH.

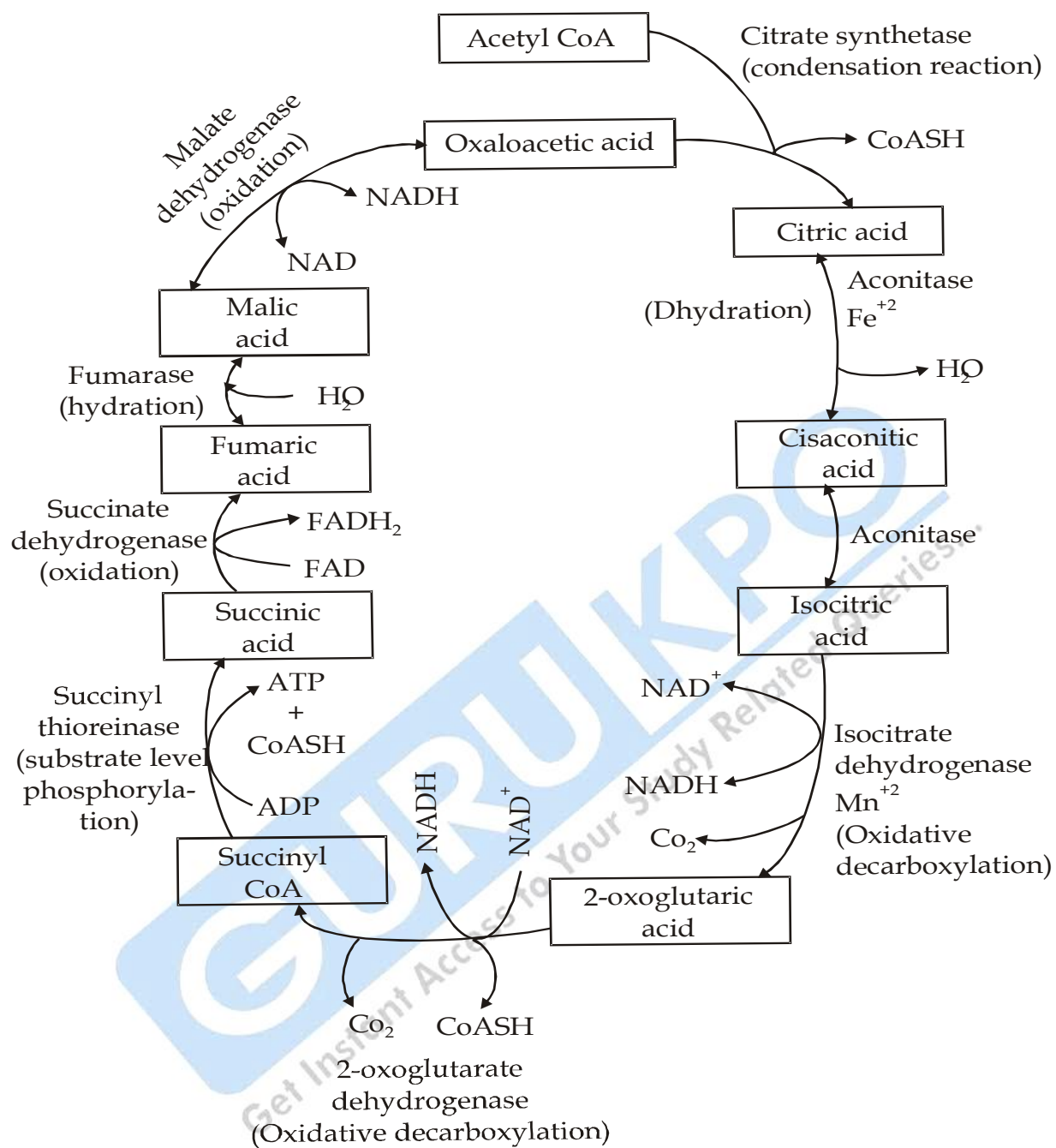
Production of oxaloacetic acid completes the cycle, as it can condense again with the acetyl CoA to produce citric acid and so on.

3 molecules of NADH, one molecule of FADH_2 , one molecule of ATP and two molecules of CO_2 are produced.

Energy yield of the Citric Acid Cycle

Step	Reaction No.	Method of ATP formation	ATP yield per mole
1	Isocitrate $\rightarrow$ α -Ketoglutarate + CO ₂	Respiratory chain oxidation of NADH	3
2	α -Ketoglutarate $\rightarrow$ succinyl - CoA + CO ₂	Respiratory chain oxidation of NADH	3
3	Succinyl- CoA + ADP + Pi $\rightarrow$ Succinate + ATP	Oxidation at substrate level	1
4	Succinate - fumarate	Respiratory chain oxidation of FADH ₂	2
5	Malate $\rightarrow$ Oxaloacetate	Respiratory chain oxidation of NADH	3

Total gain of ATP = 12

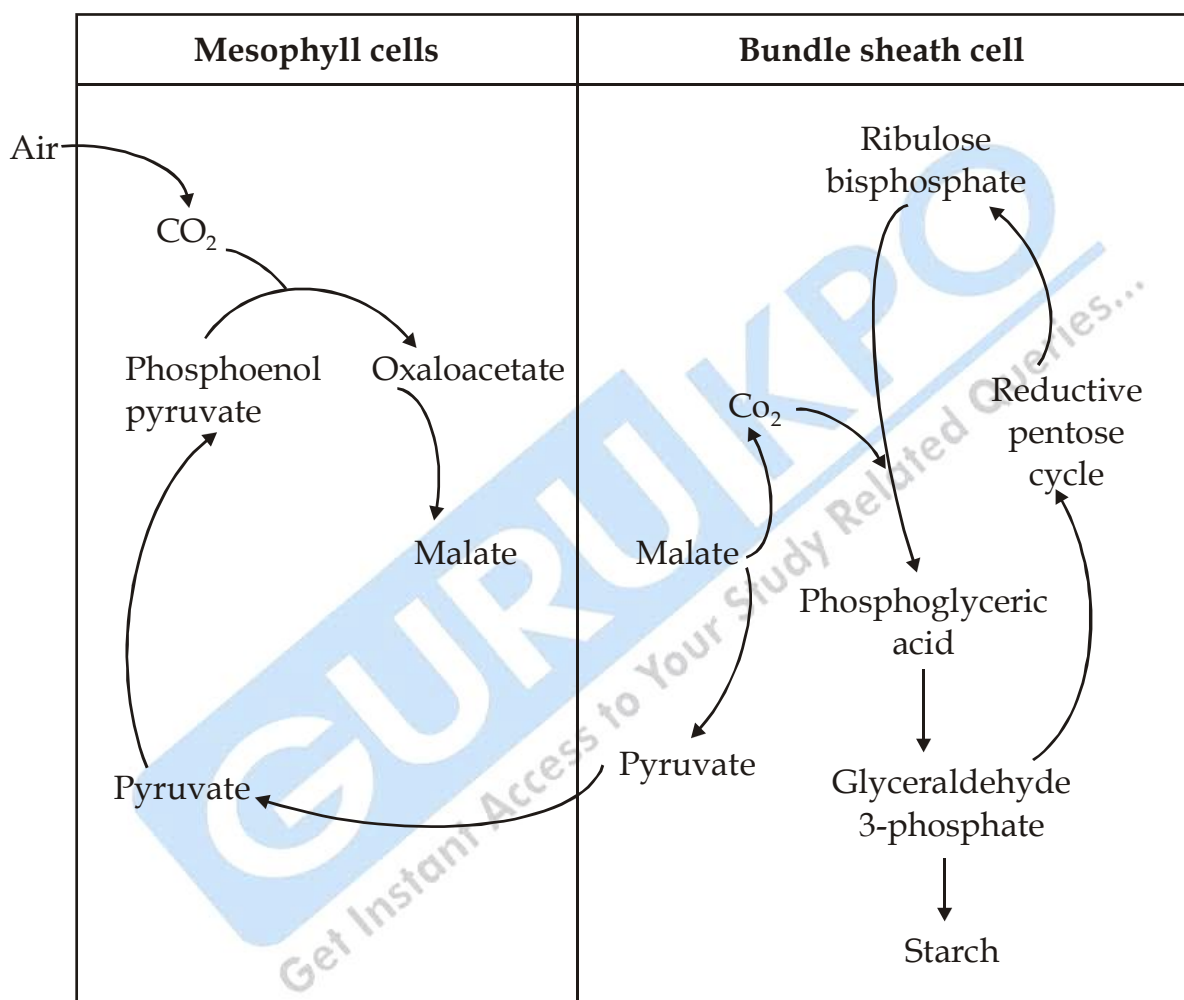


TCA Cycle / Citric Acid Cycle

Q.5. Give a detailed account of Hatch and Slack Cycle?

Ans. This cycle mainly occurs in the members of family cyperaceae (e.g. *Cyperus rotundus*), Gramineae (*Zea mays*, *Sorghum*, *Saccharum officinarum*) and some dicotyledons like *Amaranthus* and *Atriplex* etc.

This cycle is also known as α -carboxylation pathway or co-operative photosynthesis.



C₄ pathway or

Hatch and Slack Pathway (Cycle)

Details:

(1) Formation of Oxalo-acetic acid: The primary acceptor of CO_2 in C_4 cycle is a 3 carbon compound phosphoenol pyruvic acid (PEP). In mesophyll cells, the atmospheric CO_2 first combines with water to form bicarbonate ion in the presence of enzyme carbonic anhydrase.

The CO_2 acceptor PEP, combines with HCO_3^- and forms 4C acid oxaloacetic acid in the presence of enzyme PEP carboxyase which is present in the mesophyll cells in large amounts.

(2) Formation of Malic acid and Aspartic Acid: Oxaloacetate is quite unstable and is converted either into malic acid or aspartic acid. OAA is reduced to malic acid by using light generated NADPH. This reaction is catalysed by malic dehydrogenase.

Reactions occurring in bundle sheath:

(3) Formation of pyruvic acid: In bundle sheath, malic acid undergoes oxidative decarboxylation to yield pyruvic acid by NADP – Malic enzyme.

(4) The CO_2 and $\text{NADPH} + \text{H}^+$ enter into calvin cycle. The CO_2 combines with RUBP to yield two molecules of 3-PGA.

(5) In a few C_4 plants, aspartic acid undergoes transamination to form OAA which is then decarboxylated to pyruvic acid. This reaction is catalysed by aspartate transaminase.

Reaction in Mesophyll Cells:

(6) Regeneration of PEP: The pyruvic acid produced by oxidative decarboxylation is transported back to the mesophyll cells where it is phosphorylated to PEP via pyruvate – orthophosphate dikinase.

$\text{Pyruvic acid} + \text{ATP} + \text{H}_3\text{PO}_4 \xrightarrow{\text{Pyruvate-ortho P - dikanase}} \text{PEP} + \text{AMP} + \text{PPi} + \text{H}_2\text{O}$
 $\text{H}_2\text{O} - \text{Pyrophosphatase} - 2\text{Pi}$

Net Reaction:

$\text{CO}_2 (\text{Mesophyll}) + \text{ATP} + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 (\text{bundle Sheath}) + 2 \text{ADP} + 2\text{Pi}$

Chapter 2

Plant Growth Regulators

Q.1. What are auxins? Describe the physiological roles of auxins?

Ans. Auxins are the best known plant growth regulators. They have been defined as the organic substances which promote elongation of shoots when present in low concentration of less than 0.01M . The effect is more pronounced in decapitated shoots and shoot segments as compared to intact plants.

Physiological role of auxins:

(1) Cell Division: Auxins are found to be responsible for initiating and promoting cell division in certain tissues e.g. cambium. Whenever wound is caused in the plant, a swelling (wound tissue) called callus is developed because of the proliferation of the parenchyma cells. The cells formation is linked with the cambial activity which is believed to be stimulated by auxin. In tissue cultures cell division is entirely dependent upon the presence of auxin.

(2) Shortening of internodes: Lodging or falling down due to excessive elongation of the stem decreases the crop yield. Auxin prevents lodging. It produces shorter and stiffer stems. α -naphthalene acetamide is widely used to prevent lodging.

(3) Rooting: Auxins accelerate rooting in species which are grown by cuttings. They hasten rooting as well as increase the number of roots formed. Auxins are used singly or in combinations of two or more. They may be provided in solution form or as powder in talc. Elongation of roots also increases with the application of exogenous auxins. In most cases it inhibits root growth in intact plants.

(4) Apical dominance: In most seedlings the apical tip of the stem, inhibits the development of lateral buds within certain areas of the apex. If the apical bud is removed, the lateral buds develop. The inhibition of lateral bud formation by the presence of apex is called apical dominance. This inhibition is attributed to the presence of auxins in the apex.

(5) Respiration: Auxins stimulate respiration. It is connected with the enhancement of metabolic activity on the application of auxins.

(6) Prevention of Abscission layer: The formation of abscission layer results in the premature fall of leaves, flowers and fruits. A separation layer is formed at the base of the petiole, pedicel or peduncle, Abscission is due to the dissolution of the

middle lamella alone or of the middle lamella and the primary walls or the whole cell. Addicott and Lynch found that auxins gradient across the abscission zone controlled the abscission. The premature drop of fruits such as apple, pear and citrus can be prevented to a great extent by spraying the tree with a dilute solution of 2,4-D, IAA and NAA or some other auxin.

(7) Storage: NAA and related auxins are employed to increase the period of dormancy in tubers, bulbs and corms so that they can be stored for prolonged periods.

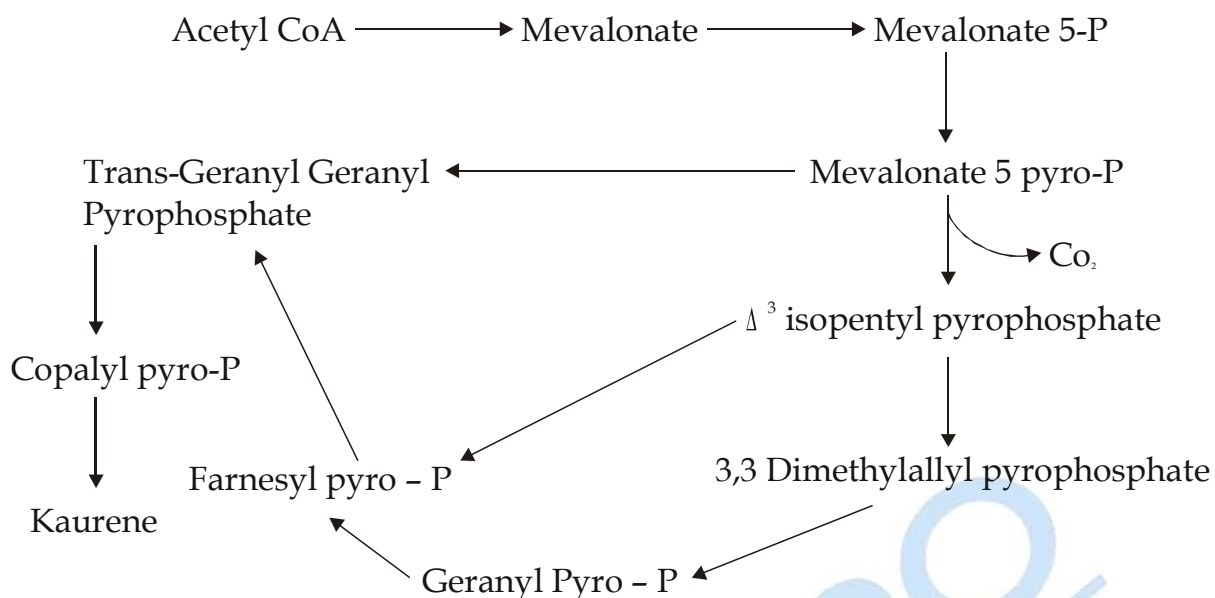
(8) Flower and fruit Thinning: Certain tree like mango form a large number of fruits in one year but give forth a subnormal crop the next year. Using auxins, such trees are thinned of their flower and fruit population in the year of abundance, so that normal crop can be had every year.

(9) Production of parthenocarpic fruits: Parthenocarpy means the production of seedless fruits. Natural parthenocarpy is present in bananas, grapes, and some oranges. Artificial application of auxins like IAA, IBA and NAA to unpollinated pistils can form seedless fruits which resemble the natural ones in all respects except for the presence of seeds e.g. Citrus, guava, figs, tomato, brinjal, etc.

(10) Eradication of Weeds: In higher proportions, auxins interfere with the normal metabolic pathways of plants and cause their death. Certain substances like 2,4-dichlorophenoxyacetic acid (2,4-D) have different toxicity towards different plants. At high concentrations the 2,4-D is very much toxic to the dicotyledons or the broad leaved plants while it is relatively non-toxic to the monocotyledonous or narrow leaved plants. The weeds are normally the unwanted dicots which grow with the cereals or grasses which are monocots. Thus in a fixed population of such plants, 2,4-D acts as a very useful selective herbicide or weed killer.

Q.2. Write about the Gibberellin biosynthesis and physiological effects of Gibberellins?

Ans. Gibberellins Biosynthesis



Physiological effects of Gibberellins

(1) Promotion of plant growth: Gibberellins stimulate extensive growth in intact plants. They enhance elongation of intact stems much more than that of excised stem segments. Gibberellins are able to overcome genetic dwarfness in some species such as maize, bean and pea. Dwarfness in such plants is due to a gene mutation, resulting into blocked gibberellin synthesis. Lack of gibberellins causes shortening of internodes and reduced height of the plant.

They also induce stem elongation in those plants which show rosette habit in the first season e.g. cabbage, henbane, sugar beet. The phenomenon is known as bolting.

(2) Breaking of Dormancy: Gibberellins are known to overcome dormancy in buds of many evergreen and deciduous trees and shrubs. The dormancy of apical buds in peach is reversed by the application of gibberellin. Gibberellins also promote seed germination in several species, which otherwise fail to germinate unless subjected to low temperature, long days or red light. Gibberellins act as a substitute for these treatments.

(3) Flowering: Gibberellin can replace cold treatment and long day photoperiodic treatment in plants such as *Hyoccyamus niger*. The influence seems to be indirect through the phenomenon of bolting which is caused by activation of sub-apical meristem. Applied gibberellins do not induce flowering in short day plants except for a few species. Gibberellin changes sex expression of plants as well e.g.

in most species, it induces the formation of male flowers such as *Cucumis sativa*, *Cannabis* etc.

(4) Mobilization of Stored food in seeds: During germination of seeds, the organic food and minerals stored in the form of macromolecules in endosperm and cotyledons are released and transported to growing root and shoot axis, until the seedling is established in the soil. Thereafter the seedling starts taking its nourishment from the soil and atmosphere. Gibberellins stimulate the hydrolysis of stored macromolecules and their transport to the embryonic axis. In cereals such as barley, the embryo is surrounded by a thin layer of food reservoir in endosperm. This endosperm layer is called aleurone layer. The aleurone cells provide hydrolytic enzymes that hydrolyse the starch, proteins, phytin, RNA and certain cell wall materials present in endosperm cells. Gibberellins promote production of these hydrolytic enzyme.

(5) Light effects: Seeds of some variety of tobacco and lettuce require red lights for their germination. Gibberellin can induce germination in these seeds in complete darkness.

(6) Fruit growth: Gibberellins induce parthenocarpy in several cases. Witheer et al found that gibberellin is 500 times more effective than IAA in inducing parthenocarpy in tomato.

(7) Prevention of senescence: Exogenous application of GA can prevent senescence of leaves e.g. in *Taraxanum*, *Rumex*, and *Tropaeolum*. The hormone has also been found to delay the senescence of fruits besides that of leaves. Gibberellins are sprayed on the fruit and leaves of oranges to prevent rind disorders that appear during storage.

Chapter-3

Seed Germination

Q.1. What are the factors that cause dormancy?

Ans. The factors responsible for dormancy of seeds are:

(1) Hard Seed Coat:

(a) Seed coat impermeable to water: The seed coats of several plants belonging to the families leguminosae, Malvaceae, Solanaceae, Chenopodiaceae are impermeable to water. These remain dormant till their outer covering are broken down by mechanical injury and microbial action. In some members of leguminosae, the seed possesses a small valve called the strophilar plug. A small aperture lies below it. The plug swells up when external moisture is more than internal hydration and blocks the entry of water.

(b) Seeds coats being impermeable to oxygen: Such seeds fail to germinate because their outer covering does not allow oxygen to penetrate the interior. It may be partially permeable to CO_2 . Non-availability of oxygen and increased concentration of CO_2 in the seed interior represses respiration and hence germination e.g. *Synapsis arvensis* and apple seeds.

(c) Mechanically resistant seed coats: In certain wild plants, the hard seed coat acts as a physical barrier to the expansion of the embryo. In pigweed (*Amaranthus retroflexus*) the seeds are readily permeable to water as well as oxygen but the resistant seed coat does not allow the embryo to grow.

(2) Immaturity of the embryo: In certain species of Orchidaceae and Orobanchaceae and of *Fraxinus* and *Ranunculus*, seeds are liberated at the stage when the embryo is not fully developed. Dormancy of this type is a natural consequence of the presence of an incompletely developed embryo in the seed.

(3) Seeds requiring a period of after ripening: These seeds gain power to germinate after a period of dormancy when kept under dry environment at normal temperature e.g. oat, wheat, barley. Seeds of apple, peach, tulip, pines and many other species would not germinate even when the conditions for germination are favourable. Such seeds germinate after they have completed a period of rest, which is called a period of after ripening.

(4) Seeds requiring chilling treatment: Seeds require a low temperature treatment before they can become capable of germination. The chilling

requirement is met under natural conditions in the winter e.g. peach, plum, cherry.

Some seeds show a peculiar behaviour. They germinate in autumn and produce a young root system. The epicotyl fails to grow till it has received a low temperature during winter. The phenomenon is known as epicotyl dormancy e.g. Oak.

Certain seeds exhibit root or radicle dormancy, along with epicotyl dormancy. They require chilling treatment twice once for breaking root dormancy and then for epicotyl dormancy. They are popularly known as two year seeds e.g. Trillium, Polygonatum.

(5) Light sensitive seeds: There are some seeds which require a specific light treatment before being able to germinate, e.g. Lettuce, *Viscum album*.

(6) Chemical inhibitors: Besides the above types, the seed dormancy can also be due to the presence of growth inhibitors. They may be of diverse types e.g. phenolic inhibitors, abscissic acid, alkaloids etc. The inhibitors can be found in the seed coats, endosperm or embryo, e.g. apple, peach etc. The inhibitors are destroyed in nature by the production of anti-inhibitors, growth hormones or their oxidation.

Q.2. What are the factors which regulate seed germination?

Ans. (I) External Factors:

(1) Water: Active metabolism can occur only when protoplasm contains abundant supply of water which accounts for 70-95% of its mass. Seeds have only 6-15% of water. Germination cannot occur unless and until the seed is provided with an external supply of water. Water performs a number of functions during the germination of seed.

- (a) It softens seed coats so that it becomes easier for the radicle and the epicotyl to come out of the seed.
- (b) Water makes the seed coats permeable. Increased permeability allows better gaseous exchange.
- (c) It is a carrier of oxygen. This facilitates switching over from anaerobic to aerobic respiration that is required for germination in most of the cases.
- (d) Water activates the protoplasm of the seed cells.
- (e) Insoluble food materials get solubilized in the presence of water. The diffusion of these mobilised food becomes easy.

(2) Oxygens: The majority of seeds depend on aerobic respiration for germination. As such oxygen is necessary for germination. Deeply buried seeds

do not germinate because oxygen does not penetrate the deeper layers of the soil to any appreciable quantity.

(3) Carbon dioxide: Very high concentration of CO_2 is normally toxic to germination but it promotes germination in the seeds of lettuce and *Trifolium subterraneum*.

(4) Temperature: Seeds normally germinate within a wide temperature range. Optimum temperature for different plant lies between $50\text{-}30^\circ\text{C}$. Certain seeds will germinate at constant temperature while others require a diurnal thermoperiodicity. The rate of seed germination increases with the rise in temperature lying within the temperature range. The rate of increase in seed germination decreases towards higher limits of temperature range.

(5) Light: Seeds of many plants are light indifferent or non-photoblastic i.e. they are not influenced in the germination by the presence or absence of light. The seeds which are affected by light are described as photoblastic. The photoblastic seeds are of two types – positively photoblastic and negatively photoblastic. The positive photoblastic seeds require light for germination. They are known as light sensitive seeds e.g. lettuce, tobacco, *Rumex*, *Viscum album*, many grasses and several epiphytes.

(6) Other factors: Many orchids and other plants exhibit seed germination only when an appropriate fungus partner is available. Seeds of some parasitic plants will similarly grow only in the vicinity of their host roots because the latter excrete certain growth hormones. Seeds of some aquatic plants germinate only at low or acidic pH (e.g. *Potamogeton*, *Sagittaria*).

(II) Internal Factors:-

(1) Vitality: The ability of a seed to germinate when provided with optimum conditions is described as vitality of the seeds. The vitality of a seed is dependent upon its stored food, size, health etc.

(2) Longevity or viability: With the passage of time, a seed loses its power to germinate. Thus each seed has a longevity or the period within which it can show renewal of growth or germination. Most of the crop plants lose their viability within 2-5 years, legumes generally retain their viability for longer periods. A no. of seeds have been found to remain viable even after 100 years, e.g. *Trifolium*, *Mimosa*, *Astragalus*. Lotus seeds more than 1000 years old have been found to germinate. The loss of viability of a seed is caused by loss of nutrients in the region of growing points or respiratory centres.

Majority of seeds exhibiting unusual high longevity have impermeable seed coats. The hard coats of these seeds do not allow water and oxygen to penetrate the interior. Thus the seeds continue to remain in suspended animation.

(3) Dormancy: The failure of a viable seed to germinate under favourable conditions is described as dormancy. It is due to the internal conditions of the

seed. It is also described as the inhibition of germination due to internal conditions in an otherwise viable seeds.

(4) Secondary Dormancy: The seeds of some plants can germinate immediately after their ripening. If germination is delayed, the seeds become dormant. This phenomenon is termed as secondary dormancy, e.g. *Taxus*. Secondary dormancy is induced in certain other seeds when exposed to high temperature or CO_2 rich atmosphere.

(5) Other conditions: Germination is influenced by the state of maturity at the time of separation from the parent plant, the presence of sufficient food reserve, certain enzymes and growth regulators, genetic potentiality.



Chapter-4

Transpiration

Q.1. What is vernalization? Explain the mechanism of vernalization.

Ans. Annuals, biennials and some perennial plants require chilling treatment before they become able to flower. They include some varieties of garden Chrysanthemum, Primula vulgaris, viola sps etc. These plants require fresh vernalization each winter.

The cold (low temperature) treatment to a plant bud or seedling in order to fulfil a specific low temperature requirement for accelerating flowering is called vernalization. Vernalization is a process which promotes flower initiation in a plant by a previous cold treatment. The term was coined by Lysenko in 1928.

Mechanism of Vernalization: A number of theories have been proposed for the mechanism of vernalization. Some of the important ones are:-

(1) Theory of phasic development: Lysenko developed and elaborated this theory. According to this theory, a plant passes through a succession of development phases, each of which has a specific requirement on the environment and that the effect produced by such a requirement is irreversible.

The theory emphasizes on the following points:-

- (1) Growth and development are the two processes.
- (2) During its overall process of development, a plant passes through a specific sequence of phases.
- (3) A next phase begins only when its previous phase has been completed.
- (4) The changes taking place are irreversible.
- (5) Every phase has its specific environmental requirements e.g. light, temperature etc.

Lysenko recognised two main phases in the development of a plant. They are thermophase and Photophase. Photophase prepares the plant to flower. The thermophase precedes the photophase.

In winter plants, the temperature requirement of the thermophase is low so that the plants must be chilled. In other plants, the cold requirement is absent because in their case the optimum temperature of thermophase is sufficiently high. It lies between 20⁰-30⁰C in case of maize and cotton.

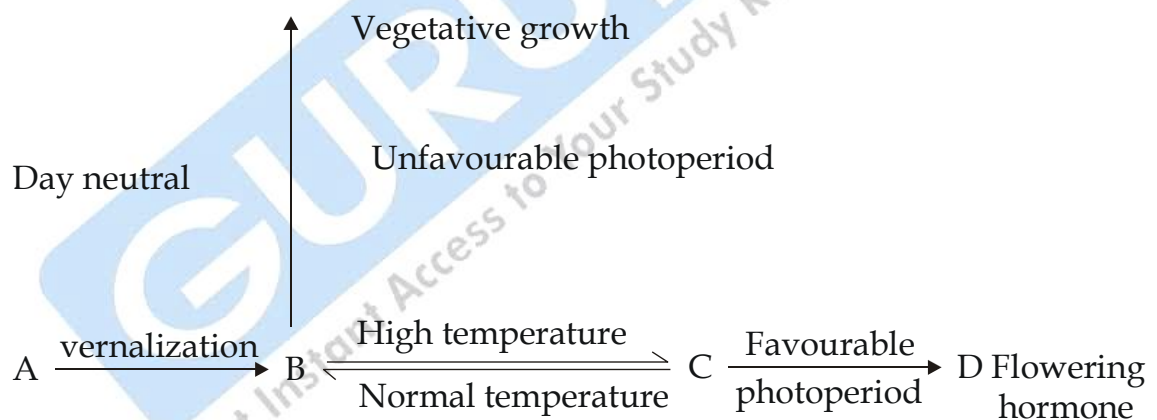
Points for non-acceptance of the above theory:

- (1) Cold requirement can be replaced by gibberellins in a number of plants.
- (2) Mckinney and Sando reported that in a number of wheat varieties flowering can occur over wide ranges of temperature and light conditions.
- (3) The phasic development theory presumes that the effects of each stage are irreversible. Almost every vernalized plant can be devernalized either by exposure to high temperature or anaerobic or dry conditions.

(II) Hormonal theory: Purvis and Gregory was the first to propose that cold treatment or vernalization produces a precursor of a hormone required for flowering. The precursor is transported to the stem apex, where it is transformed into an intermediate. The intermediate produces the flowering hormone under favourable photoperiods.

The hormone precursor has been named as vernalin by Melchers. Melchers has been able to demonstrate that a vernalized henbane when grafted to a non vernalized biennial plant transmitted its stimulus of vernalization to the latter since it came to flower with the former.

Purvis has given a scheme to explain the mechanism of vernalization.



Induction of flowering after cold treatment: The substance (A) on vernalization produces substance (B) which in turn produces (C). The substance (C) is an unstable intermediate. If it does not receive the required photoperiod or if subjected to the conditions of devernalization, then it reverts back to B. On the other hand, if it received favourable photoperiod, it changes to [D] which is the flowering hormone.

Q.2. Define Transpiration. Describe mechanism of stomatal opening and closing.

Ans. The process of loss of water in the form of water vapours from the aerial portions of the living plant is called transpiration.

Mechanism of Stomatal opening and closing: Stomatal transpiration occurs when stomata are fully open. The opening and closing of stomata is a direct response to increase or decrease in the osmotic concentration of the guard cells. The turgidity of the guard cells which is regulated by their osmotic concentration is the main cause of stomatal opening and closing. Various theories have been given to explain these turgor changes. Some of these theories are:-

(I) Photosynthesis in the guard cells: According to Von Mohl, the chloroplasts in the guard cells synthesize osmotically active substances. These increase their osmotic concentrations. The water from the surrounding cells enter the guard cells and their turgor pressure increases. This results in stomatal opening.

The theory is not acceptable as the photosynthesis in guard cells is too low to account for the accumulation of sugars.

(II) Classical Starch Hydrolysis Hypothesis: According to Lloyd the amount of starch in the guard cells is high in the dark and low in the light. He also observed that turgidity of the guard cells is dependent on their osmotic concentration, which in turn, is controlled by interconversion of starch and sugar. In the presence of light starch is converted into sugar and osmotic concentration of the guard cells increases. In the dark, sugar is converted into osmotically inactive starch which decreases the osmotic concentration of the guard cells.

Sayre indicated that interconversion of starch into sugar is determined by pH. During day time CO_2 liberated during respiration, is used in photosynthesis by the mesophyll cells. This results in increase in pH to 7-7.5. In this alkaline state, starch is converted to glucose-1-phosphate. In the dark CO_2 accumulates in the intercellular spaces as it is not utilized in photosynthesis. It lowers the pH of the guard cells to 4.2-5. In this acidic state glucose-1-phosphate is converted into starch.

Yin and Tung showed the presence of the enzyme phosphorylase in the chloroplasts. Phosphorylase favors the hydrolysis of starch to glucose-1-phosphate in the presence of inorganic phosphate at high pH. At low pH enzyme favours the formation of starch from glucose-1-phosphate. Starch is osmotically inactive while glucose-1-phosphate is osmotically active.

The above scheme was criticised by Steward. He raised two major objections:-

- (1) Stomatal closure requires ATP which is not mentioned in the above schemes.
- (2) Inorganic phosphate which is used in the formation of glucose-1-phosphate is osmotically active as glucose-1-phosphate. Hence there would be no

appreciable change in the osmotic concentration of guard cells as proposed in Sayre's and Yin and Tung's scheme.

Steward proposed the following scheme to explain the stomatal movement:-

(A) Opening of stomata in the light: It involves the following steps:-

- (1) pH of the guard cells increases to 7 due to the use of CO_2 in photosynthesis in the presence of light.
- (2) High pH favours conversion of starch into glucose 1-phosphate by enzyme phosphorylase.
- (3) Glucose-1-phosphate is further converted to glucose -6-phosphate by an enzyme phosphoglucomutase.
- (4) Glucose-6-phosphate is now converted to glucose and phosphate by the enzyme phosphatase.
- (5) Glucose being osmotically active, increases the osmotic concentration of the guard cell; hence water from surrounding cells enters them by endosmosis.
- (6) The turgor pressure of the guard cells increases and the stoma opens.

(B) Closing of stomata in the dark: It involves the following steps:-

- (i) pH of the guard cells decreases due to accumulation of CO_2 in the intercellular spaces as photosynthesis does not occur in the dark.
- (ii) Low pH favours the conversion of glucose into glucose -1-phosphate in the presence of an enzyme hexokinase. This required ATP, which is made available by respiration.
- (iii) Glucose-1-phosphate is converted into starch by phosphorylase.
- (iv) Starch being osmotically active, osmotic concentration of the guard cells decreases. Water leaves the guard cells by exosmosis. This reduces the turgor pressure of the guard cells and they become flaccid. Therefore the stoma closes.

Objections to the starch hydrolysis theory:

- (1) The rise of guard cell osmotic pressure during stomatal opening cannot be accounted entirely by the formation of glucose from starch.
- (2) In monocots e.g. onions, starch is not synthesized in the guard cells. They contain other polysaccharides.
- (3) It fails to explain the extra effectiveness of blue light during stomatal opening.
- (4) Glucose is not detectable in the guard cells at the time of stomatal opening.
- (5) Stomatal closure at mid day often takes place without any change in starch contents.

(III) Theory of Ion influxes and hormonal control or Active K^+ transport or potassium pump theory.

Two Japanese scientists, S. Imamura and M. Fujino showed the accumulation of K^+ in the guard cells during stomatal opening.

(A) Opening of stomata in light:

- (i) In light, starch in the guard cells is metabolised to Phosphoenol Pyruvate (PEP). It is later converted into organic acids, particularly malic acid. This reaction is catalyzed by an enzyme phosphoenol pyruvic carboxylase.
- (ii) Malic acid dissociates into malate and hydrogen ions (H^+) in the guard cells.
- (iii) H^+ from the guard cells, are transported to epidermal cells and K^+ from epidermal cells are absorbed into the guard cells through the agency of hydrogen potassium ion exchange pump in the plasma membrane.
- (iv) In the guard cells, K^+ are balanced by malate anions. Besides small amount of Cl^- ions are also absorbed which neutralize a small percentage of K^+ .
- (v) The process of K^+ exchange requires ATP and thus is an active process.
- (vi) Increased K^+ and anion concentration in the guard cells increases their osmotic concentration. Hence water enters the guard cells by endosmosis.
- (vii) Turgor pressure of the guard cells increases due to endosmosis and the stoma opens.

(B) Closing of stomata in the dark: Closure of stomata in the darkness is the result of the following reactions:-

- (i) As CO_2 is not utilized in photosynthesis during night, hence its concentration in the sub-stomatal cavity increases.
- (ii) An inhibitor hormone-Abscissic acid (ABA) functions in presence of CO_2 . It inhibits K^+ uptake by changing the diffusion and permeability of the guard cells.
- (iii) The K^+ are transported back to the epidermal or subsidiary cells from the guard cells.
- (iv) The osmotic concentration of the guard cells decreases. This results in the movement of water out of the guard cells (exosmosis) which now become flaccid.

This causes stomatal closure.

Scotoactive opening: In succulents, stomata open during night and close during the day. This is called as scotoactive opening. This mechanism can be explained on the basis of potassium pump:-

- (1) In succulents (*Opuntia*), starch is incompletely broken down into organic acids, particularly malic acid, in the dark under anaerobic conditions.
- (2) This results in acidification of the cell sap of the guard cells. Under these conditions malate ions from guard cells are exchanged with K^+ from the surrounding epidermal or subsidiary cells.
- (3) The osmotic concentration of the guard cells increases. Water enters them by endosmosis, turgor pressure increases and the stoma opens.
- (4) As soon as the stoma opens, aerobic oxidation sets in acidification is removed, starch is no more converted to organic acids, hence osmotic concentration decreases and the K^+ are exported to the neighbouring cells.
- (5) Turgor pressure of the guard cells decreases due to loss of water and stomata closes.

The process of alternation of opening and closing of stomata during night is called as scotoactive movement and is characteristic of succulent plants, especially the members of the family Crassulaceae.

Q.3.. What are micronutrients? Describe the role of micronutrients and their deficiency symptoms in plants life?

Ans. Besides the 10 macronutrients, there are a few other elements which are essential for plants but are required in very small amounts. These are called micronutrients or minor nutrients. These include copper, zinc, boron, molybdenum, chlorine etc.

(1) Boron: Boron is present in soil in very small amount. It occurs in highly complex forms such as boric acids, borosilicates, and calcium and manganese borates.

Functions:

- (1) Boron is involved in the absorption and translocation of carbohydrates.
- (2) It affects flowering and fruiting, active salt absorption etc.
- (3) It facilitates the formation of calcium pectate and thus affects the cell division.
- (4) Boron stimulates the growth of the pollen tube in some species, though its exact mechanism is not yet known.
- (5) It is involved in the production of root nodules in legumes.

Deficiency:

- (1) In Boron deficiency all areas of high metabolic activity like root and stem tips, get killed and fall down.
- (2) Flowering is little and fruits are fewer and smaller.
- (3) Leaves show white stripes, necrosis and various other type of distortions.

(4) Some of the common deficiency symptoms of boron are :

- (a) Browning of cauliflower.
- (b) Heart rot of sugar beet.
- (c) Brown heart or water core of turnip.
- (d) Internal cork of apple.
- (e) Monkey face of olives.
- (f) Stem crack of celery.

(II) Copper: Copper occurs in almost every type of soil in the form of complex organic compounds.

Functions:

- (1) Copper is a constituent of certain enzyme systems such as ascorbic acid oxidase, phenolases and cytochrome oxidase.
- (2) It is also a part of plastocyanin, which acts as an electron carrier in the photosynthesis reaction.
- (3) Since copper has a variable valency (i.e. Cu^{2+} and Cu^{+}); it can take part in electron transport system.

Deficiency:

- (1) Cu^{+} deficiency is accompanied by the appearance of dark green colour in young leaves, followed by chlorosis, leaf distortions and necrotic spots.
- (2) Two common deficiency diseases are exanthema and reclamation.
- (3) In exanthema, the tree barks show deep slits from which gum exudes.
- (4) In the reclamation disease, the tips of young leaves undergo chlorosis. So it is called as leaf tip disease. The tip soon becomes necrotic.
- (5) Necrosis spreads to margins and gives a withered appearance to the leaves. Growth is stunted. Fruits are fewer. They may show necrosis and skin splitting.

(III) Zinc: Bivalent form of zinc (Zn^{2+}) is exchangeable and is readily available in the soil.

Functions:

- (i) Zinc is essential for the synthesis of tryptophan, a precursor of auxins.
- (ii) It is an activator of several enzymes viz. Carbonic anhydrase, alcohol dehydrogenase, pyridine nucleotide dehydrogenase, carboxy peptidase, etc.
- (iii) Zinc plays an important role in protein synthesis, because in its absence there is substantial increase in soluble nitrogenous compounds. Its action on protein synthesis is related to the RNA production.

- (iv) Phosphorus metabolism may also get disturbed in zinc deficiency, because it is a constituent of alkaline phosphatases.

Deficiency: Zinc deficiency gives rise to various kinds of effects:-

- (i) Interveinal chlorosis occurs first in the older leaves. Chlorosis starts on the tips and margins. It is followed by necrosis.
- (ii) Various types of leaf distortions occur due to lack of cell differentiation.
- (iii) Terminal bud dies and stem growth is reduced. Therefore, leaf rosettes are formed. (e.g. Apple and Peach).
- (iv) In case of acute Zn-deficiency (the young leaves are completely chlorotic. This deficiency symptom is called as white bud (e.g. maize).
- (v) In mild deficiency, deciduous fruit trees develop smaller leaves. So, Zn deficiency produces little leaf disease of these plants.
- (vi) Seed and fruit formation is reduced.

(IV) Molybdenum: Mo occurs in soil in three forms – dissolved, exchangeable, and non-exchangeable forms.

Functions:

- (i) Molybdenum is essential for nitrogen fixation and nitrate reduction. It seems to be either an activator or constituent of some dehydrogenases.
- (ii) Molybdenum has some role in the synthesis of ascorbic acid and hydrolysis of organic phosphates.

Deficiency:

- (i) Chlorosis is caused in the older leaves as in nitrogen deficiency. But unlike the nitrogen deficient plants, the cotyledons stay healthy and green.
- (ii) Molybdenum causes Whiptail disease in crucifers, in which the young leaves become distorted and have long midrib with poorly developed blades.
- (iii) The flowers show pre-mature fall. In cauliflower, the inflorescence loses its compact form. It develops bracts and forms several separate groups.
- (iv) Though many nodules are present in a Mo-deficient legume plant, the latter shows, nitrogen deficiency. This is because of the loss of the capacity to fix nitrogen by the bacteria present in the nodules.

(V) Chlorine: It is absorbed from the soil as chloride ions. It is required in very small amounts.

Functions:

- (1) The best known function of chlorine in plants is its stimulation of photosynthesis.
- (2) It acts as an enzyme activator for one or more reaction in which water is split and oxygen is released.

- (3) In some plants, bromine can substitute per chlorine, although at much higher concentrations.

Deficiency:

- (a) Leaves become wilted, chlorotic and then necrotic. They ultimately develop bronze colour.
- (b) Root growth is stunted but swelling is generally near the tips.
- (c) Fruit formation is retarded drastically and photosynthesis is also inhibited.

(VI) Manganese: Manganese occurs in the soil in various forms (e.g. bivalent, trivalent and tetravalent), but it is absorbed largely in the manganous form (Mn^{+2} , bivalent).

Functions:

- (1) Mn is known to be a specific activator of some enzymes like oxidases, peroxidases, dehydrogenases, kinases and decarboxylases. Mn can take part in electron transfer.
- (2) It is concerned with the formation of chlorophyll and maintenance of lamellar structure of chloroplasts.
- (3) It is essential for photolysis of water and evolution of oxygen. It helps in transferring electrons from OH^- or O^{--} to chlorophyll Mn. Therefore it plays an important role in carbohydrate metabolism, both photosynthesis and respiration.
- (4) It is essential for nitrogen metabolism by being required for nitrite and hydroxylamine reductases.

Deficiency:

- (i) Symptoms of Mn deficiency first appear in the leaves. Chlorotic patches are in the form of specks or reticulations in the dicots and strips in the monocots.
- (ii) Necrosis may also follow. In cases of severe deficiency, leaves show premature fall or do not develop at all.
- (iii) Both stem and root experience stunted growth. Their apices may die back.
- (iv) Flowers are sterile

Chapter-5

Enzymes

Q.1. What are enzymes? Give the characteristics of enzymes, discuss about the mechanism of enzyme action?

Ans. The term enzyme (en = in, zyme = yeast) means 'in yeast'. This was referred to denote the reaction where in the production of ethyl alcohol and carbon-dioxide by the enzyme, zymase present in yeast takes place. This reaction is popularly known as yeast fermentation.

Summer and Myrback defined enzymes as simple or compound proteins that act as specific catalysts.

They are soluble, colloidal molecules, which are produced by living cells. All enzymes are globular proteins with a complex 3-'D' structure, capable of binding substrate molecules to a surface.

Characteristics of Enzymes:

(1) Colloidal nature: Enzyme molecules are of giant size. Their molecular weights range from 12,000 to over 1 million. Due to their large size, the enzyme molecules possess extremely low rates of diffusion and form colloidal system.

(2) Catalytic Nature: Enzymes act like catalysts and accelerate the rate of chemical reaction. The catalytic property of an enzyme is measured by the turn-over number or molecular activity which is defined as the number of substrate molecules converted into product per unit time, when the enzyme is fully saturated with substrate. Carbonic anhydrase catalyses the hydration of carbon dioxide to produce carbonic acid.

(3) Specificity of Enzyme Action: Enzymes are specific in their action, with few exceptions. Four types of enzyme specificity have been recognized:-

(a) Absolute specificity.

Some enzymes are capable of acting only on one substrate.

e.g. Urease, carbonic hydrase.

(b) Group specificity: Some enzymes catalyze the reaction of a structurally related group of compounds.

e.g. Lactic dehydrogenase catalyses the interconversion of pyruvic acid and lactic acid and also a number of other structurally related compounds.

(c) Optical specificity: A particular enzyme will react only with one of the two optical isomers, L or D e.g. Arginase acts only on L-arginine and not on its D-isomer.

(d) Geometrical specificity: Some enzymes exhibit specificity towards the cis and trans forms e.g. fumarase catalyses the inter-conversion of fumaric and malic acids. It does not react with malic acid which is trans isomer of fumaric acid or with D-malic acid.

(4) Thermolability (Heat sensitivity): Due to proteinaceous nature enzymes are very sensitive to heat. The rate of an enzyme action increases with rise in temperature, the rate of action increases 2 to 3 times for a rise in temperature of 10°C i.e. the value of temperature quotient or Q_{10} is 2 to 3. But at higher temperature, the value of coefficient does not remain constant and decreases rapidly. Above 60°C , the enzymes coagulate and thus become inactivated due to irreversible changes in their chemical structure.

The optimum temperature range for most enzymes varies between 30 and 45°C . Most enzymes are thermolabile and begin to denature at temperature between 45°C and 50°C .

(5) Reversibility of a Reaction: The enzymes are capable of bringing about reversion in a chemical reaction. The digestive enzymes catalyze hydrolytic reactions which are reversible.

e.g. Lipase which catalyzes the synthesis of fats from glycerol and fatty acid, can also hydrolyze them into their component units.

(6) pH sensitivity: The pH of the medium controls the activity of an enzyme to a great extent. This sensitivity is related to the degree of dissociation to the electric charge of enzyme and to the formation of enzyme substrate complex.

(7) Each enzyme acts best in certain pH value which is specific to it and its activity slows down with any remarkable change in the H^+ ion concentration.

pH optima for various enzyme are:-

Enzyme	Optimum pH of the medium	Nature of the medium
(1) Pepsin	1.5-1.6	Highly acidic
(2) Amylase	4.6-5.2	Acidic
(3) Maltase	6.1-6.8	Acidic
(4) Catalase	7.0	Neutral
(5) Urease	7.0	Neutral

Mechanism of Enzyme Action: A simple enzymatic reaction can be written as:-



Where E = Enzymes

S = Substrate

P = Product

ES = Enzyme substrate complex

EP = Enzyme product complex.

In ground state, any molecule contains a specific amount of free energy, known as the standard free energy ΔG° .

When the free energy of the ground state of P is lower than that of S, ΔG° for the reaction is negative and the equilibrium favours P. This reaction is not affected by any catalyst. A favourable reaction does not mean that $S \rightarrow P$ conversion is fast.

There is an energetic barrier/energy hill between S and P, that molecules must overcome. At the top of energy hill is a point at which decay to the S or P state is equally probable. This is known as transition state. The difference between the energy levels of the ground state and the transition state is called the Gibbs free energy of activation or activation energy and is symbolized by $\Delta G^{\ddagger}$. The double dagger ($\ddagger$) denotes a thermodynamic quantity of a transition state.

The catalysts affect the reaction rates, not the reaction equilibria. The enzyme are the biological catalysts that catalyze the reaction $S \rightarrow P$ and also catalyzes the reverse reaction, $P \rightarrow S$.

Active Sites: Substrate molecules are comparatively much smaller than the enzyme molecules. There are some specific sites or regions on the enzyme for binding with the substrate. Such sites of attachment are known as active sites or catalytic sites or substrate sites.

(I) Fischer's Lock and Key Model: It was proposed by Emil Fischer in 1898. According to this model, the union between the substrate and enzyme takes place at the active site in a manner in which a key fits a lock and results in the formation of an enzyme substrate complex. Since the two molecules, the substrate and the enzyme are involved, this hypothesis is also known as concept of intermolecular fit.

The enzyme substrate complex is highly unstable which immediately decomposes to produce the end products of the reaction and to regenerate the free enzyme. This complex union results in the release of energy.

The existence of an ES complex has been proved by various techniques like electron microscopy and x-ray crystallography.

(II) Koshland's Induced fit Model: An important but unfortunate feature of Fischer's model is the rigidity of the active site. The active site is presumed to be pre-shaped to fit the substrate.

Koshland proposed a modified model to explain the enzyme properties more efficiently. According to this model, the enzyme molecule does not retain its

original shape and structure. But the contact of the substrate induces configurational or geometrical changes in the active site of the enzyme molecule. Consequently, the enzyme molecule is made to fit completely the configuration and active centres of the substrate.

Michaelis-Menten Hypothesis: Michelis and Menten proposed this theory for enzyme kinetics reaction. This theory is based on the following assumptions:-

- (i) Only a single substrate and a single product are involved.
- (ii) The concentration of the substrate is much greater than that of the enzyme in the system.
- (iii) An intermediate enzyme substrate complex is formed.
- (iv) The rate of decomposition of the substrate is proportional to the concentration of the enzyme substrate complex.

The theory postulates that the enzyme (E) forms a weak-bonded complex (ES) with the substrate (S). This enzyme substrate complex (ES) on hydrolysis, decomposes to yield the reaction product (P) and the free enzyme (E).



Chapter-6

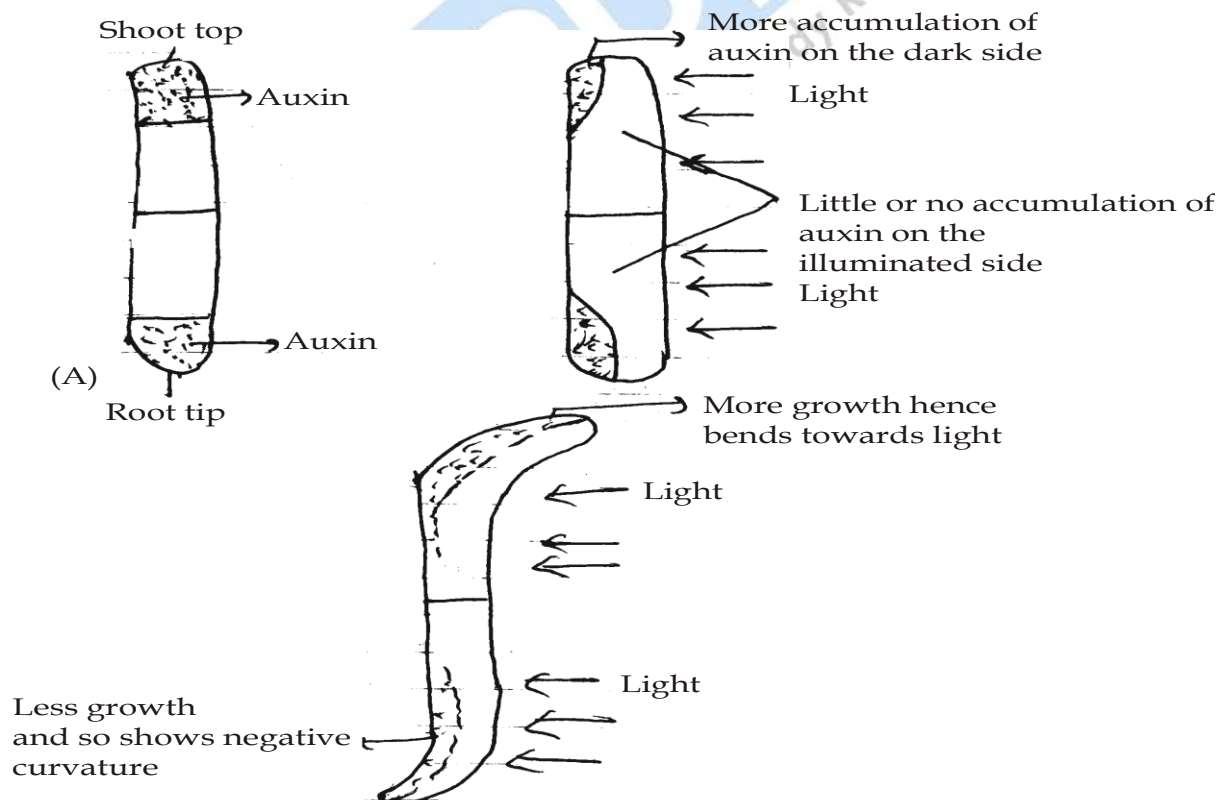
Plant Movement

Q.1 What do you understand by tropic movements? Describe the types of tropic movements and the mechanism that governs each one of them?

Ans. Paratonic movements of growth:

These movements of growth due to external factors are called tropic movements and are shown by fungi, bryophytes, ferns and all seed plants. Movement of stem petiole, floral axis are good examples of paratonic movements.

(i) Phototropism (heliotropism): The curvature induced in plant organs in response to the unidirectional light is called phototropism. The unidirectional light causes the stem apex to move towards the source of light and in root away from it. Thus stem shows positive phototropism and the root negative phototropism.



The positive and negative curvature shown by stem and root respectively is the result of the unequal growth of the illuminated and shaded (non-illuminated) sides of the apex. In case of stem, growth is more on the shaded side while in the root growth is more on the illuminated side.

Phototropism: Effect of light on auxin accumulation and root-shoot growth.

Greater growth of the shaded side of the stem is due to more accumulation of auxin on the shaded side than the illuminated side. Greater accumulation of auxins on the shaded side is due to following two reasons:-

- (1) Translocation of auxins from illuminated side to the shaded side.
- (2) Inhibition of auxin synthesis in the illuminated side.

Phototropism can be demonstrated in laboratory by keeping a potted plant in a phototropic chamber. It is a small wooden box and one of its sides can be slided up and down. The box has a small opening on one side to provide light. A potted plant is kept inside the chamber. Within a few days, the stem bends towards light and finally comes out of the opening through which light is available.

(ii) Geotropism: The growth and orientation of stems and roots in response to the force of gravity is called geotropism. The stem shows negative geotropism i.e. it grows away from the force of gravity and root shows positive geotropism i.e. it grows in the direction of gravity. Thus if a plant is kept on a horizontal plane, its stem will bend upwards and roots in the downward direction.

Geotropism can be explained on the basis of auxin concentration. In a horizontally placed plant, the auxin accumulates on the lower side of the stem and root apices due to gravity. In the stems higher concentration of auxin on the lower side of the apex, stimulates its growth on that side. As a result, the stem grows in upward direction, showing negative geotropism.

In case of a root, the higher concentration of auxin on the lower side inhibits the growth on that side and there is greater growth on the upper side than on the lower side. So, the root apex grows downwards i.e. shows positive geotropism.

Different parts of a plant respond differently to the stimulus of gravity:

Accordingly they may be of following three types:-

(1) Orthogeotropic: Primary roots grow towards the force of gravity and are called positively orthogeotropic. The main stem which grows away from the gravity is called negatively orthogeotropic.

(2) Plagiogeotropic: Secondary roots and branches arising from the main stem usually place themselves at an angle of 45° (90° - 180° in case of stem) from the main axis. Thus these organs are placed obliquely to the force of gravity. Such organs are called plagiogeotropic.

(3) Diageotropic: Tertiary roots, horizontally placed branches and leaves arise at an angle of 90° from the main axis. They are placed horizontally to the force of gravity and are said to be diageotropic.

Geotropism can be demonstrated in the laboratory by an apparatus called clinostat.

Clinostat consists of a rod which is attached to a motor at one end and has a disc at the far end. A flower pot containing a seedling is fixed horizontally to the disc.

The motor rotates the rod at a fixed speed. In a vertically placed pot, shoot always grows upwards (away from the force of gravity) and root downwards towards gravity. To demonstrate geotropism two clinostats with horizontally placed plants are required. In one of the clinostats the pot is continuously rotated, while in other it is kept stationary at one position. After sometime, it is observed that the plant in the first clinostat (which is rotated) does not show geotropism. It is because in this case all the sides of the plant are equally stimulated by gravity. On the other hand, the stem of the plant attached to a stationary clinostat shows negative geotropism and the root shows positive geotropism. It is because in this case one side of the plant axis gets greater gravitational stimulation than the other. Gravitational stimulus takes about a week before geotropic response is seen.

(iii) Hydrotropism: Growth movements in plant organs in response to variation in the amount of moisture are known as hydrotropism. The curvature of organ concerned is due to unequal growth on its two sides. Roots are positively hydrotropic i.e. they grow towards water.

It can be easily demonstrated by germinating seeds in a shallow sieve containing saw dust or sand. Within a few days, when seeds germinate the radicles come out of the pores of the sieve due to positive geotropism. But soon the radicles bend towards the moist surface of the sieve and continue to grow parallel to the bottom of the sieve. This shows that positive hydrotropism of roots is stronger than positive geotropism.

(iv) Chemotropism: The movement of plant organs due to unilateral stimulus of chemicals is called chemotropism. Such movements are shown by the fungal hyphae which tend to grow towards area rich in nutritive chemicals like sugars, proteins, amino acids, etc. Chemotropic movements are also shown by pollen tubes. The pollen tubes advance through the style and reach the ovules present in the ovary under the influence of chemicals secreted by them.

(v) Thigmotropism or Haptotropism: The growth movement of plant organs in response to unilateral stimulus of touch is known as thigmotropism. It is most obvious in tendrils and tendrillar parts that help the weak stems to climb. It is found in twining stems and aerial roots of some plants. The response to the stimulus of contact can vary from plant to plant, e.g. tendrils of some plants like *Bignonia* develop suckers only when their tips happen to meet the support. In majority the contact stimulus results in decreased growth on the stimulated side. Contact is facilitated by nutatory or circumnutatory movements of the thigmotropic organs.

Young roots show thigmotropism. In the soil, they are observed to bend around small stones. Since roots bend away from the source of contact, the response produced is negative.



Chapter-7

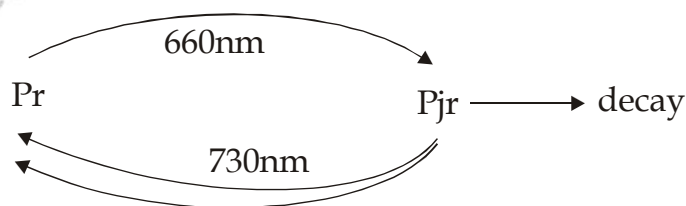
Photoperiodic

Q.1. What do you understand by photoperiodic induction? Give the mechanism of photoinduced flowering?

Ans. Photoperiodic induction: It is not necessary that the appropriate photoperiodic treatment of a plant be continued till blossoming occurs. It is seen that only a short exposure to appropriate photoperiod is required for the production of flowers. The phenomenon is known as photoperiodic induction or photoperiodic after effect, e.g. *Xanthium strumarium* requires only one photo-inductive cycle to initiate the floral primordia.

Mechanism of Photoinduced flowering: Several theories have been proposed to explain the mode of operation of photoperiodic induction in causing flowering:-

(1) Phytochrome Theory: Phytochrome is a special photoreceptor that influences several physiological processes. It has two forms- P_{730} (Pfr) and P_{660} (Pr) where the subscripts fr and r stand for far-red and red respectively. P_{730} is formed from P_{660} when the latter receives appropriate light waves. The phenomenon is called as pigment shift or conversion. P_{730} or Pfr is unstable. It is either destroyed or changed back to Pr. This can be shown as:-



Dark reversion

It is generally presumed that Pfr form of phytochrome is the active form. It suppresses flowering in short day plants while it promotes the same in long day plants. On the other hand Pr helps the short day plants to flower. As Pfr changes

to Pr in dark, darkness favours flowering in short day plants. This is the reason that all short day plants require a minimum or critical dark period. In the case of long day plants an uninterrupted long dark period is inhibitory. This can be met by producing more Pfr by exposing plants to longer photoperiods.

(2) Bunning's Hypothesis (Endogenous or circadian Rhythm): According to this theory, there is an internal physiological circadian rhythm with a 24 hour cycle in all organisms. In this rhythm or oscillation a cell passes periodically from a high synthetic capacity to a high catabolic capacity. They correspond to two phases each lasting for about 12 hours. The two phases are:

- (i) Photophilic phase (i.e. light loving phase)
- (ii) and scotophilic phase (i.e. dark loving phase).

Light given to the plant during the photophilic phase stimulates flowering while it inhibits the same during scotophilic phase. Bunning supposes that in short day plants photophilic phase is present immediately after dawn so that they require a shorter day length. In long day plants, the photophilic phase begins late in the day and therefore they require a longer day length to complete this phase.

During the photophilic phase, a substance A is produced in the leaves. It is changed into another substance B in the scotophilic phase. If this phase is interrupted before or about the middle. B is changed back to A because it is unstable in light. The substance B is transported to the meristem where it is converted to a light stable compound (which can induce flower formation) Bunning's hypothesis is supported by the fact that in artificial photo-induction maximum response is observed at particular intervals which are multiples of 24.

(3) Cajlachjan's Hypothesis: Cajlachjan, the original propounder of flower inducing compound florigen proposed in 1958 that florigen is a complex of two types of substances, gibberellins and anthesins. According to him, gibberellins are essential for the formation and growth of stems while

anthesins are required for flower formation. Cajlachjan assumes that flowering in all annual seed plants requires two phases:-

- (i) Floral stem formation phase and
 - (ii) Flower formation phase.
- (i) Floral stem formation phase requires intensive carbohydrate metabolism and respiration which are mediated by metal containing enzymes, increased content of gibberellin in leaves and auxin in stem buds.
 - (ii) The flower formation phase requires intensive nitrogen metabolism, increased residual respiration, higher content of anthesins in leaves and nucleic acid metabolites in stem buds. Long day conditions favour the first

phase while short day conditions stimulate the second phase. In long day plants, gibberellins are critical while anthesins are critical in short day plants. This theory is still in the realm of speculation as anthesins have not yet been proved to exist.

Q.16. Define photoperiodism. Give a brief account of the types of plants on the basis of their photoperiodic responses?

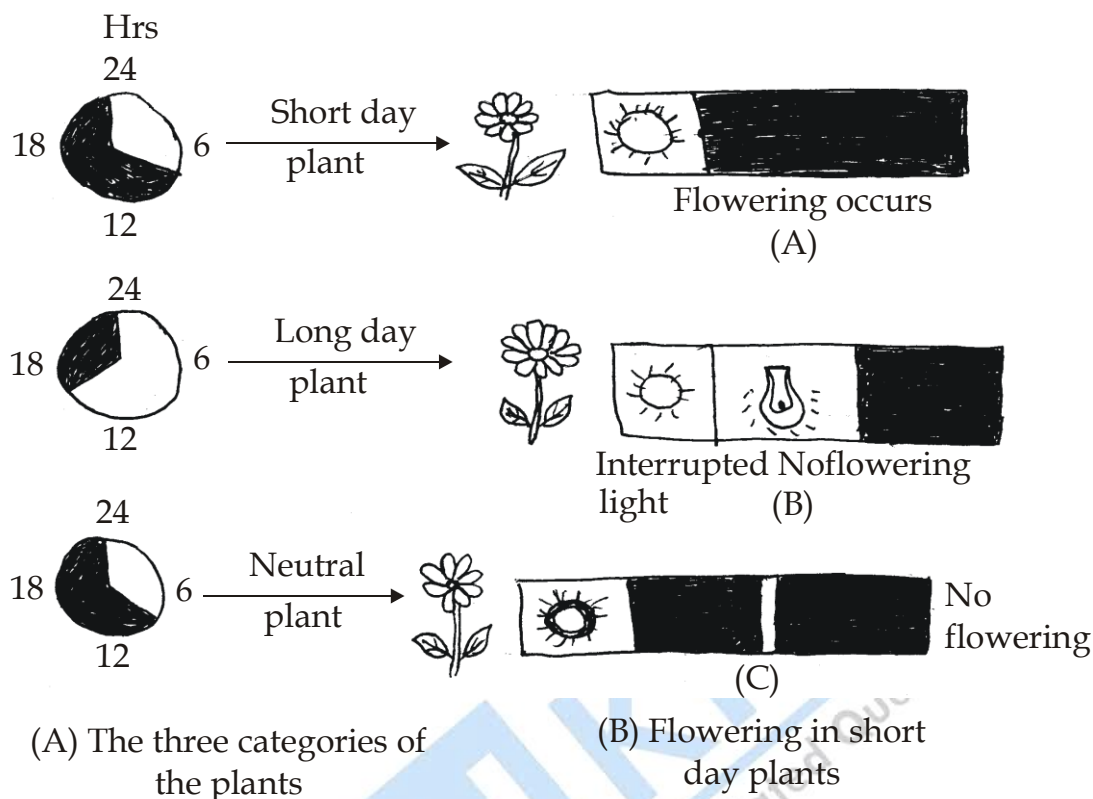
Ans.

Photoperiodism: The term photoperiodism is derived from two greek words, photos and periodos which mean respectively light and length of time. It was studied in detail for the first time by Garner and Allard. They came to the conclusion from a number of observations that the length of day time has something to do with flowering. Garner and Allard saw that the giant tobacco variety 'Maryland Mammoth' did not flower in the summer even when it reached 10-15 feet in height while in winter it could flower before reaching a height of 5 feet. They could achieve the same result artificially. The plant could remain vegetative if during the winter it was given extra light hours from electric lamps. In the summer the plants came to flower when light hours were reduced by darkening the plants for sometime during the day. Thus it became clear that flowering was controlled by the relative length of the day. They introduced the term photoperiod to denote the length of the day favourable for a plant to flower. The response of the individual plant to the relative length of the day or photoperiod is termed as photoperiodism by them. The photoperiodic response appears only at a definite period in the development of a plant and after receiving a definite dose of light hours in a definite sequence. Therefore, Hillman has defined photoperiodism as a response to the timing of light and darkness.

Types of plants on the basis of their light requirements:

Garner and Allard classified the plants into 5 categories on the basis of their photoperiodic response.

- (1) Short Day Plants
- (2) Long Day Plants
- (3) Day neutral plants
- (4) Short -long day plants
- (5) Long- Short day plants



(A) The three categories of the plants.

(B) Flowering in short day plants.

(1) Short Day Plants (SDP): They are those plants which flower below a critical day length or photoperiod. Photoperiods longer than the critical one keep the plants in vegetative phase. Short day plants are also known as long night plants since they require an uninterrupted dark period for flowering. Some common examples of short day plants are:-

(i) Tobacco (autumn and winter variety), *Xanthium strumarium*, Aster, *Glycine max* (Soybean), Coleus, Zea mays (maize), *Chenopodium rubrum*.

(2) Long Day Plants (LDP): They generally require long photoperiods which might be above a critical length of light hours. A brief light exposure during dark period or providing light for longer periods stimulates flowering. Flowering is also stimulated by alternative short night and still shorter dark periods. Therefore, they are called short night plants. Under normal conditions, the long day plants show only vegetative growth below their critical day length, e.g. *Hyoscyamus niger* (Henbane), *Beta vulgaris* (Beet root), *Brassica campestris* (mustard), *Raphanus sativus* (Radish), winter wheat, winter barley etc.

(3) **Day neutral plants:** These are the plants with least response to day length for flowering. They can blossom over a wide range of photoperiods e.g. Tomato, pepper, cotton, cucumber, Helianthus.

(4) **Short-Long Day Plants:** These plants require short photoperiods for floral initiation followed by long photoperiods for blossoming, e.g. Trifolium repens (white clover).

(5) **Long-Short Day Plants:** These plants flower only when they experience long photoperiods followed by short photoperiods. The two photoperiods are required for floral initiation and blossoming, e.g. Aloe bulbifera, Bryophyllum.

(6) **Intermediate Day Plants:** These plants can flower only within a definite range of light hours, above and below which no flowering occurs, e.g. Chenopodium album, Coleus hybrida, Andropogon furcatus.

(7) **Amphiphotoperiodic Plants:** These plants behave just opposite to intermediate plants, flowering both above and below an intermediate day length, e.g. Setaria verticillata, Madia elegans.

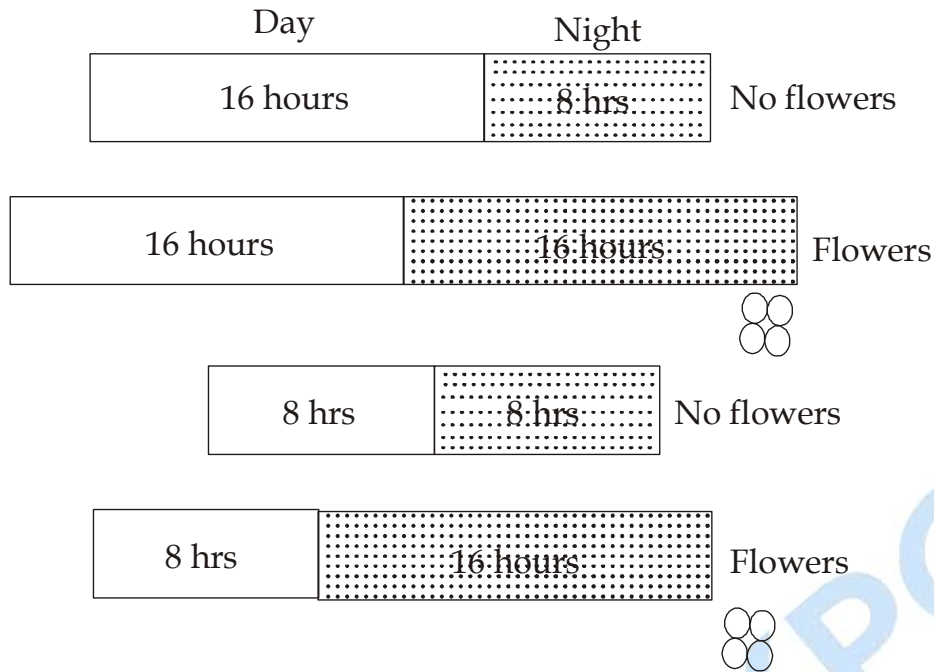
Photoperiodism is a specific or varietal character. Tropical plants are mostly short day or day neutral plants. Plants belonging to the high altitudes show long day photoperiodic response. Short day plants flower either in spring or autumn while long day plants blossom in the summer season.

The above classification does not mean to imply that all short day plants flower under photoperiods that are shorter than the photoperiods inducing flowering in long day plants.

e.g. Xanthium has a critical day length of $15\frac{1}{2}$ hours and flowers if this critical value is not exceeded. Hyoscyamus has a critical day length of 11 hours and flowers when this critical value is exceeded. *Xanthium*, a short day plant and *Hyoscyamus*, a long day plant will flower if subjected to a photoperiod of 13 hours.

Importance of the Dark Period:-

To begin with, it was believed that in a photoperiodic response it was the length of the day which determined the response. Experiments by K.C. Hamner and J. Bonner demonstrated that in fact, it was the length of the night (dark period) that was critical, e.g. *Xanthium strumarium*, a short day plant flowered when the dark period in its daily cycle was more than 9 hours, regardless of the day length.



Effect of long and short night on flowering in *Xanthium strumarium*

It was also shown that even a brief exposure to light during dark period nullified the effect of darkness. This indicated that the plant was sensing the uninterrupted night period and not the light period.

Effect of light and dark interruption on flowering in long and short day plants.

Chapter 8

Water Relations and Cytokinins

Q1 Write Short notes on :

- (a) Diffusion
- (b) Osmosis
- (c) Plasmolysis

Ans

(a) Diffusion:

The movement of gas , liquid and solute molecules or ions from their region of higher concentration to the region of their lower concentration is known as **diffusion**. The exchange of carbon dioxide , oxygen and water vapour between leaf and the external atmosphere is due to the process of diffusion. It continues till the regional differences in the concentration of substance disappear.

Characteristics of diffusion:

The following are the important characteristics of diffusion:

- (i) **The molecules (or ions) diffuse from the region of their higher concentration to the region of lower concentration.** This can be shown by various examples. If a crystal of copper sulphate is put in water , the copper sulphate molecules diffuse throughout the liquid , giving it a uniform colour. Similarly sugar diffuses uniformly when put in water giving it a uniform sweet taste.
- (ii) **The diffusing molecules move randomly towards all the region of their lower concentration.** This continues till the molecules get evenly distributed in the space available. The movement of molecules is due to their kinetic energy.
- (iii) **The direction of diffusion of one substance is independent of the movement of another substance.** The 2 gases can diffuse in the same or opposite directions at the same time independently without affecting the movement of another. The diffusion of CO_2 and O_2 through the stomata takes place simultaneously in opposite direction. The diffusion of CO_2 is controlled by the concentration of its own molecules and is not affected by the concentration of O_2 molecules. Similarly , the diffusion of O_2 molecules depends upon the concentration of its own O_2 molecules only and not CO_2 molecules .

- (iv) The rate of diffusion of molecules is dependent upon their kinetic energy, their size, the density of medium through which they move and the gradient of concentration over which they diffuse.

Factors affecting the rate of diffusion:

In terms of free energy, net diffusion of gases or other substances like water, is from the region of its greater free energy (or total molecular activity) to a region of its lesser free energy. Therefore all such factors that bring about changes in free energy, affect diffusion. Some of these are given below.

1. Temperature:

The rate of diffusion increases with increase in temperature. This is because free energy increases with rise in temperature and the molecules move towards the region of low temperature.

2. Size of molecules:

In general, larger is the molecule, slower is the rate of diffusion. This can be deduced from **Graham's Law of diffusion** which states that the rate of diffusion of gases is inversely proportional to the square root of their densities.

(density = mass per unit volume)

3. Medium in which diffusion occurs:

The rate of diffusion would be slower if the medium is concentrated i.e. increase in the number of foreign molecules causes the rate of diffusion to decrease. Thus a gas would diffuse more rapidly in vacuum than in air.

4. Diffusion pressure deficit (DPD) :

It is the difference in concentration of diffusing molecules between one area and another over a specific distance. The steeper is the diffusion pressure gradient, the faster the rate of diffusion.

5. Solubility of solutes:

Diffusion of solute molecules in a particular solvent depends upon their solubility in that solvent. Greater is the solubility of molecules in a solvent, higher would be the rate of diffusion.

(b) Osmosis :

Osmosis may be defined as the passage of solvent molecules from a region of their high concentration to a region of their low concentration through a differentially permeable membrane. The solvent in all bio systems is water. Thus in other words **osmosis is the diffusion of solvent**

molecules through a differentially permeable membrane. The term osmosis was proposed by Nollet.

Depending upon the movement of water into or outward of the cells, osmosis is of two types as under:-

(i) Endosmosis:

When a cell is placed in a hypotonic solution, water will enter into the cell from the outer (hypotonic) solution. It is because the cell sap is more concentrated (has less water molecules). Diffusion of water into the cell from outside is known as endosmosis. It will result in increase in volume of the cell.

(ii) Exosmosis :

When a cell is immersed in hypertonic solution, water will diffuse out of the cell because the concentration of water molecules in the cell is more than in the outer solution. This process is described as exosmosis.

The phenomenon of osmosis can be demonstrated quite readily by the following experiments.

1. Demonstration of osmosis by potato osmoscope:

Take a medium sized potato tuber. Peel off the skin and cut it flat at one end. Scoop a cavity from the other end reaching short of the flat end. Pour some sugar solution into the cavity and place the tuber in petridish containing water. Mark the level (A) of sugar solution in the cavity with the help of pin. After keeping it undisturbed for a couple of hours mark the level of sugar solution again. A rise in the level (B) will be noticed.

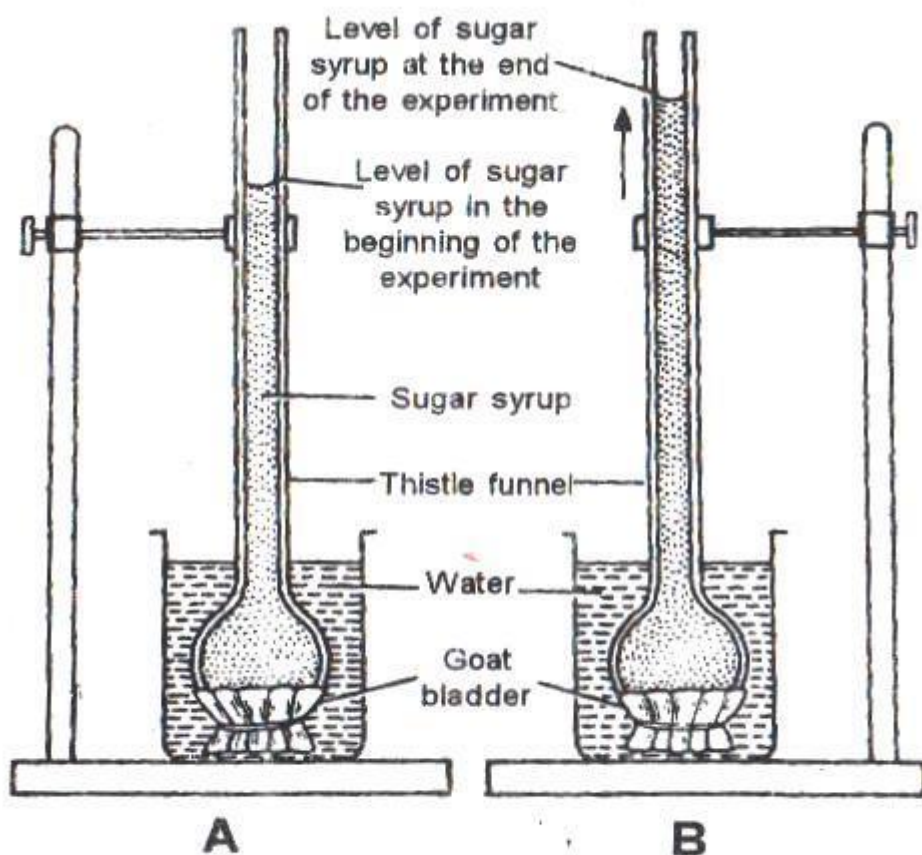
The rise in the level of sugar solution is due to osmosis. The potato cavity contains sugar solution with low diffusion pressure. It is separated from water (high diffusion pressure) in the dish by potato cells. The protoplast of the separating potato cells functions as a single semipermeable membrane. Following the principle of osmosis, water from the dish moves to the sugar solution. This results in a rise in the level of the sugar solution.

Demonstration of osmosis by Thistle Funnel Experiment:

Take a long - stemmed thistle funnel and fill it with sugar solution (say 10%) in water or fill it with concentrated sugar solution or syrup. Close tightly its mouth with semipermeable animal membrane (sheep or goat bladder). Tie it firmly with

waxed thread. Scissor the free edges of the membrane and apply rubber solution to seal it airtight. Invert the funnel in a beaker containing water so that the membrane is completely immersed. Mark the level (A) of sugar solution in the stem of the funnel. Leave the apparatus undisturbed for a couple of hours.

A rise in level (B) of sugar solution will be noted. The water in beaker does not taste sweet. The rise in the level of the sugar solution in the stem of thistle funnel is due to osmosis. Water in the beaker has higher diffusion pressure compared to water in the sugar solution in the funnel. The two are separated by a semipermeable membrane. Hence only water diffuses from its higher pressure zone to its lower pressure zone. As a result level of the solution rises. Because the membrane checks the passage of sugar molecules, water in the beaker does not taste sweet.



Osmosis : Demonstration by thistle funnel.

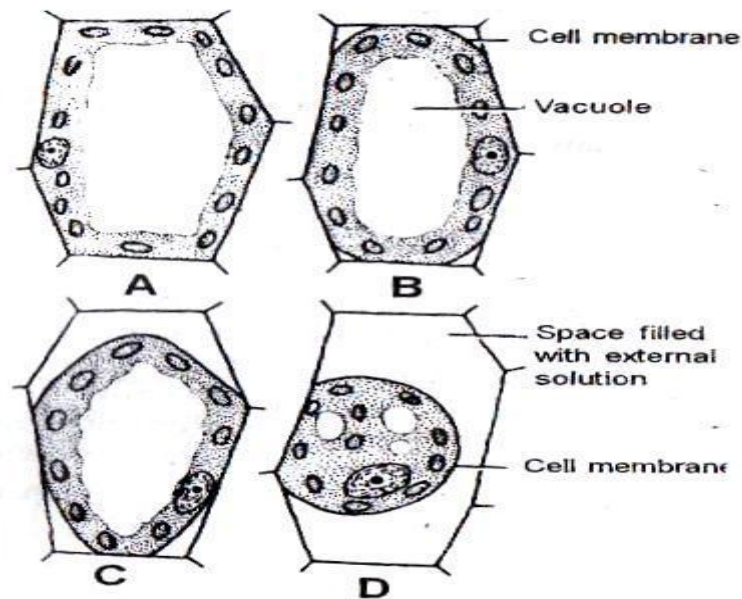
A. Before osmosis and B. After osmosis.

Importance of Osmosis for plants:

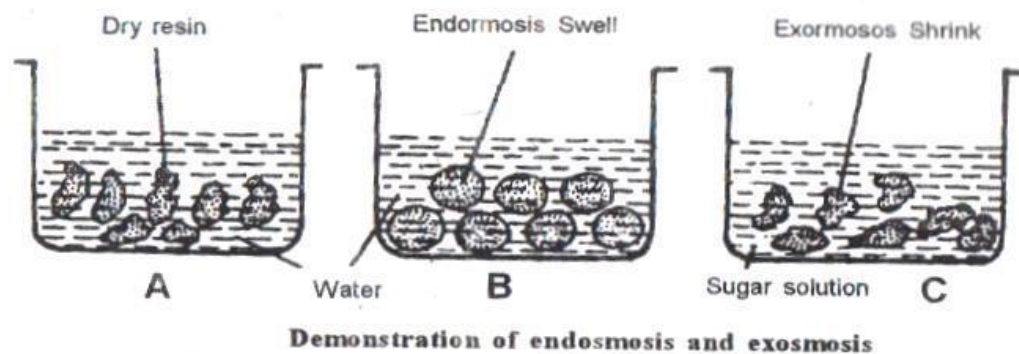
- (1) Absorption of water from the soil by root hairs is due to osmosis.
- (2) Osmosis helps in maintaining the turgidity of plant organs.
- (3) The opening and closing of stomata is dependent on the turgidity of guard cells which is due to osmosis.
- (4) Growth of young cells by increase in their size is due to the osmotic pressure and turgor pressure.
- (5) Due to higher osmotic concentration of the cells, these become resistant to freezing and desiccation.

Plasmolysis :

In hypertonic solution, water comes out of the cell due to exosmosis. As a result of continued exosmosis, the protoplasm shrinks and pulls away from the cell wall. This phenomenon is called as Plasmolysis and the cell is said to be plasmolysed. In a plasmolysed plant cell, the space between the contracted protoplasm and cell wall remains filled with external solution.



A-D. Plasmolysis : A. Normal cell; B. Incipient plasmolysis; C-D. Stages of permanent plasmolysis.



Plasmolysis can be demonstrated in the epidermal peel of **Rhoeo discolor** leaf. The cell contents are purple due to the presence of anthocyanin pigments. When the epidermal peels are placed in hypertonic solutions exosmosis begins. Hence the purple cell contents start pulling away from the cell wall and the cell begins to lose turgidity. Thus a colourless space can be seen between the cell wall and the purple contracted protoplasm. This initial stage of plasmolysis is called as **incipient plasmolysis**. If a cell showing **incipient plasmolysis** is immersed in water or in a solution whose concentration is less than that of the cell sap (i.e. hypotonic solution), the cell regains its turgidity as endosmosis takes place. However, if the exosmosis continues, it causes **permanent plasmolysis**. Such a cell cannot regain turgidity even after it is transferred to a hypotonic solution. It eventually dies.

Q2 Describe Vernalization and the mechanism of Vernalization .

Ans

Besides biennials and annuals, some perennial plants also require chilling treatment before they become able to flower. They include some varieties of garden Chrysanthemum, Primula vulgaris, Viola sps etc. These plants require fresh Vernalization each winter.

This cold (low temperature) to a plant bud or seedling in order to fulfil a specific low temperature requirement for accelerating flowering is called Vernalization. In other words Vernalization is a process which promotes flower initiation in a plant by a previous cold treatment . Chourad has defined Vernalization as the acquisition of the ability to flower by a chilling treatment.

Sites of Vernalization:

The perception of stimulus of cold treatment is normally received by the **shoot apex** (**in contrast to leaves in photoperiodism**) . **Chrysanthemum** can be vernalized if its shoot apex is put to low temperature, In seeds , the stimulus is perceived by the embryo and in the embryo , the site of Vernalization is the **plumule**. Recently it has been observed that not only the stem apical meristem but all dividing cells whether of stem , root or leaves are potential sites of Vernalization.

Factors affecting Vernalization:

Some of the factors which are responsible for bringing about successful cold treatment in a plant are as follows :

- (1) Low temperature
- (2) Required duration of exposure
- (3) Age of the plant
- (4) Aerobic respiration i.e. presence of O₂
- (5) Water

(1) Low temperature :

Low temperature required for Vernalization is usually between 0° - 10°C but it can be as high as 17°C . At temperature below 0°C , the effectiveness of Vernalization decreases . A temperature of -6°C is completely ineffective.

(2) Required duration of Exposure :

Flowering response to Vernalization is dependent not only on the temperature but also the duration of Vernalization period. Lang's work with Henbane in 1951 illustrates the relationship between temperature and time of exposure. He exposed the cold requiring henbane to different temperatures ranging from 3°C to 17°C for varying periods of time. Lang found that all temperatures from 3°C - 17°C are effective if the period of Vernalization is 105 days. But if the period of Vernalization is reduced to 15 days, then a temperature of 10°C was found to be most suitable while if the Vernalization period was extended to 42 days , the most effective temperatures were found in the range from 3°- 6°C .

(3) Age of the plant:

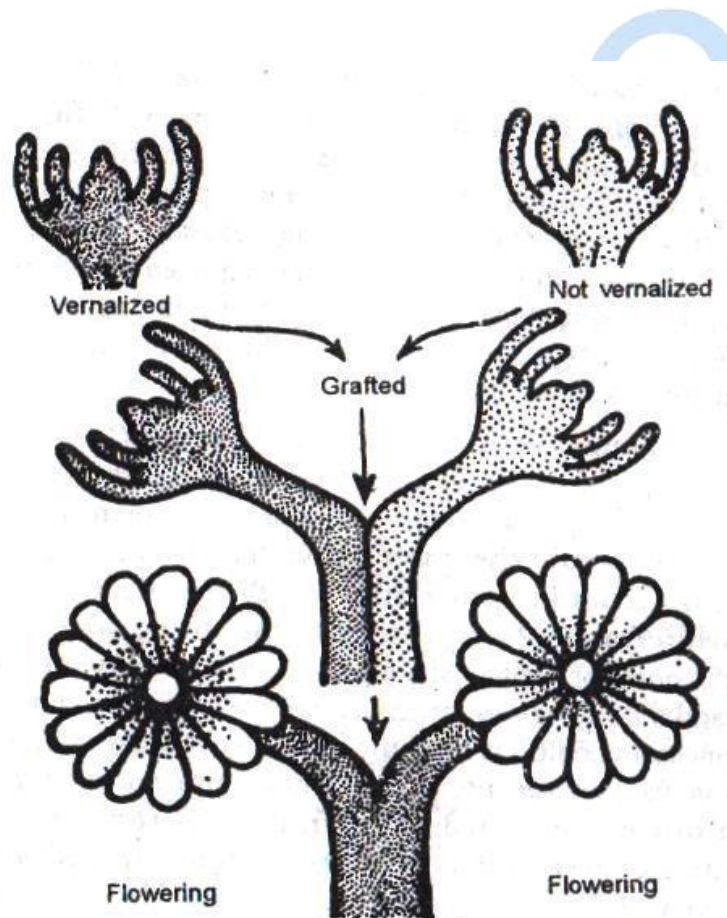
The age at which a plant is sensitive to Vernalization is quite different in different species. For example , in cereals , low temperature treatment can effectively vernalize the germinating seed and may even vernalize the embryos developing in the mother plant. In contrast some plants need a certain period of growth , before they become sensitive to low temperature treatment. e.g. *Hyoscyamus niger* must be in the rosette stage and have completed at least 10 days of growth before its sensitivity to Vernalization may be observed.

(4) Aerobic Respiration or Presence of O₂:

Vernalization is an aerobic process. Grains kept in an atmosphere of pure Nitrogen, although provided with adequate supply of water, are unresponsive to low temperature treatments. The oxygen requirement although low is **absolute**. O₂ is also necessary for the Vernalization of whole plant such as Henbane.

(5) Water :

Active embryo or shoot require the presence of optimum quantities of protoplasmic hydration. Water is also essential for optimum aerobic respiration and other metabolic reactions of Vernalization. Also the Vernalization of dry seeds is impossible unless the seeds have imbibed some moisture.



**Flowering stimulus may pass from one plant to a grafted unvernialized partner.
(As in Henbane)**

Mechanism of Vernalization:

The mechanism of Vernalization has not yet been understood properly. A number of theories have been proposed. Some of the important ones are:

(1) Theory of Phasic Development:

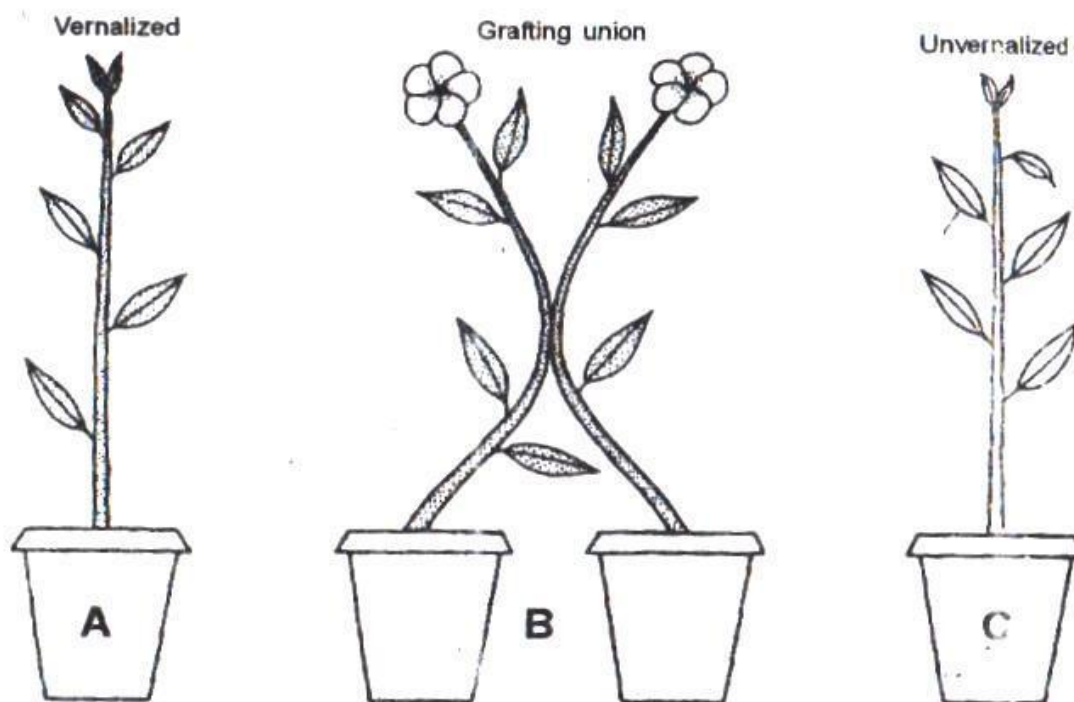
Lysenko developed and elaborated this theory. According to this theory, a plant passes through a succession of development phases, each of which has a specific requirement on the environment and that the effect produced by such a requirement on the environment and that the effect produced by such a requirement is irreversible. In simpler words, the theory emphasizes on the following points:

- (i) **Growth and development are two different processes.**
- (ii) **During its overall process of development a plant passes through a specific sequence of phases.**
- (iii) **A next phase begins only when its previous phase has been completed.**
- (iv) **The changes taking place are irreversible.**
- (v) **Every phase has its specific environmental requirements e.g. light, temperature etc.**

Lysenko recognized two main phases in the development of a plant. They are **Thermophase** and **Photophase**. **Photophase** prepares the plant to flower. The Thermophase precedes the Photophase.

There is no doubt that the timing of flower production is influenced by temperature, light intensity and light duration. However, the theory of Phasic development does not seem to be correct. The following points suggest the basis for non acceptance of this theory.

- (i) **Cold requirement can be replaced by gibberellins in a number of plants.**
- (ii) **Mckinney and Sando reported that in a number of wheat varieties flowering can occur over wide ranges of temperature and light conditions.**
- (iii) **The phasic development theory presumes that the effects of each stage are irreversible. Almost every vernalized plant can be devernalized either by exposure to high temperature or anaerobic or dry conditions.**



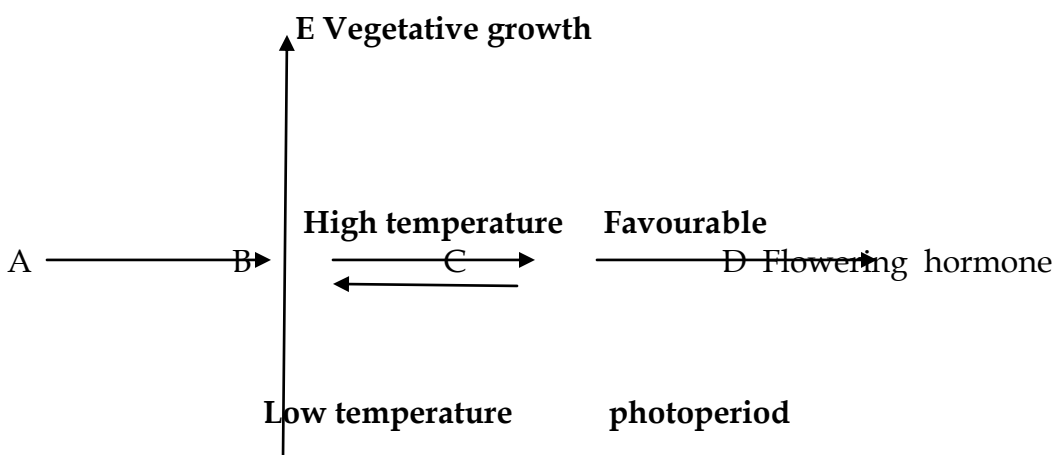
Experiments to demonstrate that flowering stimulus can translocate from vernalized plant to unvernallized plant through graft union in Henbane.

(2) Hormonal Theory:

Purvis and Gregory were the first to propose that cold treatment or Vernalization produces a precursor of a hormone required for flowering. The precursor is transported to the stem apex where it is transformed into an intermediate. The intermediate produces the flowering hormone under favourable photoperiods.

The hormone precursor has been named as **Vernalin** by Melchers . Melchers has been able to demonstrate that a vernalized henbane when grafted to a non-vernalized biennial plant transmitted its stimulus of Vernalization to the latter since it came to flower with the former.

In general , it is thought that cold treatment gives rise to a substance which is then transformed to another chemical that expresses the Vernalization effect . Purvis has given a scheme to explain the mechanism of Vernalization:



Induction of flowering after cold treatment (Purvis)

The substance (A) on vernalization produces substance (B) which in turn produces (C). The substance (C) is an unstable intermediate. If it does not receive the required photoperiod or if subjected to the conditions of devernalization, then it reverts back to B. On the other hand, if it received favourable photoperiod, it changes to (D) which is the flowering hormone.

Q3 Describe the mechanism of translocation of Assimilates ?

Ans Transport of Organic substances

All cells require food for their activity. In some lower plants (e.g. Ulothrix . Spirogyra) all the cells of the thallus are green and thus capable of manufacturing their own food. Therefore, in such plants, there is no need of transportation of food from one part of the plant to another. But in most higher plants, the green cells are restricted to the leaves and young stems and all non-green cells of the plant (e.g. those of roots, stems, fruits) are dependant for organic food upon these green cells. Thus the food has to be transported from green cells to non-green cells of the plant body. **This movement of prepared food from one organ of the plant to another is known as translocation, transport (if passive) or conduction of solutes(if active).** The organic nutrients synthesized in the leaves are often termed as **assimilates**.

Many theories have been proposed to explain the mechanism of translocation of solutes in higher plants. But none of these is perfect. Some important theories are briefly described here:

(1) Diffusion:

Mason & Maskeli thought that organic solutes move from one part of the plant to the other by simple diffusion through the living cells. The movement is along the concentration gradient. However, the actual rate of solute transport is very fast as compared to the normal diffusion. It is 10-150 cm. per hour or 2000-40,000 times the rate of diffusion.

(2) Activated diffusion hypothesis:

According to Mason and Phillis there is an activation of the diffusion process inside the phloem because:-

- (i) Cell to cell movement of organic compounds is enhanced by the supply of ATP.
- (ii) assimilates move from mesophyll cells into the sieve tubes against concentration gradient.
- (iii) Sieve plates do not seem to favour mass flow.
- (iv) Passage of solutes from phloem tubes into the storage cells or sink is again favoured by the presence of ATP.

Activation involves metabolic or respiratory energy. Kursanov and Turkina have observed a high respiratory activity in the vascular bundles of petioles in sugar beet and **Plantago major**. Respiratory energy can be used in three ways – by activating the diffusing assimilate molecules, by decreasing the resistance offered by the protoplasm of sieve tubes or , in providing translocation carriers.

(3) Protoplasmic streaming hypothesis:

This was first put forward by de Vries in 1885 and later developed by Curtis . Hugo de Vries suggested that streaming of the protoplasm (also called **cyclosis**) might be the mechanism of **solute translocation**. The streaming involves the circulation of protoplasm along the periphery of sieve element. This movement is similar to that of a conveyor belt. As cytoplasm rotates , the solute molecules are transported from one end of the sieve tube to another. Transfer of solute from one sieve tube to another takes place by diffusion. The theory was supported by many workers as it can explain the simultaneous upward and downward translocation.

The only evidence in favour of this theory is that those conditions which inhibit the rate of protoplasmic streaming inhibit the rate of protoplasmic streaming , also retard the flow of solutes.

One of the criticism against this hypothesis is that the observed rate of protoplasmic streaming are too low and therefore , can not account for the known linear rates of sugar translocation in sieve tubes. Moreover , streaming occurs only in young sieve tube elements.

(4) Contractile protein :

This theory was proposed by the Canadian plant physiologist **Fensom** in 1974. He observed a network of interlinked microfibrils in the lumen of sieve tubes. These fibrils were composed of contractile threads of p- proteins. Fensom

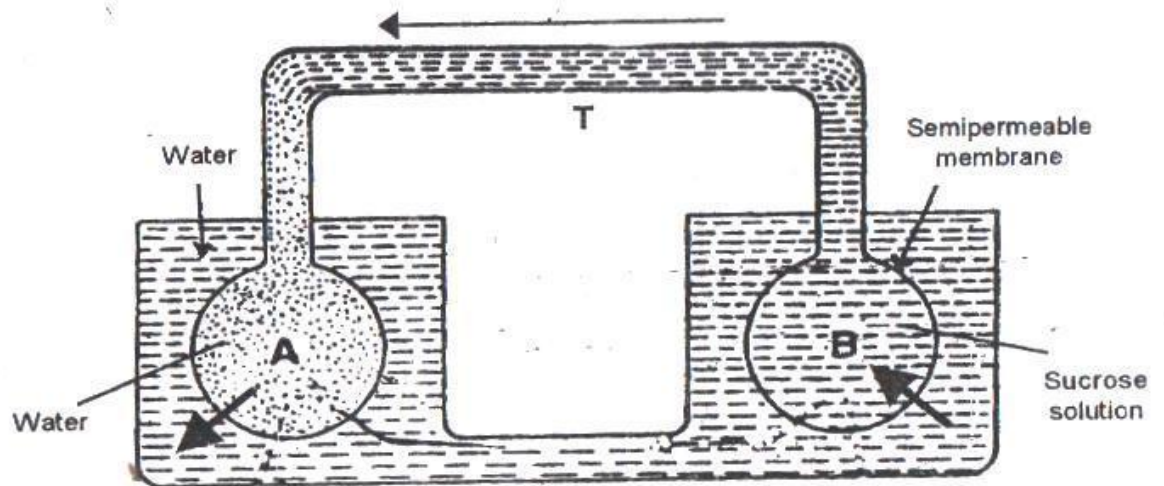
observed that particles attached to microfibrils moved with a bouncing motion resembling Brownian movement but were several times more rapid. Besides, he also found movement in the fluid of sieve tubes in which microfibrils were immersed. The latter movement is much slower (than those of the particles attached to microfibrils) and thought to be induced by contractile p- proteins.

(5) Mass flow or pressure flow hypothesis:

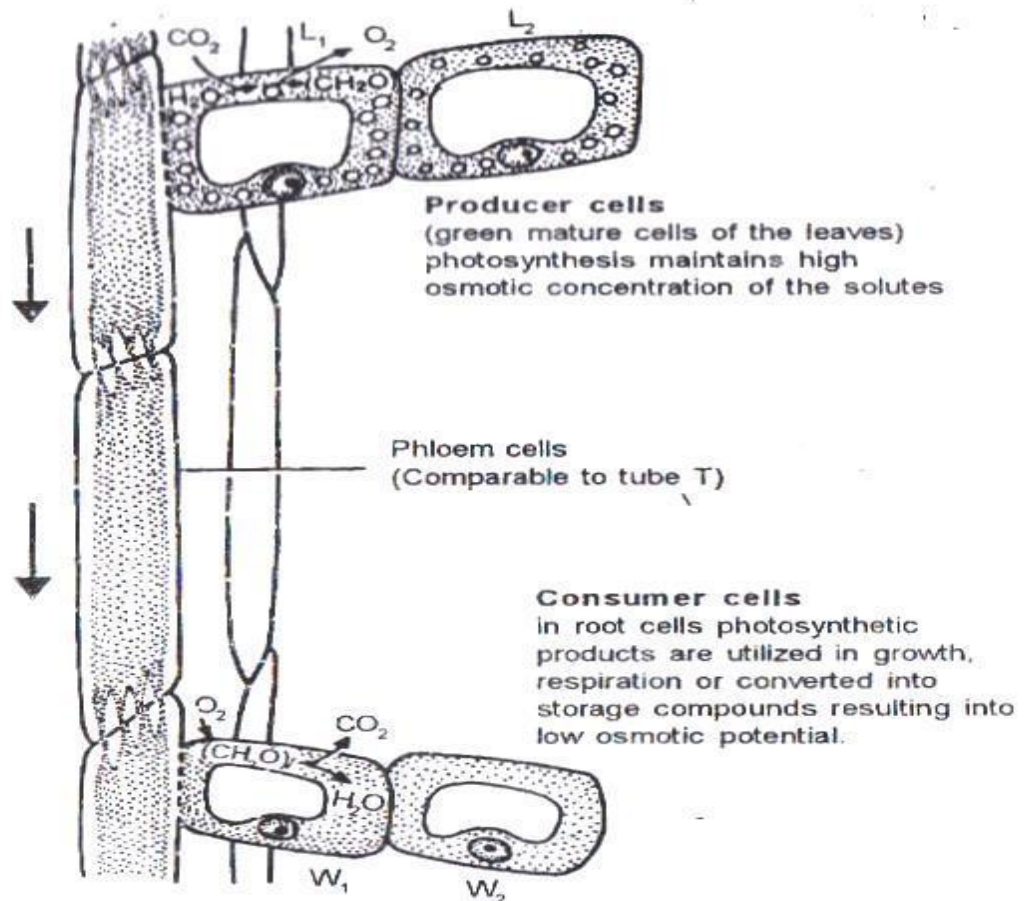
The theory was proposed by **Ernst Munch** in 1928. It is variously known as **Mass Flow** or **Solution Flow** or **Pressure Flow theory** or **Munch Hypothesis**. He proposed a physical model that behaved very much like the phloem system in a living plant. It consists of two bulbs A and B made up of semipermeable membranes and connected with each other by a tube T. Bulb B is filled with sucrose and bulb A contains only water. Both bulbs are immersed in water. In doing so, water immediately begins to enter into the bulb B (which contains sucrose solution), since it has a lower water potential. As a result, pressure increases in this chamber and it forces some of the solution to rise into the connecting tube T and eventually flow across to the other bulb A taking some of the sucrose along. When the sucrose solution enters bulb A, it forces some of the water there to flow out through the membrane into the water bath. The flow of solution from B to A continues until the solution in both the bulbs becomes equally concentrated. At such a moment both the bulbs would have equal water potential.

However, if the above model is modified in such a way that the sugar in the bulb A is readily converted in insoluble form (or removed), then the process of flow would continue indefinitely. A system similar to this indeed exists in plants. Corresponding to the bulb B are the green cells of the leaves which serve as the **source** because the sugars are produced in the green leaf cells. The root cells correspond to the bulb A. These cells serve as the **sink** because the assimilates synthesized in the source are transported to these regions. The tube T, connecting the bulbs, A and B can be compared with sieve tubes. The water bath in the Munch's model is represented by xylem.

The concentration of sugar is high in the leaf cells due to their photosynthetic activity. In root cells, the sugar concentration is low because :- (i) it is used up in the metabolism, and (ii) it is converted into insoluble form for storage.



Experimental representation of Munch mass flow hypothesis.



Mass flow theory as applied to plant.

The cells of the leaf and also of the roots are in aqueous environment . Now according to the above scheme, there would be a downward mass flow of sugars from the leaves to the roots. During the flowering season and other periods when vegetative activity is intense at the apex of the plants, sugars in leaves will be readily consumed in metabolic activities. Thus there would be a reduction in the turgor pressure of the leaf cells. At such times , the insoluble carbohydrates and other stored food in the root would be converted into sugar. As a result , the turgor pressure of root cells would increase . Under these conditions , therefore , the upward translocation would take place.

Mass flow hypothesis is a simple and attractive hypothesis. Several physiological evidences have been presented in favour of this hypothesis. The following observations specifically support this hypothesis.

- (1) Translocation of solutes from sources (leaf) to sink (root) is proportional to the concentration gradient in the phloem.
- (2) When the phloem tissues are excised , the sap from the cut end is exuded under pressure. Turgor pressure of significant magnitude is recorded in the sieve tube.
- (3) Source – sink distribution pattern as envisaged by the hypothesis has been observed by using radioactive tracers of various assimilates.
- (4) All the substances dissolved in the symplast solution of sieve tubes are found to move with the same velocity with minor differences caused by their individual concentration gradients. The volume of flow is such that a sieve tube element should be refilled 3-10 times every second. This rate can be achieved only by mass flow.

Objections to the mass flow hypothesis:

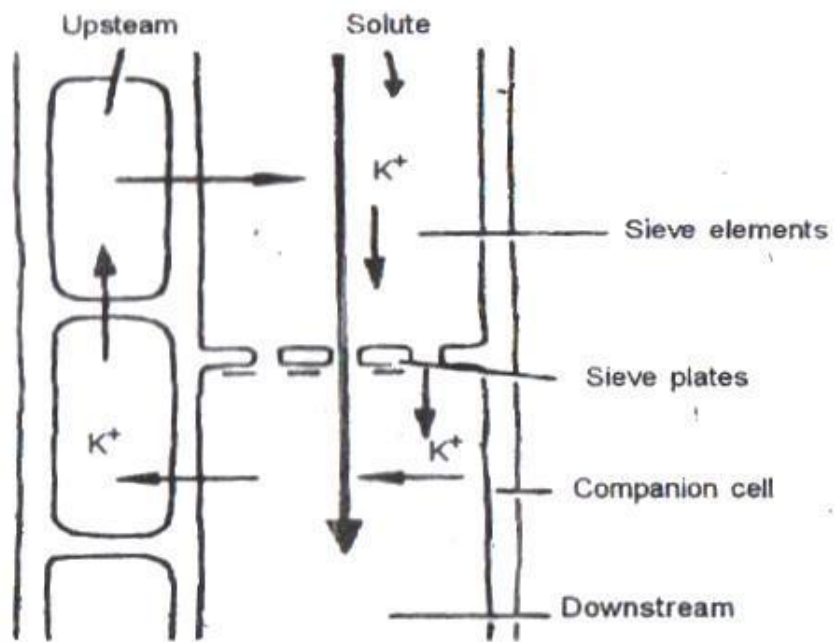
Though the mass flow hypothesis is simple , attractive and has been favoured by many plant physiologists , yet some objections have been voiced against it.

- (i) Plasmodesmata and intervening cytoplasmic inclusions are likely to offer a good deal of resistance for rapid flow of solutes.
- (ii) In members of Dioscoreaceae , sieve tube elements are not continuous from one internode to another. Large parenchyma cells are present at the nodes which separate the sieve tubes elements of one internode from another.
- (iii) The cells at the source end of the mass flow should always be turgid. In actual observation , it is seen that the supply cells of sprouting tubers , bulbs, corms and seeds are generally flaccid.
- (iv) The osmotic pressure of the receiving cells has often been found to be higher than the supplying cells.
- (v) Nitrogenous solutes of phloem do not always show a gradient . Rather they may be concentrated towards the sink end.

- (vi) There is some metabolic component involved in the phloem transport of solutes since dinitrophenol (an inhibitor) decreases the rate of translocation.
- (vii) Some recent workers have found evidence with the help of micropipettes that bidirectional movement of solutes can occur in the same sieve tube. This is impossible under mass flow hypothesis.

(6) Electro - osmotic hypothesis:

One of the objections against mass flow hypothesis is that it may not be able to account for the flow of solution through sieve pores. The sieve pores are small and at least partially occluded with P- protein and strong pressure will be required to force the solution through them. To overcome this problem , the electro - osmosis hypothesis has been proposed independently by Fensom and Spanner. According to this hypothesis , the solute moves in the positive direction of the electrical gradient along with K^+ ions . Important features of the mechanism are as follows:



Model to illustrate electro-osmosis hypothesis.

- (i) The sieve plates are negatively charged , hence they repel negatively charged anions.

- (ii) There is a high concentration of potassium ions (K^+) in the sieve tube solution. These and other cations can pass through the sieve plate.
- (iii) An electrical gradient builds up across the sieve plate in the direction of flow; when the solute is moving in downward direction, anions begin to accumulate below the sieve plate and cations above it (when solutes move in an upward direction, K^+ may accumulate below the sieve plate).
- (iv) A current of K^+ passes through the sieve pores by electro-osmosis, and sugar and water molecules adhered tightly to K^+ are carried along with them. Thus each sieve plate is an '**electro osmotic pumping station**' which induces mass flow of solution along with the movement of K^+ ions. The energy for this movement is supplied by ATP from companion cells and sieve tubes.
- (v) The K^+ from the downstream side of the sieve plate are pumped back to the upstream side of the sieve plate through adjoining companion cells.

The evidence in support of electro osmosis are as follows:

- (1) High concentration of K^+ is found in the sieve cells.
- (2) Role of companion cells in supplying ATP has been established.
- (3) The charged porous surface of the sieve plates is suitable for the flow of solutes by osmosis.

The hypothesis has, however, been rejected on the following grounds:

- (1) The hypothesis fails to explain the bidirectional transport of metabolites in phloem.
- (2) Considerable energy would be required to maintain a continuous circulation of K^+ ions.

Q4 What are Cytokinins ? Describe the various physiological effects of Cytokinins
Ans Cytokinins

Skoog reported that a substance present in the vascular tissue was responsible for causing cell division in the pith cells. Miller was first to isolate the crystals of a cell-division inducing substance from autoclaved sperm DNA. Later Miller et al found this substance to be very effective in causing cell divisions even in very low concentrations (1 part per billion) when auxin was also present in the medium. Since the substance had specific effect on Cytokinesis, it was named **kinetin**. Later on the name **kinin** was suggested to include kinetin and other substances having similar properties. Presently the term **cytokinin** proposed by Letham is the most acceptable term.

Skoog and Miller have defined **cytokinins as chemicals, which regardless of their other activities, promote cytokinesis in cells of various plant organs.**

Physiological Effects of Cytokinins:-

In most of their physiological activities, cytokinins require the presence of auxins, cytokinins are very active in very minute activities. Their major roles are:

(1) Cell Division:

Of all the growth hormones cytokinins have been found to be a 'true cell division factor' in a number of lower and higher plants. While a number of tissues from meristematic regions are capable of multiplication in absence of cytokinins, auxins etc. 'the non-meristematic tissues isolated from higher plants require a cytokinin and auxin for in vitro growth.

(2) Cell Enlargement :

A number of workers have found evidences to prove that cytokinins are equally good in causing cell expansion like auxins and gibberellins. According to Arora et al, the kinetin caused enlargement of cortical cells of tobacco roots upto four times their normal size.

(3) Morphogenesis:

Morphogenesis is controlled by an interaction between auxin and cytokinin. Skoog and Miller report that a pith culture of tobacco produced buds that grew into shoots when kinetin was present in excess of auxin while roots appeared when their ratios were reversed. The bud initiating effect of cytokinins is wide spread though not universal. Although cytokinins are produced by roots, the formation of root itself has been found to be inhibited in the former case above probably because of supra-optimal level of cytokinins. Cytokinins inhibit elongation of primary root but promote increase in its diameter. They can also stimulate the formation of interfascicular cambium.

(4) Counter Action of Apical dominance: Cytokinins counteract the influence of apical dominance over the lateral buds provided they are applied directly over them. Application of cytokinins even a few mm away from the lateral bud is ineffective. In the intact plant the apical bud causes a preferential movement of root manufactured cytokinins towards it, thus causing the starvation in lateral buds.

(5) Delaying of Senescence or the Richmond - Lang Effect :

Richmond and Lang reported that senescence (chlorophyll disappearance and protein degradation) was delayed, in the detached leaves of Xanthium, for several days if they were treated with kinetin. This effect of cytokinin in retarding ageing is called the **Richmond - Lang** effect. It prevents the breakdown of chlorophylls, proteins and nucleic acids and causes retention of nutrients and greater flow of amino acids and auxins towards the leaf and other organs.

(6) Promotion of Seed Germination :

Application of cytokinins can promote termination and even break dormancy in some seeds. Lettuce (*Lactuca sativa*) seeds are particularly responsive to

cytokinins. The seeds germinate only in light and fail to germinate in the dark . Like , gibberellins. Cytokinins can replace the light requirement in such seed germination.

(7) Accumulation of solutes:

Cytokinins help the plant cell to accumulate solutes very actively. A cell synthesizing or containing cytokinins can, therefore deprive its neighbours of essential metabolites. When certain fungi that cause rust diseases infect the leaves , necrotic areas of dead and dying cells are produced. These areas are often immediately surrounded by several green and starch – rich cells even when the rest of the leaf has become yellow and senescent . These **green islands** are rich in cytokinins , probably synthesized by the fungus. These cytokinins presumably help maintain food reserves for the fungus.

(8) Anthocyanin Synthesis :

Anthocyanins are flavonoid pigments , which are responsible for the red , pink , purple and blue colours in plants. Cytokinin treatment increases Anthocyanin content in many cultured cells and tissues and in parts of intact plants.

Mechanism of Cytokinin Action:

The exact Mode of cytokinin action is not yet fully understood. Some cytokinins occur as constituent of t-RNAs. They determine the conformation of anticodon(Ghosh and Ghosh, 1970) . However Kende and Tavares observed that a slight change in cytokinin structure prevented its incorporation into t-RNA without influencing its growth promoting action. It is just possible that cytokinins influence growth through two forms – one incorporated inside t- RNAs and the other in free state. The tRNA contained cytokinins control the synthesis of proteins and hence enzymes. The free cytokinins may protect such t-RNAs from ribonucleases or actually help in the synthesis of new tRNA.

Q 5 Define Ascent of Sap and describe the mechanism of ascent of sap?

Ans

Once the water is absorbed by the root hairs , it is translocated to various parts of the plant. The fluid that moves upwards in the stem is not pure water but a dilute solution of mineral ions absorbed from the soil and is referred to as sap. **The process of translocation of sap from the roots to the top of the plant is called as ascent of sap .**

Mechanism of ascent of sap:

Many theories have been proposed to explain the mechanism of ascent of sap. These theories are placed in the following three categories.

- (I) Vital theories
- (II) Root pressure theory
- (III) Physical theories

(I) Vital theories :

According to the vital theories , living cells are responsible for the ascent of sap . These theories , once considered to be the major explanations , have now only academic interest because the role of tracheary elements (dead cells) in ascent of sap is well established.

- (i) **Westermaier (183 -84)** suggested that tracheary elements held water by capillarity and acted as a reservoir.
- (ii) **Godlewski's theory (1884) or Relay pump theory:**

According to this theory , the rhythmic changes occur in the osmotic pressure of the living cells of the xylem parenchyma and medullary rays. The process involves absorption of water from a vessel by a living cell(of xylem parenchyma or medullary ray) when its osmotic pressure increases. The entry of water into a living cell now lowers its osmotic pressure. Therefore water is now pumped by this living cell into the xylem element placed above it. In this way , water continues to climb upwards.

The theory was found unacceptable because of the following points:

- (1) Water moves upwards even in the absence of living cells.
- (2) Metabolic inhibitors affecting the living cells do not change the rate of ascent of sap .
- (3) Wood anatomy does not show the arrangement of living and xylem cells as proposed in Godlewski's theory.

(iii) Janse's theory:

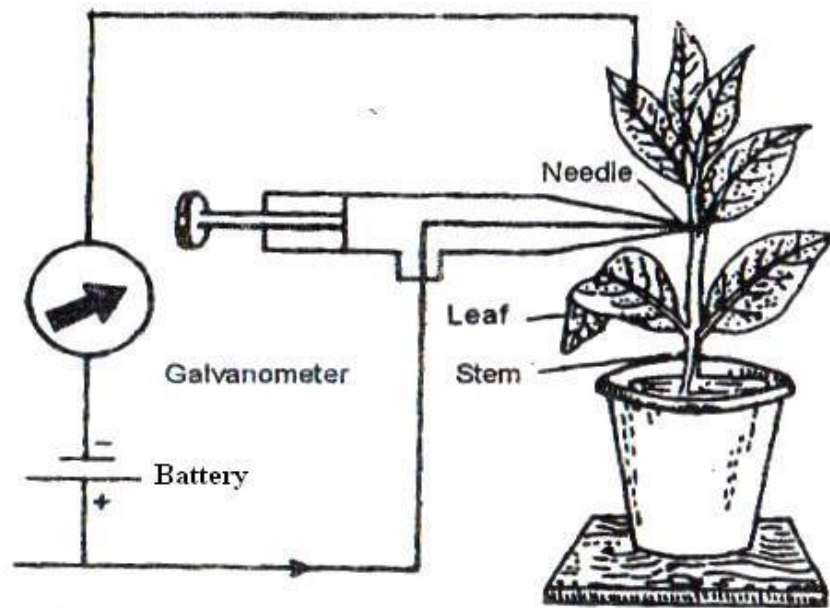
Water is absorbed by the medullary ray cell from the xylem vessels on one side and is given over at a higher level to vessel on other side. The transport inside the living cells is carried out by protoplasmic streaming.

(iv) J.C. Bose's pulsation theory (1923) :

According to Sir J.C. Bose, the living cells of the innermost layer of the cortex lying just outside the endodermis show regular pulsation which cause upward movement of water.

He constructed an apparatus to demonstrate this activity. It consisted of an electric probe, a galvanometer , an electric circuit and a plant. One of the terminal of galvanometer was connected to a probe (needle) and another with some point in the plant. When the probe was inserted into the stem , a few oscillations could be seen in the galvanometer. The oscillations become violent

when needle reached the innermost layer of the cortex. This led Bose to believe that the cells of this layer show pulsations i.e. they contract and expand alternately. The cell gets expanded by absorbing water from the cell below it. It then contracts and pumps the water into the cell placed above it. No such pulsatory activity could be seen in the xylem. Thus he concluded that xylem acts only as a reservoir and the pulsatory cells carry the water upwards.



The electric probe of Sir J.C. Bose.

The theory was criticized because:

- (i) There was no relation between the pulsatory activity and rate of translocation.
- (ii) The actual rate of sap flow is 8000 to 30,000 times as rapid as would be possible under the theory proposed by Bose.
- (iii) Some workers have suggested the activity of living cells in the ascent either through :- (a) the creation of bio- electric potential of xylem walls or (b) osmotic relations between living cells and newly differentiated xylem elements.

(II) Root pressure theory:

If a stem is cut below the first leaf or near its base, the xylem sap can be seen to flow out from the cut end. This is called **exudation** or **bleeding**. According to **Priestley**, exudation was due to the hydrostatic pressure. It can be shown experimentally. A potted plant is watered profusely. The stem is cut near the base, below the lowest leaf. A mercury manometer is attached to the cut end of the stump with the help of rubber tubing. After sometime the level in the manometer rises, indicating that water is being forced upward from the cut end of the stump.

Stocking described root pressure as a pressure developing in the tracheary element of the xylem as a result of the metabolic activity of the root.

Root pressure can be observed in many plants, but it alone does not seem to be a force that can translocate water to the top of the tallest plants. In this connection, the following objections are put forward:

- Root pressure can generate about **2 atm** of pressure while of about 20 atm would be needed for raising the water to height of 125m.
- In some plants like conifers, root pressure has never been observed.
- In temperate region, root pressure is generally low during summer, when the rate of transpiration is high.
- Ascent of sap continues even in the absence of root pressure.

(III) Physical theories:

(1) Capillarity theory:

Water rises in the narrow tubes due to the forces of surface tension. This phenomenon is called as **capillarity**. The theory was put forward by **Boehm**. He believed that as xylem vessels are narrow, the ascent of sap may be caused by it. The various objections to this theory are:

- (i) The value of capillarity is very small. Water can only rise to a height of little more than one metre in the normally occurring vessels having a diameter of 0.03mm.
- (ii) The vessels do not have the uniformity of the lumen on account of presence of different types of thickenings.
- (iii) Capillarity cannot operate in plants having tracheids in the xylem due to the presence of endwalls.

(iv) Soil water is not connected with the xylem vessels directly.

(2) Jamin's Chain theory :

Jamin thought that air and water were present in regular alternate layers in xylem. The water was pushed upwards when the air expanded. The theory could not be accepted because it does not account for the rapid unidirectional flow of water.

(3) Imbibition theory:

Imbibition is the adsorption of water by the hydrophilic colloids . Unger was first to suggest that the upward movement of water takes place by imbibition . Sachs advocated the theory. According to him the water moves upwards due to imbibitional force between the cell wall of the xylem and the water column. The theory did not gain any importance because it is known that water moves through the lumen of xylem elements and not along their walls.

Atmospheric pressure theory:

When water is lost by transpiration , the pressure at the transpiring surfaces falls below that of the atmosphere. If the atmospheric pressure acts on water from below , water will rise upwards to fill up the gap in pressure. The main objections to this theory are :

- (i) There is no free surface at the lower end of the plant which is necessary for the operation of atmospheric pressure.
- (ii) Even if complete vacuum is created at the transpiring end, the maximum height to which water can rise is just over 10m.

(5) Transpiration pull or tensile strength of water:

This is the most widely accepted explanation for the ascent of sap. It was proposed by **Dixon** and **Jolly** in 1894. This theory is based essentially upon the following three facts:

(i) Cohesive force or tensile strength of water :

The water molecules have a strong mutual attraction i.e. they tend to stick to each other. This property is called as **cohesion**. They also tend to stick to the wall of the xylem elements : this is called **adhesion**. A high cohesion of water molecules means that a relatively large tension is required to break a column of water.

(ii) Continuity of water column:

Water in the tracheids and vessel of leaf veinlets is continuous from the leaves to the roots. The cohesive and adhesive forces are very great and do not allow the water column to break or pull away from the walls of the xylem. In fact , the cohesive force or the tensile

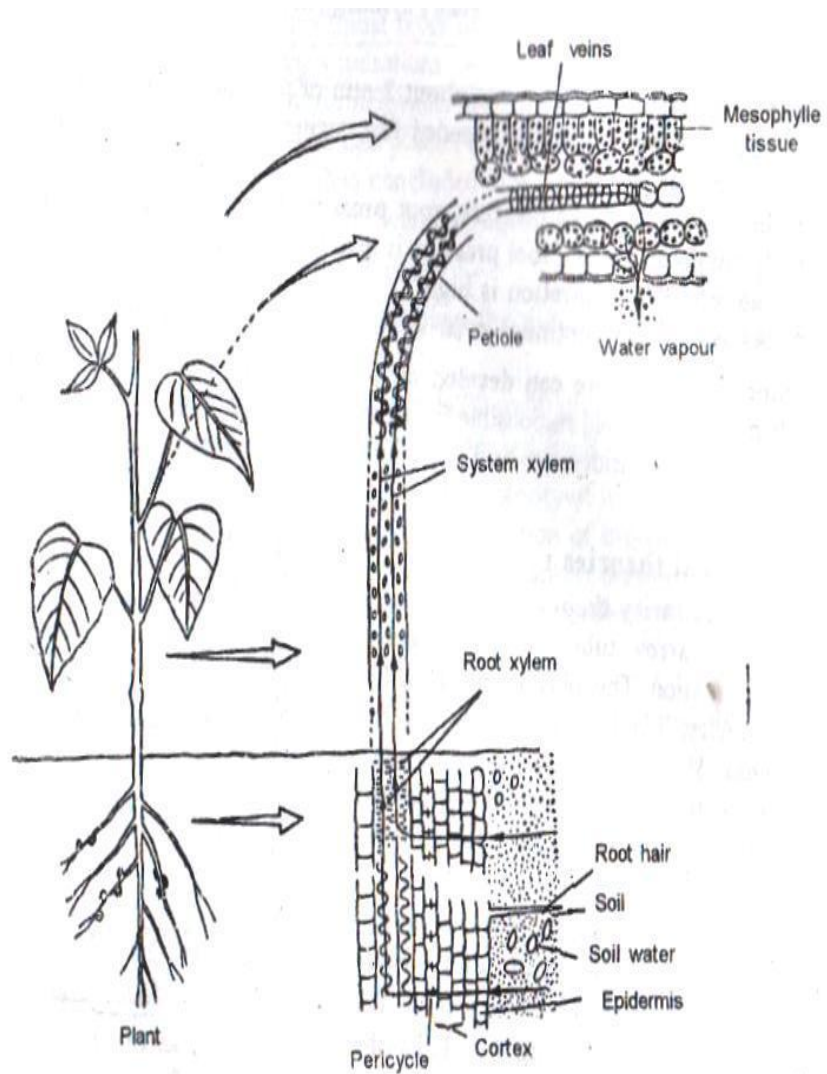
strength of sap in the xylem vessels and tracheids may stand tension greater than 300atm.

(iii) Transpiration pull:

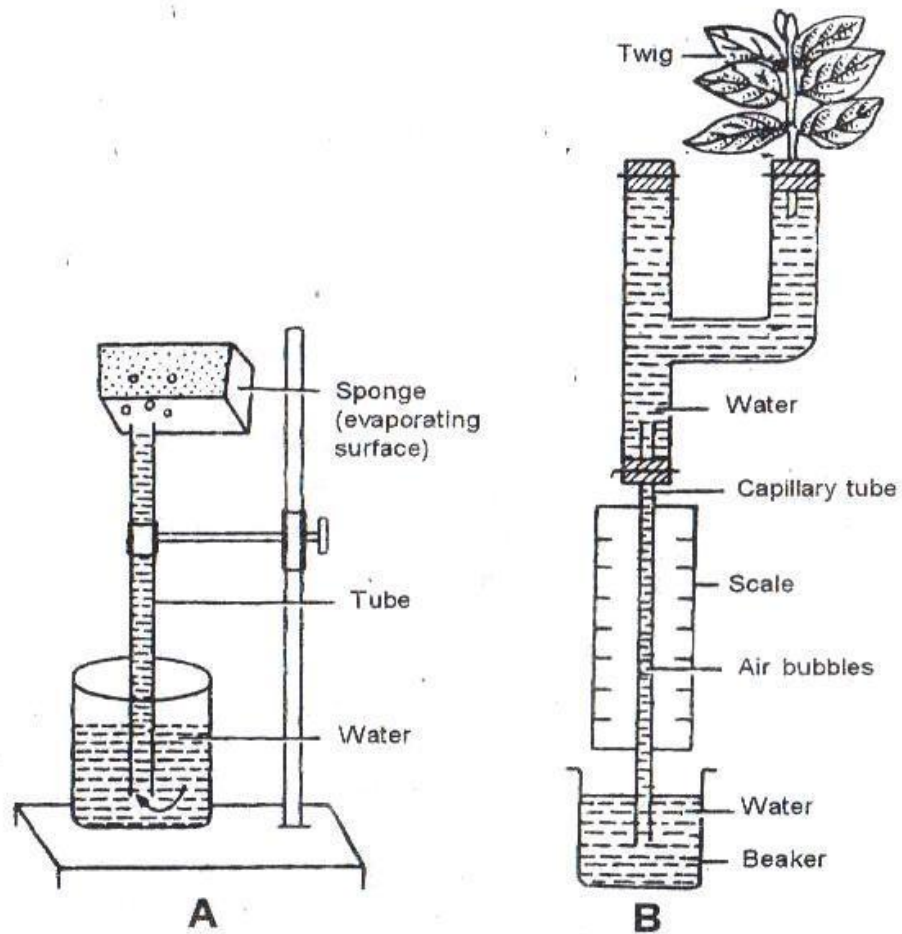
Water evaporates from the mesophyll cells of the leaf due to transpiration. This results in an increase in their diffusion pressure deficit (DPD) or suction pressure. Since the water in the mesophyll cells is in contact with the xylem sap of stem and roots through tracheids in the veins the DPD gradually passes down to the xylem of the root and water is pulled up. Thus due to transpiration there is a constant pull or tension in water column of xylem due to transpiration.

To demonstrate transpiration pull, imagine a physical system consisting of a long hollow glass tube, one end of which is dipped in a beaker of water. The tube is also filled with water and now a piece of sponge thoroughly soaked in water is placed at the top of this tube. Thus there is an unbroken water column between the sponge and the beaker. As the water evaporates from the sponge, it is pulled from the beaker. The continuity of water column in the tube does not break even under greater pull due to cohesive and adhesive properties of water.

An almost similar system exists in the plants. The sponge can be compared with the transpiring surface (i.e. leaves), water filled glass tube with glass tube with xylem and beaker with the soil which serves as a water reservoir. The steps involved in ascent of sap can be summarized as follows:



Ascent of water due to transpiration pull



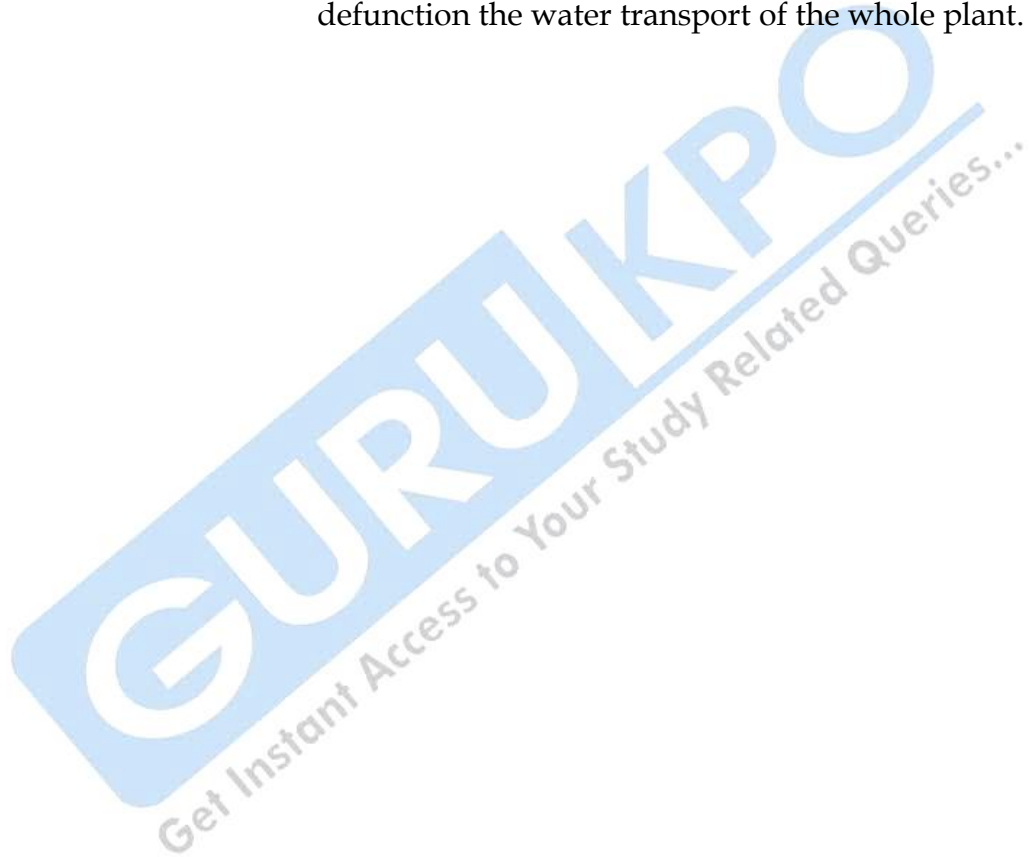
Demonstration of transpiration pull : A. In a physical system; B. As in plants.

- The evaporation of water from the walls of the mesophyll cells of leaves results in an increase in their diffusion pressure deficit (DPD).
- These cells therefore pull water from the neighbouring cells and this pull gradually passes to the xylem of the leaf.
- Since the xylem of the leaf is in continuity with the xylem of the stem and root, the transpiration pull is transmitted down to the root which regulates absorption. This results in the upward movement of water in continuous liquid columns in the xylem.

- The continuity of water column is maintained even under conditions of considerable strain (pull) because of the strong cohesive forces between water molecules.

Objections to Dixon's Theory:

- (1) The theoretical value of cohesion force of water is much higher than the experimental value. This decrease is due to the presence of dissolved gases.
- (2) Air bubbles are frequently found in the tracheids and vessels. They increase in size and number with increase in temperature and tension. As such they should defunction the water transport of the whole plant.



Chapter 9

Biomolecules

Q1 Describe in detail about the Primary Structure , Secondary Structure and tertiary structure of protein in detail, giving a brief account of the properties of proteins.

Ans

Proteins

The term protein was suggested by **Berzelius** and used by **G.J. Mulder** for the first time . Proteins are present in each cell in one form or the other. Proteins account on average about one fourth to one third of the total dry weight of any living organism. Proteins take part in the structural organization of several cellular components.

The Chemical Structure of Proteins

The proteins are big molecules built up from several smaller units. Backbone of all protein molecules is made up of linear chains of polypeptides. These polypeptides are made up of amino acids. The smallest protein known is insulin which has 51 amino acids. Examples of some bigger proteins are chymotrypsin (246 amino acids) and human haemoglobin (574 amino acids) . Union between amino acids involves amino ($-NH_2$) and carboxyl ($-COOH$) groups and releases water . Such bonds ($=CO-NH$) are called peptide bonds. At the end of one polypeptide chain , there is a free or acetylated amino group called N- terminus and at the other end a free or amidated carboxyl group called C-terminus , is present.

Polypeptides are the simplest proteins. Most of the natural proteins , are complex polypeptides and have different configurations.

Polypeptides are named according to the amino acids constituting them. For example , if Glycine , alanine and serine combine in the following manner , a tripeptide named glycyl, alanyl , serine will be formed.

Naming of the polypeptide starts with N-terminal and proceeds one amino acid at a time to the end of the chain (C-terminal) . Each amino acid except the last one is named as an acyl group.

The Physical Configuration of Proteins:

The linear chains of polypeptides are arranged in space in various ways. For example, these may stick together to form a sheet or may bunch up randomly into a ball or arrange in a highly ordered screw. These arrangements give rise to the secondary structure of proteins. The details of the secondary and tertiary structures of proteins were worked out initially by two American scientists, L. Pauling and R.B. Corey with the help of X-ray crystallography.

Primary Structure :

Arrangement of amino acids in a polypeptide chain is the primary structure of a protein. For determining the primary structure of a protein, the number and sequence of amino acids in the polypeptide is to be known. The covalent polypeptide bond is the only type of bonding involved at this level (primary) of protein structure. The peptide bond determines the three dimensional structure of the protein, because this bond is a resonance between single bond and double bond.

Actual structure of the peptide bond is a compromise between above two structures:

As a result of double bond, characteristic of the C- N there is no free rotation about this axis. However, the polypeptide chain can rotate on either side of this peptide bond that is on C- C or N- C axis. The amount of rotation at the bond between the nitrogen and the α -C atom (N- C) of the chain is called Phi (ϕ) and the rotation at the one end between α -C atom and the carbonyl carbon atom (C- C) is called Psi (ψ). The configuration of polypeptide chain can be determined if the value of ϕ and ψ for each amino acid residue are known.

In a fully stretched polypeptide chain $\phi = \psi = 180^\circ$. G.N. Ramchandran recognized that an amino acid residue in a polypeptide chain cannot have any pair of values of ϕ and ψ angles. Certain combinations are not possible due to steric hinderances. The possible ranges of ϕ and ψ can be predicted and visualized in a graphic diagram called Ramchandran plot.

For determining the amino acid sequence of a polypeptide chain various methods have been developed during the last 25 years. Amino acid sequence of the smallest protein known, that is of insulin, was studied by Fredrick Sanger in 1954. Insulin consists of two chains, A and B, joined together by two disulphide bonds. A disulphide bond is present in A chain also.

One of the larger protein links analysed for amino acids is chymotrypsinogen, which is composed of 246 amino acids. The linear chain is held in a definite shape by 5 disulphide (S-S) bonds.

Normal human haemoglobin is composed of 4 polypeptide chains and 4 haeme groups, of two identical pairs where each pair consists of an α - and a β - chain. The α -chain is made of 141 amino acid residues and contains relatively more acidic groups than the β -chain. (with 146 residues). The two chains are held together by ionic and hydrogen bonds. Myoglobin with 153 amino acids contains only one polypeptide chain. Both Myoglobin and haemoglobin contain the prosthetic group haeme, a metal organic compound closely related to porphyrin component of chlorophyll. The oxygen combining properties of Myoglobin and haemoglobin are due to the presence of haeme.

Secondary Structure :

The polypeptides are held together to give rise to a definite shape of the protein molecules by some secondary forces such as hydrogen bonds. Hydrogen bonds are formed between the carbonyl oxygen and imide hydrogen along the polypeptide backbone ($\text{N}-\text{H} \cdots \text{O}=\text{C}$). The hydrogen and oxygen atoms are separated apart by a distance of $2.8\text{\AA}$ and the energy of these bonds is about 1400 cal / mole . The second feature of the secondary structure is the bending and folding of polypeptide chain.

The most common type of secondary structure is alpha helix or alpha structure. This is formed by the bending of the polypeptide chain to form hydrogen bonds in the same chain. The bending of the chain is very regular and chain assumes the shape of a helix. It has been revealed by X-ray analysis that large sections of polypeptide backbone of Myoglobin are folded into α -helices. This type of secondary structure is found in many other proteins of wool, hair and other fibrous structures also. In the α -helix of Myoglobin and probably in others too, each amino acid is away from the other at a distance of $1.47\text{\AA}$ and there are 3.7 amino acids in one turn. Distance of **one turn of the helix**, which is $(3.7 \times 1.47) . 5.4\text{\AA}$ is the **identity period of α -helix**.

Screwing of the helix may be right handed or left handed and side chain may stick out of the chain proper. But almost all naturally occurring proteins have right handed alpha helix. Sometimes the side chains interact with each other, and the regularity of the chain is disturbed.

Pauling and Corey have considered an alternate type of secondary structure. In this case the polypeptide chains are laid linearly either parallel or antiparallel with respect to one another. This structure is called Beta structure or β -pleated sheet. β -structures are quite common in nature and are favoured by the presence of amino acids, glycine and alanine. Silk and certain synthetic fibres such as nylon and orlon are composed of β -structures.

Tertiary structure:

Three dimensional configuration of proteins is very common in the biological systems. This is the tertiary structure of proteins. The helices and other regular arrangements are disturbed in the nature due to the following reasons (1) since an amino group is absent from proline, its presence in a chain does not allow normal hydrogen bonding. (2) disulphide bridges between cysteine molecules join various parts of the polypeptide chain and regular helix is disturbed. And (3) side chains have different configurations. They can be linked together or with the solvent molecules and cause the chain to fold.

These phenomena result in the tertiary structure of the protein which is energetically most stable form and is represented by most of the biologically active proteins. The only covalent linkage involved in tertiary structure is disulphide bond formed by the oxidation of two cysteinyl groups.

residues. However, there are several non covalent interactions such as salt linkages, hydrogen bonds, dipole – dipole interactions stabilizing the tertiary structure.

Quaternary Structure:

Quaternary Structure of proteins concerns interactions by which two or more polypeptide chains are joined to form an ordered biologically active protein. Many of the enzymic proteins have this kind of structure. The individual polypeptides are called sub- units or protomers and ordered total protein is called oligomer. The forces involved in binding the protomeric sub – units are same as those in tertiary structure. For example, hydrophobic interactions and electrostatic attractions are quite common in oligomeric proteins. If the sub –units in a oligomer are identical, the protein is said to be homogeneous. However, if the sub-units are different, the protein is heterogenous. For example, haemoglobin is a heterogenous oligomeric protein as it consists of two alpha chains and two beta chains.

High temperature or some other unnatural conditions like high acidity and high alkalinity, etc breakdown the native 3 –dimensional form to give randomly oriented, biologically inactive proteins. It is denaturation of the protein. When the denatured chains are carefully returned to their original environment, some of them can resume their native configuration with full biological activity (renaturation). Denaturation is specially reversible in proteins containing some disulphide bonds.

Properties of Proteins:

Solubility :

Earlier the proteins were classified on the basis of their solubility. **Albumins** are soluble in distilled water whereas **globulins** are soluble only in dilute salt solutions of neutral pH. **Leucosin** in the seeds of the cereals and ricin in castor oil seeds are examples of plant albumins while **legumin** from peas and tuperin from potato tubers are examples of **globulins**. Some proteins are soluble in acidic or basic solutions, for example, **glutenin**, which is the most common protein of cereals. **Prolamines** is another class of proteins which are soluble in alcoholic solutions. **Gliadin** of wheat and zein of maize are examples of this class. **Scleroproteins**, which are abundant in horns, nails and hair are relatively insoluble and are hydrolyzed only in special conditions. Most of the digestive enzymes are also unable to hydrolyse this protein and that is why it is not digested by most of the animals. Some insects and fungi are able to utilize it as food material.

There are some basic proteins also which are rich in basic amino acids ; arginine, and lysine. They are combined with nucleic acids to form nucleoproteins and are thus formed in the nucleus of the plant and animal cells. Histones and protamines are examples of this class.

Zwitterion formation by protein:

Like amino acids , proteins are also charged either positively or negatively and thus migrate either to anode or cathode of an electric field. At isoelectric point (pI) they are electrically neutral and do not move towards any pole. The isoelectric pH of the proteins depend upon the relative number of acidic or basic groups, which are rendered by amino acids . Serum albumin, which contains many acidic amino acids has an isoelectric pH of 4.7. Gliadin with many basic amino acids has 9.0 isoelectric pH. Isoelectric pH of certain other proteins are given in table below:

Isoelectric pH of some proteins

Protein	PI	Protein	PI
Casein	4.6	Cytochrome C	9.8
Avidin	10.0	Lysozyme	11.0
Pepsin	2.7	Tetanus toxin	5.1
Serum globulin	5.4	Edestin	6.9

Denaturation:

Under certain conditions the spatial arrangement of polypeptide chains within a protein molecule is changed from native form to a disordered arrangement . This has been termed as denaturation of protein by Kauzmann . Thus during denaturation , only primary structure of the protein is retained. Physical and chemical properties of such proteins are different than the native proteins and they lose most of their biological activities. During denaturation , the soluble globular proteins are changed into insoluble fibrous proteins. Commercial fibres are obtained from globular proteins by treating them with various denaturing agents.

There are several chemicals which can denature the proteins. Mineral acids and alkalies , acetone , alcohols . salts such as ammonium sulphate, urea and tungstic, picric and trichloroacetic acids are common denaturing chemicals. Hydrostatic pressure , ultraviolet and ionizing radiations also cause denaturation., these agents cleave the hydrogen and / or salt linkages and thus the polypeptide chain is unfolded.

Hydrolysis of proteins:

Proteins can be hydrolysed stepwise to their ultimate building units , the amino acids. This hydrolysis can be brought about either by an acid , a base or

hydrolytic enzymes. During stepwise hydrolysis following products are obtained from a protein-

Protein $\rightarrow$ denatured protein $\rightarrow$ metaprotein $\rightarrow$ peptone $\rightarrow$ polypeptide $\rightarrow$ dipeptide $\rightarrow$ amino acids

Complete hydrolysis with about 20% boiling HCl or H₂SO₄ yields free amino acids and their breakdown products. Alkali hydrolysis of the protein is carried out with 20% NaOH solution at 120°C in the presence of nickel. Complete hydrolysis yields a mixture of amino acids and their breakdown products. **Threonine, serine, arginine and lysine** may be partially hydrolysed during alkali hydrolysis.

Hydrolysis of proteins by enzymes is quite common in nature. Proteases split proteins stepwise into amino acids. The organisms later on absorb and metabolise these amino acids. Several proteolytic enzymes are used commercially to prepare amino acids, from the digestion of natural proteins.

Oxidation of proteins:

Proteins are oxidized by putrefaction process. The products are amino nitrogen compounds, CO₂ and water depending upon conditions employed. The bad smell of the dead and decaying bodies is due to putrefaction of the protein.

Q2 Give a detailed account of the various physical and chemical properties of carbohydrates. Also write in brief about the structure of different carbohydrates.

Ans

Carbohydrates are synthesized from CO₂ and H₂O in chlorophyll containing plants during photosynthesis. They are made up of carbon, hydrogen and oxygen.

Monosaccharides:

Monosaccharides have the empirical formula C_n (H₂ O)_n. The value of n ranges from 3 to 8. Glyceraldehyde and dihydroxy acetone are the smallest monosaccharides abundant in the living cells.

Properties of Monosaccharides:

Physical :

Monosaccharides are sweet tasting colourless solids. They are soluble in water, sparingly soluble in alcohol and insoluble in ether. They contain asymmetric carbon atom and exist in different isomeric forms. For example, glyceraldehyde may exist in two forms which are mirror images of each other.

When a polarized light (light vibrating in one plane) is passed through a solution of these carbohydrates, the plane of the light is rotated to either right or left. All monosaccharides which contain a D- glyceraldehyde unit are called D-sugars. L- monosaccharides contain a L-glyceraldehyde unit in their structure.

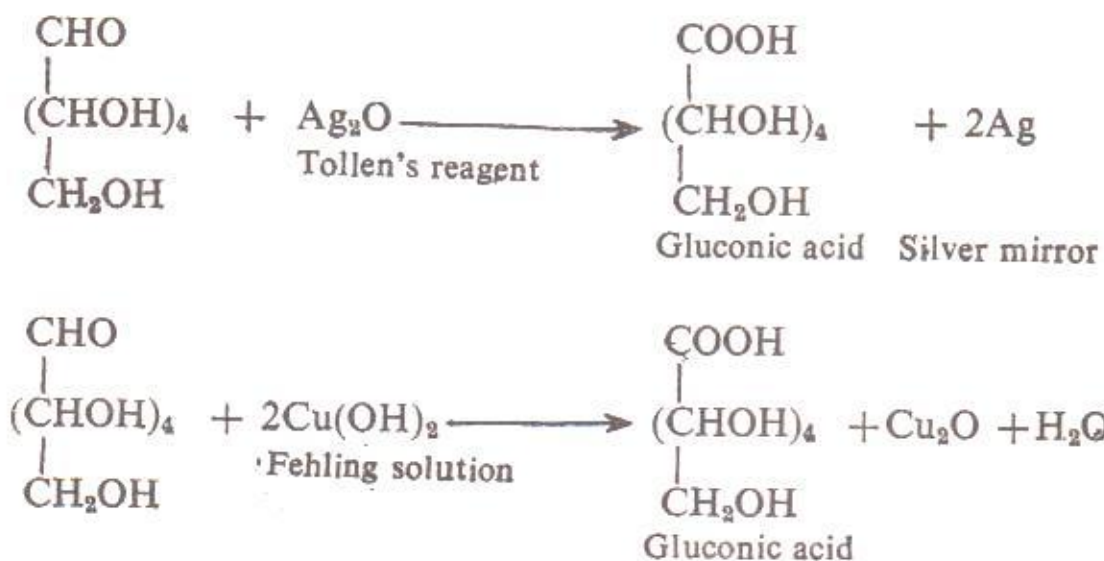
Carbohydrates isolated from living system are a mixture of several isomers. The degree of optical rotation may change due to interconversions of isomers. Fresh solution of glucose gives an optical rotation of $+112^\circ$ which changes to $+52.7^\circ$ on standing. Similarly, fructose gives an optical rotation of -113° which changes to -92° . This change in optical rotation is called mutarotation.

Chemical:

The properties of monosaccharides are based on $-CHO$ or $=CO$ and $-OH$ groups present in them.

(a) Oxidation:

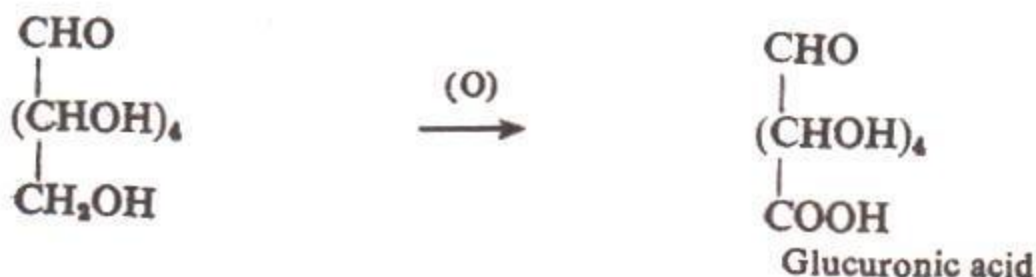
Monosaccharides are easily oxidized by the oxidizing agents. Glucose yields gluconic acid after oxidation with mild reagents such as Tollen's reagent (ammoniacal Ag_2O) or Fehling solution (alkaline $CuSO_4$).



The reduction of Tollen's reagent yields silver as polishing on the surface of the tube whereas with Fehling solution red precipitate is obtained. Similarly, potassium ferricyanide can be reduced to ferrocyanide. These reactions are used in the elimination of glucose and other sugars containing free carbonyl groups. Such groups are called reducing sugars.

When strong oxidizing agents like conc. HNO_3 are used, gluconic acid is ultimately oxidized to dicarboxylic saccharic acid.

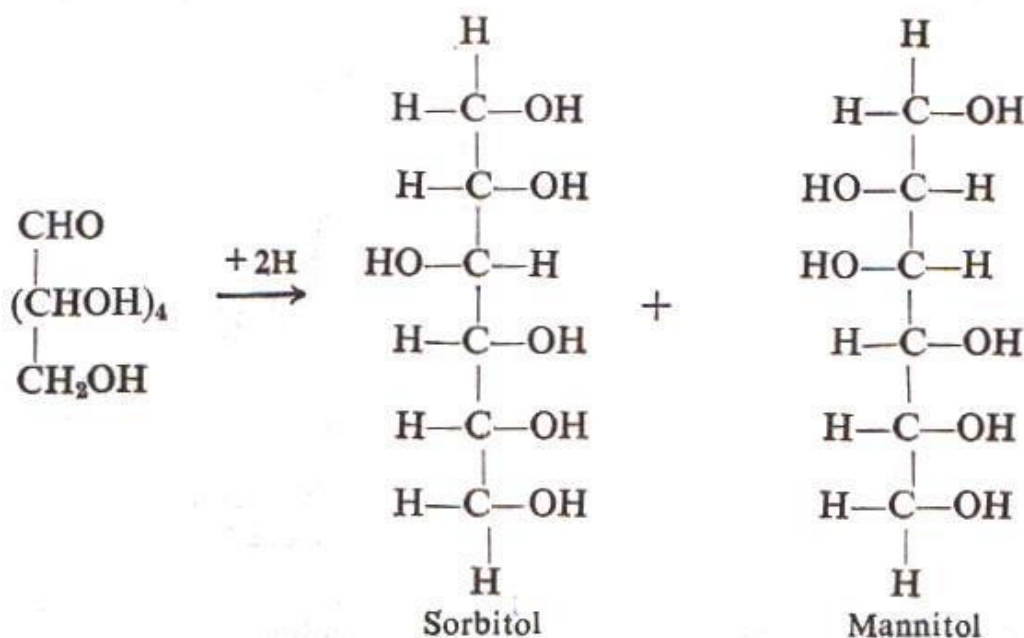
In the animal body, slow oxidation of glucose may yield glucuronic acid. In glucuronic acid, aldehyde group is intact and the primary alcohol group on carbon 6 is oxidized to a carboxylic group.



Glucuronic acid in the animals combines with hormones and also with some toxic substances and the conjugation products are excreted out. It is also a major component of hyaluronic acid, heparin, chondroitine, and mucoitin sulphate which are found in blood, skin and cartilage.

(b) Reduction:

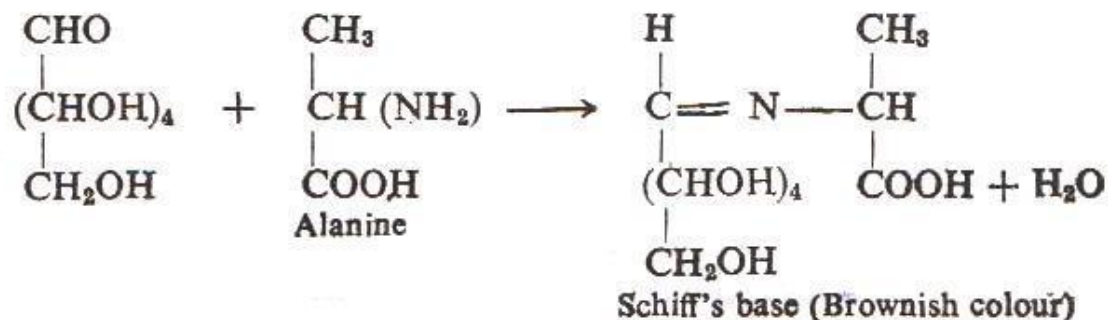
Free aldehyde and ketone groups of monosaccharides are reduced to alcoholic hydroxyl groups by sodium - mercury amalgam and water. Thus, D- glucose after reduction yields a mixture of polyhydroxy alcohols; sorbitol and mannitol.



Electrolytic reduction or catalytic reduction with hydrogen and nickel also yield sorbitol and mannitol. Sorbitol is used to prepare surface active agents, as humectant and mannitol is used in the manufacture of explosives.

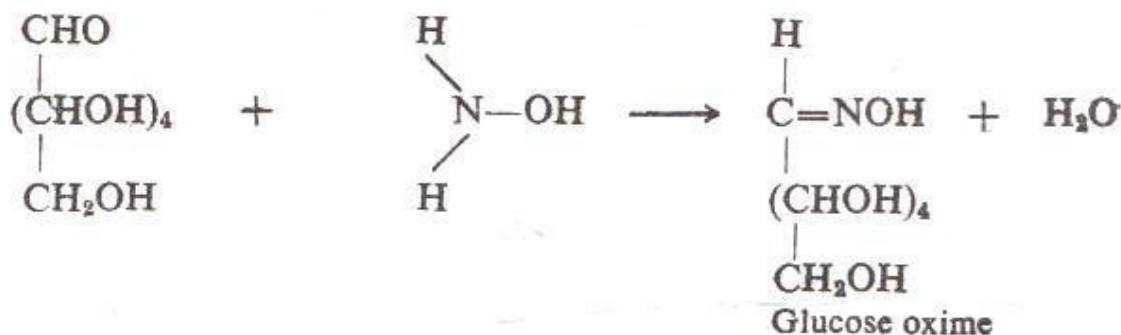
(c) Condensation reactions:

Aldehyde groups of monosaccharides condense with primary amines to form a Schiff's base.



When treated with hydroxylamine, glucose forms glucose oxime.

Free aldehyde and ketone groups of sugars react with phenylhydrazine to form a special type of Schiff's base, hydrazone, which is then converted to a double Schiff's base, osazone. The osazones of reducing sugars are yellow crystalline, insoluble in water.



(d) Reaction with HCN :

Addition of HCN to sugars containing free aldehyde or ketone groups yields cyanohydrin.

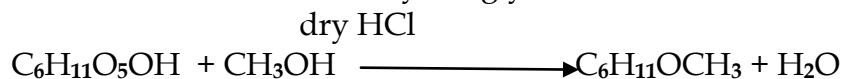
(e) Esterification:

The hydroxyl groups of alcohols in the carbohydrate may be converted to esters by treatment with the appropriate acetylating agents. For example, when D-glucose is treated with acetic anhydride in the presence of pyridine or anhydrous ZnCl_2 , penta-acetyl glucose is formed.

Glucose can be esterified with phosphoric acid also; yielding glucose 1-phosphate or glucose 6-phosphate. The phosphate esters of glucose and fructose are important compounds in carbohydrate metabolism.

(f) Methylation:

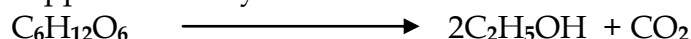
Methylating agents such as $\text{CH}_3\text{I} + \text{Ag}_2\text{O}$, $\text{CH}_3\text{OH} + \text{HCl}$, CH_3Cl , $(\text{CH}_3)_2\text{SO}_4$ etc react with monosaccharides to yield glycoside.



The glycosides may further react to form tetramethyl esters. Polysaccharides also undergo Methylation.

(g) Fermentation:

Monosaccharides like glucose and fructose undergo alcoholic fermentation by micro-organisms such as the yeasts and produce ethanol and CO_2 . The yeast supplies the enzyme for fermentation.

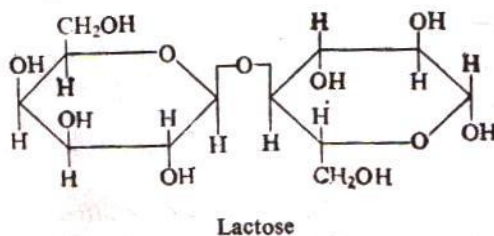


Structure of Monosaccharides:

Naturally occurring sugars exist in true structural forms : either straight chain or ring. About 1 to 10 per cent of the total sugars occur as straight chain whereas the remaining bulk in the form of a closed ring. The property which lead to the confirmation of straight chain structure in glucose was , its conversion to polyhydroxy heptylic acid via cyanohydrin. Since, during this reaction HCN was added to terminal carbon which was then hydrolysed to a $-\text{COOH}$ group; the carbonyl group of glucose must occupy a terminal position and hence it would be an aldehyde. The acetylation properties of glucose have proved it to be a pentahydroxy molecule. The correct position of asymmetric carbon in a glucose molecule was determined by Emil Fischer in 1886. These methods were used for the determination of structure of other monosaccharides also. The structural formula (linear chain) of some important monosaccharides are as follows:

Lactose :

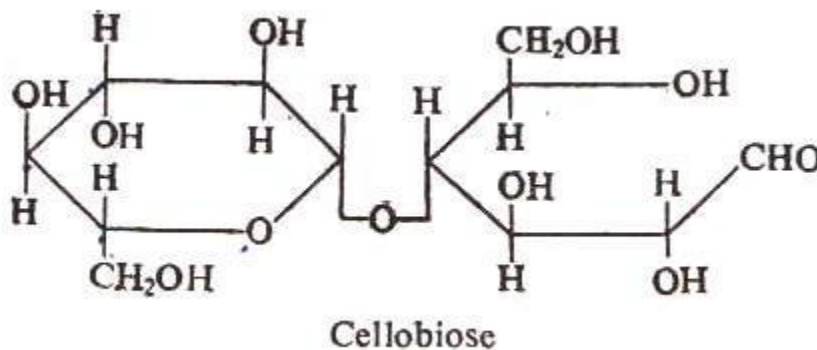
Lactose is made up of glucose and galactose units joined together through carbon 1 of galactose and carbon 4 of glucose. It is also a reducing sugar because open chain configurations also exist. Lactose occurs naturally in the milk of mammals up to 5%. It has been detected in the flowers of some plants (*Forsythia sapotacea*) also. Hydrolysis of this sugar yields galactose and glucose.



Cellobiose :

Cellobiose is composed of glucose units (1-4 linkage) , It may also exist in reducing form with a free aldehydic group. Alternately the ring may close to form

α - or β -cellobiose. Cellobiose is present only in trace amounts in nature. The cellulases present in micro-organisms and growing seedlings are able to hydrolyse plant cellulose to cellobiose. Its further hydrolysis yields D-glucose units.



Polysaccharides :

Polysaccharides are composed of several monosaccharide units joined together by glycosidic bonds. The most frequent monosaccharide unit in polysaccharides is D-glucose, although D-fructose, D-galactose and other hexoses also occur. Starch, cellulose and glycogen are examples of homopolysaccharides while hyaluronic acid, pectins and chitins are heteropolysaccharides.

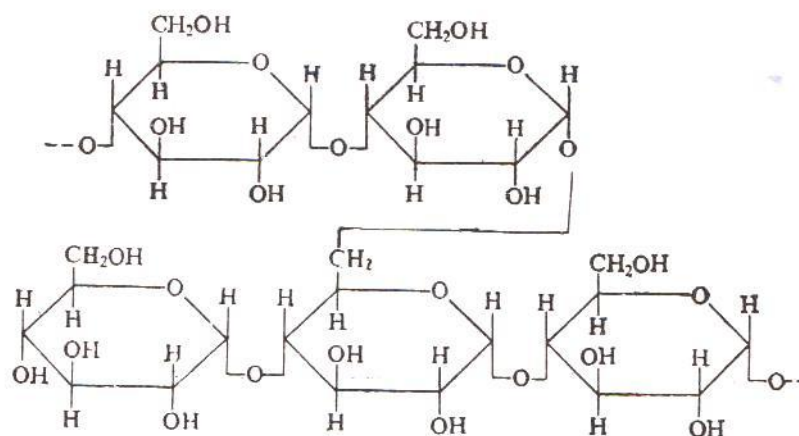
Polysaccharides are tasteless, and colourless amorphous powders which are little soluble in water, although some may form colloidal solutions. They are easily hydrolysed to their monosaccharide units.

Starch :

Starch is a high molecular weight polysaccharide made up of two components, amylose, and amylopectin. The amylose is an unbranched chain of 300 to 1000 α -D-glucose units with 1-4 glycosidic bonds. The amylopectin is also made up of α -D-glucose units but is branched like a tree. The main chain has 1-4 linkage with 1-6 linkage at branching points.

The ratio of amylose to amylopectin in starch is different for different tissues. The starch from normal maize seed contains about 20% amylose and 80% amylopectin. Waxy maize, a recently developed variety of maize, contains almost 100% amylopectin.

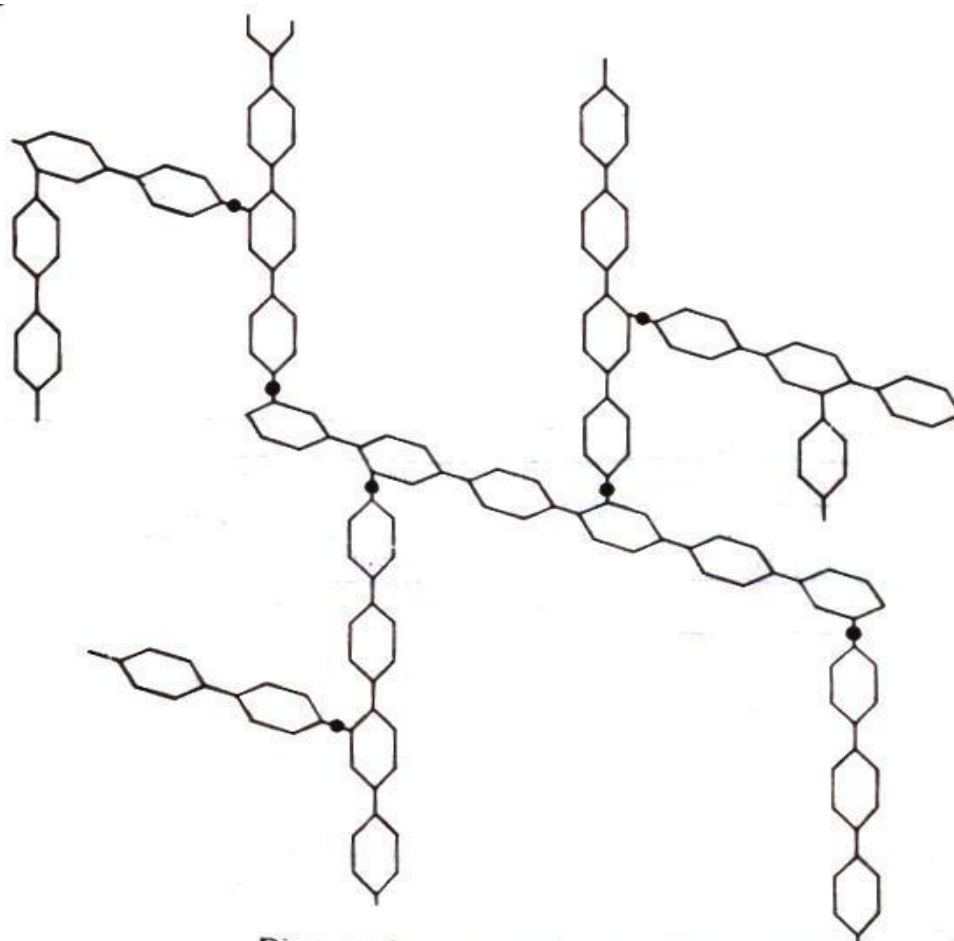
Starch is the storage carbohydrate of plants. It occurs as grains of 1μ (e.g. rice) to 100μ (e.g. potato) size in the chloroplasts or amyloplasts. The grains contain about 20% water, about 10% of which is chemically bound to starch. The shape of the grains may be oval, spherical, lens shaped or irregular. The starch in the grains is contained in the form of layers. Potato starch grains are covered with thin layers of cellulose.



Amylopectin

Glycogen :

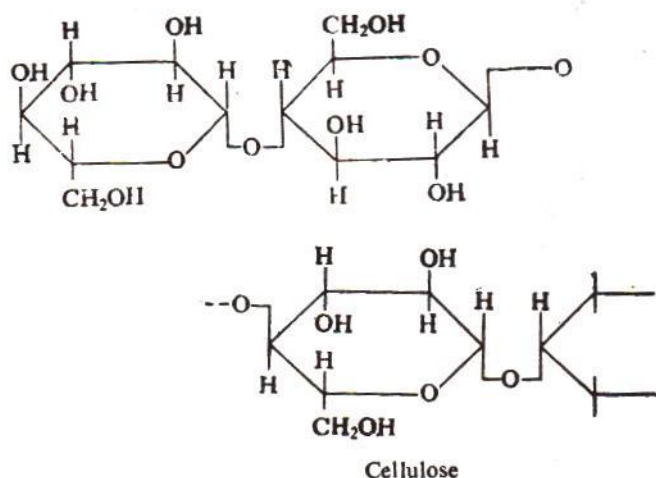
The storage carbohydrate of animals is glycogen. It is similar to amylopectin in its structure except that the molecular weight is lower and the chains are shorter. The branching is also extensive, each branch containing about 12 glucose units. Glycogen is also found in the liver and the muscles. In the liver it is split to glucose to maintain its (glucose) proper concentration in the blood. It dissolves in water to yield opalescent solution and is easily hydrolysed to α -D- glucose.



Diagrammatic representation of glycogen molecule.
Each hexagon represents a gluco-pyranose unit.

Cellulose :

Cellulose molecule is composed of 1600 to 2700 β -D glucose units with 1-4 glycosidic linkage. It is a long thread like molecule. Cellulose is the major structural polysaccharide of plants. Careful acid hydrolysis of cellulose yields a series of oligo - saccharides such as cellobiose , cellotriose , cellotetrose etc. Complete hydrolysis permits recovery of about 95% of the weight of cellulose as glucose. Enzymatic hydrolysis of cellulose is brought about by cellulase which is present in some protozoans and mites. Some of the bacteria in cattle and other herbivores also secrete cellulase that enables the cattles to digest the herbal cellulose to glucose.



Inulin:

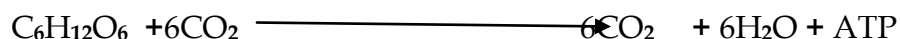
Inulin is an unbranched molecule of about 30 -35D -fructose units , m.w. 5000 and the glycosidic linkage is between carbon 1 and 2 of the fructose units. Inulin is obtained commercially from Jerusalem artichoke rhizome. It is found naturally with other short chain fructans. The enzyme inulase hydrolyses inulin to fructose.

Biological Significance of Carbohydrates:

Carbohydrates are involved in the structural organization of many tissues , both in plants and animals . Cellulose , hemicelluloses (xylans and mannans) and pectic substances (galactouranans , arabans and cellulose) are common structural carbohydrates in plants. The primary cell wall in plants contains about 43% of cellulose. Lignin is also present in the cell walls of the older tissues. Chitin , hyaluronic acid and chondroitin sulphates are important structural polysaccharides of animals. They are present in the shells of lobsters , crabs and insects and in the cartilage , adult bones , heart valves and cornea.

Carbohydrates are stored as reserve products in many tissues, Normally the starch is stored in seeds , tubers and rhizomes. In some plant families , such as Compositae, Campanulaceae , the stored carbohydrate in rhizomes and tubers is inulin. Sucrose , (sugarcane , beet) , glucose (grapes) and fructose (fruits) are also stored. Glucose is the most common carbohydrate of human blood where it is present in a concentration of 1g /l. In animal cells , glycogen is the storage polysaccharide.

The most important role of carbohydrates is the production of energy in the form of ATP both in plants and animals. The polysaccharides such as starch and glycogen are converted to glucose by enzymes. The glucose is finally broken down to CO₂ and H₂O and yields NADH which is eventually oxidized to produce ATP.



In overall oxidation of 1 molecule of glucose , 38 ATP molecules are generated.

Q3 Write a detailed account of Nitrogen Fixation?

Ans

The commonly used term 'nitrogen' correctly refers to the nitrogen atom (N) . The molecular nitrogen symbolized as N_2 or $N = N$, is actually a dinitrogen. The atmospheric nitrogen represents molecular nitrogen. It is an inert gas and cannot be utilized directly by higher plants. They utilize it only when the nitrogen is fixed. i.e. it is converted into nitrogenous salts or nitrate and ammonium ions. The mechanism by which the molecular nitrogen is converted into fixed form of nitrogen either by reacting with certain elements or compounds or by any means , is called nitrogen fixation.

The nitrogen fixation in nature is of the following two types:

(i) Non biological (Physical / Chemical)

(ii) Biological

(i) Non - Biological (Physical) :

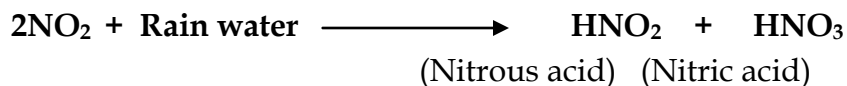
Non -biological N_2 - fixation is usually found in rainy seasons during lightening, thunder storms and atmospheric pollution. It occurs in several steps and starts with combination of atmospheric nitrogen with oxygen under the influence of electric discharge and thunder to produce nitric oxide.



This nitric oxide is then oxidized to nitrogen peroxide in presence of O_2 .



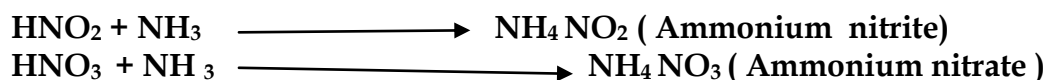
During rains , NO_2 combines with rain water to form nitrous acid and nitric acid which come to ground along with rains.



On ground the alkali radicals of the soil react with nitric acid to produce nitrites and nitrate which are soluble in water and which can be absorbed by the plants through roots.

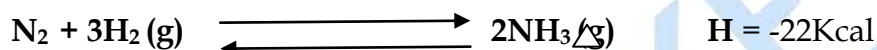


NH_3 present in the soil may also react with HNO_2 or HNO_3 to form ammonium nitrite or ammonium nitrate respectively.



(ii) **Non- biological (Chemical) N_2 - Fixation:**

Nitrogen can also be fixed by chemical processes and this was quite important during the first world war., when German scientists had to develop this method due to unavailability of nitrate for their explosives. This is known as Haber's process and commercially it is important as the most economical method . In this process , synthetic NH_3 is made by passing a nitrogen hydrogen mixture through a bed of catalyst consisting essentially of iron oxides.



By using a temperature of about 500°C and a pressure of about 1000atm , there is about 50% conversion of N_2 to NH_3 .

Besides Nitrogen can also be fixed chemically in nature through electrical discharges. Lightening discharges initiate the otherwise slow combination of N_2 and O_2 to NO which in turn is oxidized to NO_2 .



Biological N_2 Fixation:

Conversion of elemental nitrogen or gaseous nitrogen into nitrogenous compounds or salts by certain microorganisms like bacteria , blue green algae , fungi etc. is called **biological nitrogen fixation**.

Biological N_2 -fixation is mainly carried by two types of micro -organisms (i) free living or non -symbiotic and (2) Symbiotic .

Free -Living N_2 - Fixing Micro Organisms :

- (iii) **Bacteria :** The soil contains a special type of bacteria called nitrogen fixing bacteria.

They convert free N_2 of the soil into soluble compounds which are absorbed from the soil by plants. These can be classified into following categories:

- (a) **Strict anaerobic bacteria:**
Clostridium pasteurianum , Clostridium butylicum , Desulfovibrio. , Methanosarcina bakere.
- (b) **Facultative anaerobic bacteria :**
Klebsiella pneumoniae , Escherichia coli.
- (c) **Aerobic bacteria**
Azotobacter , Derxia
- (d) **Photosynthetic bacteria :**
Rhodopseudomonas . Rhodospirillum , Chromatium
- (i) **Blue Green Algae / Cyanobacteria :**
About 40 species of Blue green algae including **Nostoc** , **Anabaena** , **Oscillatoria** **Tolypothrix**, **Cylindrospermum** etc are present freely in the soil where they fix free N₂ into nitrogenous and ammonium compounds.
- (ii) **By Free Living Fungi:**
e.g. **yeasts and Pullularia** . The yeast present in the soil is called **soil yeast**. A few species of soil yeasts are involved in N₂ fixation . **Pullularia** fungus is also involved in nitrogen fixation.

Symbiotic N₂ fixation:

The fixation of free nitrogen of the soil by microorganisms living symbiotically inside the plants is called **symbiotic biological N₂ - fixation** . The term 'symbiosis' was coined by De bary in 1879. The symbiotic biological N₂ fixation may be grouped under following three categories:

(1) Nitrogen fixation through Nodule Formation in leguminous plants:

About 2500 species belonging to family Leguminosae including common plants like **Pisum** , **Cicer arietinum** , **Cajanus** , **Glycine max** etc produce root nodules containing Rhizobium spp, and Bradyrhizobium spp. These bacteria can fix nitrogen only when they are present inside the nodules. If these bacteria are present outside the nodules in rhizosphere , they will not fix nitrogen. The association of bacteria inside the nodules is symbiotic because the host plant (especially roots) provides space to live and carbohydrates as food for bacteria and in turn bacteria supply fixed nitrogen to the host plants.

When the crop is harvested the root nodules remain buried inside the soil. The bacteria present in the root nodules convert the free nitrogen of air into nitrates. Thus, the fertility of the soil is increased. When the plants of the next crop are grown in this soil, they absorb these soil nitrates in the form of manure through roots. Thus, the soil where leguminous plants are grown remain always fertile and it needs no manuring.

(2) N_2 - Fixation Through Nodule Formation in Non -Leguminous Plants:

In addition to legumes there are many plants specially trees and shrubs belonging to families other than Leguminosae which produce root -nodules. Some of the non - Leguminous plants producing root nodules for N_2 -fixation are:

- (i) **Casuarina** : - It contains bacterium **Frankia** . a member of actinomycetes.
- (ii) **Myrica**: - associated with Frankia .
- (iii) **Parasponia** - associated with Rhizobium.
- (iv) **Sometimes nodules** are also produced in the roots of certain gymnosperms e.g. Podocarpus and in the leaves e.g. **Pavetta zinumermanniana** and Chomelia.

(3) N_2 - Fixation Through Non- Nodulation:

It includes those plants where root nodules are not formed but symbiotic N_2 - fixation takes place. Some of the examples are as follows :

- (i) **Lichens**: They show symbiotic association of fungi and algae.
- (ii) **Anthoceros**: It is a bryophyte which contains Blue Green algae Nostoc inside mucilage cavities present on ventral side of the thallus.
- (iii) **Azolla**: A fern contains bga **Anabaena azollae**.
- (iv) **Cycas**: A gymnosperm which contains **Anabaena** or **Nostoc** colonies in the algal zone of the coralloid roots.
- (v) **Gunnera macrophylla** : An angiosperm whose stem contains Nostoc.
- (vi) **Digitaria, Maize and Sorghum** : Grasses whose roots show symbiotic association with **Spirillum notatum**.
- (vi) **Paspalum notatum**: An angiosperm whose roots show symbiotic association with **Azotobacter paspali**.

Associative Symbiotic N_2 - Fixation:

When the bacteria live in close association with the roots of cereals and grasses and fix nitrogen, the association is of loose mutualism type and is called **associative symbiosis** whereas this nitrogen fixation is called **associative symbiotic nitrogen fixation**. In such association the bacteria live in rhizosphere, a transition zone between soil and root. The bacteria may remain in rhizosphere or may enter into the roots. The bacteria fix nitrogen and supply to the roots and

in return the roots provide carbohydrates for nourishment of bacteria. Some of the examples of associative symbiotism are as follows:

- (i) **Azotobacter paspali** , a N_2 fixing bacteria living in the rhizosphere of a tropical grass - *Paspalum notatum*.
 - (ii) **Azospirillum brasilense** , a bacteria living in the rhizosphere of cereal roots.
 - (iii) **Beijerinckia** , a bacterium living in the rhizosphere of sugarcane.
- Symbiotic N_2 - Fixation In Leguminous Plants:**

In leguminous plants the symbiotic nitrogen fixing bacteria are found in small , knob -like protuberances called root - nodules on the roots of these plants . The root - nodules vary in their size and shape. They may be spherical , flat , finger like or elongated in shape and form pin - head to one centimeter in diameter in size. The genus *Rhizobium* , formerly called *Bacillus radicola*, is mainly found in the nodules.

Depending upon growth the genus *Rhizobium* has been divided into two major groups:

- (i) **Bradyrhizobium** : It is a generic name which includes all fast growing species.
- (ii) **Rhizobium**:

It is also generic name which includes all slow growing species.

The symbiotic N_2 - fixing leguminous bacteria are gram positive , non -spore forming and micro- aerobic. They show a degree of specificity in regards to their host plants. These bacteria can fix N_2 in presence of only specific host. None of these two partners (bacteria and host) alone can fix atmospheric nitrogen. These two partners recognize each other with the help of a chemical substance lectin which are **phytoagglutinins** (a type of carbohydrates containing plant proteins).

Formation of Root Nodules in Leguminous Plants:

The root nodules in Leguminous plants are produced due to infection of bacterium *Rhizobium*. It is free living soil bacteria which usually grows near the roots of legumes and is unable to fix nitrogen in this free condition. The bacterium fixes nitrogen only when it enters into the root and is present inside root- nodules.

The roots of leguminous plants secrete some growth factors like Indole - 3 acetic acid (IAA) or chemical substances in the rhizosphere which help in multiplying the bacterium very fast near the roots. For example, the roots of **Pisum sativum** (pea) secrete homoserine growth factor. In addition to this , the roots also secrete some special carbohydrate containing proteins , called lectins over their surfaces. The lectins help in recognition and attachment of rhizobial cells to the roots. The

rhizobial cells contain carbohydrate receptors on their surfaces. The lectins present on the surfaces of root hairs interact with the carbohydrate receptors of rhizobial cells.

The bacteria either penetrate the relatively soft root hair tip or invade damaged or broken root hairs. First, a **tubular infection thread** is formed in the root hair cell and then the bacteria enter into this thread. After the entry of bacterial cell into the root hair, a new cell-wall is formed which separates the bacteria from the contents of the host cells. The tubular infection thread contains mucopolysaccharide in which the bacteria get embedded and start multiplying. The infection thread containing bacteria grows fast and from root hair cells, reaches to the inner layers of the cortex. The infection thread progresses through cortical tissues to the immediate areas of the endodermis and pericycle.

Leghemoglobin:

The dissection of a root nodule will reveal the presence of a red pigment that is remarkably similar in properties to the hemoglobin of red blood cells. The red pigment of the nodules is called leg-hemoglobin and appears to be product of the Rhizobium - legume complex, since the pigment is not present in either organism grown alone. It is reddish pigment found in the cytoplasm of host cells. As the available space in the host cells is completely filled, the bacterial cells become dormant and are called '**bacteroids**' which now float in leghemoglobin. Leghemoglobin is an oxygen carrier and an efficient O₂ scavenger. Oxygen is necessary for the electron transport chain of the Rhizobium bacteroid. Because of its very high affinity for oxygen, leghemoglobin provides oxygen to the root nodule bacteria quickly. even at very low levels of free oxygen and stimulates ATP production released for nitrogen fixation.

Biochemistry of Nitrogen Fixation:

Although the biochemistry of N₂-fixation is not fully known but the use of isotopic nitrogen i.e. labeled dinitrogen by Schneider et al and Carnahan et al in nitrogen fixing cell free preparations have confirmed the conversion of nitrogen into ammonia.

Biochemistry of Nitrogen Fixation:

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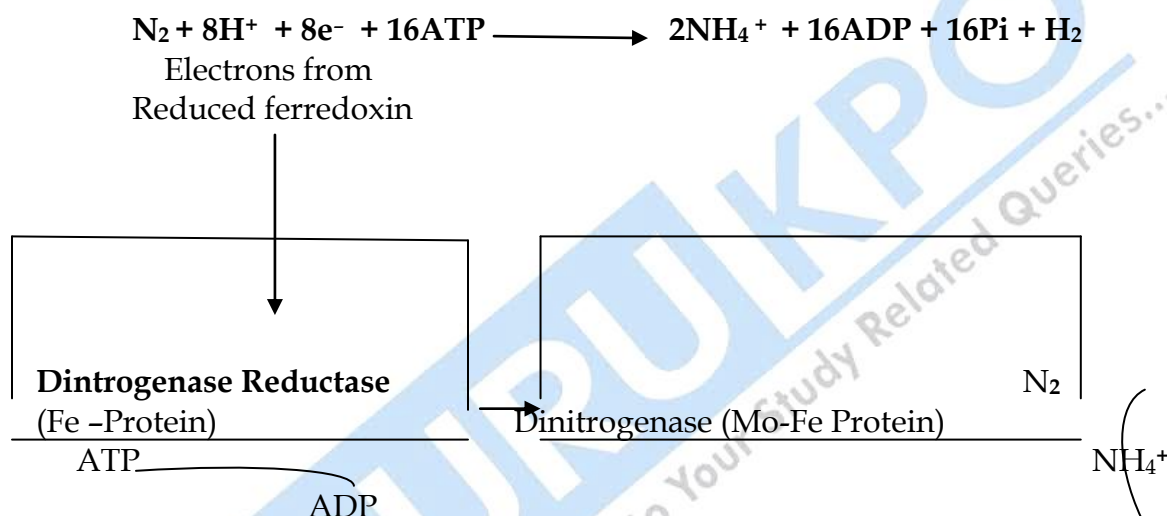
(i) Nitrogenase Complex -Key Enzyme in N₂ - Fixation

The conversion of nitrogen to ammonia is a reduction reaction which is exergonic in nature.



The $\text{N}\equiv\text{N}$ triple bond which has a bond energy of 942KJ/mol , is highly resistant to chemical attack. Lavoisier named it 'azote' meaning without life because it is quite unreactive. The industrial process for nitrogen fixation, derived by Fritz Haber in 1910 and currently used in fertilizer factories, is typically carried out over an iron catalyst at about 500°C and a pressure of 300 atm on N_2 and H_2 to provide the necessary activation energy. Biological nitrogen fixation must occur at 0.8 atm of N_2 , and the high activation barrier is partly overcome by the binding and hydrolysis of ATP.

The stoichiometry of the overall process of Nitrogen fixation can be written as :



Biological fixation of nitrogen is carried out by a highly conserved complex of proteins called **nitrogenase complex**. The nitrogenase complex comprises two protein fraction or components. Component I or dinitrogenase and component II or dinitrogenase reductase; neither of the two components is active in the absence of the other and their sizes vary with the microbial source. The structure and mechanism of nitrogenase complex was first studied by Carnahan et al at University of Wisconsin (USA).

Dinitrogenase, also known as Mo-Fe protein is a tetramer with four identical peptide chains each with an Mo-Fe coenzyme of unknown structure and an $\text{Fe}_4\text{-S}_4$ group. Its molecular weight, MW is approximately 2,10,000-2,40,000 daltons. Its redox centres have a total of 2Mo, 32 Fe and 30 S per tetramer.

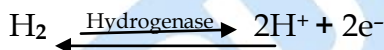
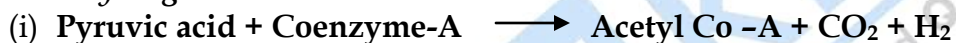
Dinitrogenase reductase or Fe protein (MW =55,000 to 60,000) is a dimer with two identical chains, each having an $\text{Fe}_4\text{-S}_4$ group can be oxidized and reduced by one electron. It also has two binding sites for ATP.

The reductase component provides electrons with high reducing power and the nitrogenase uses these electrons to reduce N_2 to NH_3 / NH_4^+ . Nitrogen fixation is carried out by a highly reduced form of dinitrogenase and it needs 8 electrons; 6 for the reduction of N_2 and 2 to produce one mole of H_2 as an obligate part of the reaction mechanism.

Dinitrogenase is reduced by the transfer of electrons from dinitrogenase reductase. Dinitrogenase has two binding sites for the reductase. The required 8 electrons are transferred to Dinitrogenase one at a time, with the reduced reductase binding and the oxidized reductase dissociating from dinitrogenase in a cycle.

Steps:

- (1) First phosphoroclastic breakdown of pyruvic acid, an end product of glycolysis, takes place resulting into production of acetyl phosphate; CO_2 and H_2 . This reaction is completed in two steps. First step requires enzyme *pyruvic acid dehydrogenase* and second step requires enzyme phosphotransacetylase. Hydrogen is broken into protons and electrons in presence of enzyme *hydrogenase*.



- (2) Acetyl phosphate reacts with ADP and is converted into acetate. One molecule of ATP is produced which acts as a source of energy in nitrogen fixation.



The ATP produced during oxidative or photo-phosphorylation as in certain photosynthetic bacteria like **Chromatium** and in blue green algae, can also be used as a source of energy for nitrogen fixation.

- (3) The electrons released during breakdown of pyruvic acid are accepted by electron carriers ferredoxin / flavodoxin (Fd). Thus ferredoxin is reduced.
- (4) The reduced Fd donates electrons to Fe - Protein component (dinitrogenase reductase). Thus Fe-protein is reduced.
- (5) The reduced Fe -protein combines with ATP in the presence of Mg^{2+} to become activated and to reduce Mo-Fe protein component of nitrogenase complex.
- (6) The reduced Mo -Fe protein subunit donates electrons to N_2 has been completely reduced to NH_3 .

Reduction of N_2 to NH_3 is a stepwise process. It's a sequential reduction by nitrogenase complex. It was proposed by Chatt et al and Hroneley et al.

Nitrogen ($N=N$) is first reduced to a dimide ($NH=NH$) (imide is any compound derived from ammonia, by replacement of two hydrogen atoms); then dimide is reduced to hydrazine (NH_2-NH_2) and ultimately to NH_3 , ammonia.

Two important characteristics of the nitrogenase complex are:

- (1) The ATP seems to play a catalytic role, rather than thermodynamic. It may be recalled that ATP, besides contributing chemical energy through the hydrolysis of one or more of its phosphodiester bonds, can also contribute binding energy through noncovalent interactions that can be used to lower the activation energy. In the reaction carried out by dinitrogenase reductase, both ATP binding and ATP hydrolysis brings about protein conformational changes that help overcome the high activation energy of nitrogen fixation.
- (2) Another feature of nitrogenase complex is that it is extremely labile when oxygen is present. The reductase is inactivated in air, with a half-life of 30 seconds. The dinitrogenase has a half-life of 10 min in air. Free living bacteria, that fix N_2 avoid or solve this problem by diverse means.

Both components of nitrogenase complex are irreversibly poisoned by oxygen. The apparent function of leghemoglobin (= legume hemoglobin) supplied to the nodules of **Rhizobium** in the legumes, is to bind O_2 and maintain PO_2 below **0.001 mm Hg** so that it cannot interfere with nitrogen fixation.

Regulation of Nitrogenase complex activity:

The activity of nitrogenase complex is controlled in two ways- one is a *coarse control*, in which synthesis is repressed by excess of ammonia. The other is a *fine control*, in which the activity of the nitrogenase is regulated by ADP. When the amount of ADP increases to about twice that of ATP, further utilization of ATP by nitrogenase is completely inhibited.

Ammonia Assimilation:

NH_3 is the end product of N_2 - fixation which is in gaseous form and has to be trapped by some mechanism inside plants and bacterial cells. Ammonia of whatever origin can be converted (assimilated) into organic forms by 3 major reactions that occur in all organisms. These reactions result in the formation of glutamine, glutamate, and carbamoyl phosphate. Out of these products glutamate and glutamine are mostly used in amino acid synthesis.

(1) Reductive amination:

Released NH_3 usually combines with Krebs cycle intermediate α -ketoglutarate which gets aminated (nitrogen of NH_3 is transferred to this α -ketoacid) into an amino acid glutamate. This reaction is catalysed by the enzyme glutamate dehydrogenase, a widespread enzyme in plants. This reaction requires a strong reductant i.e. $\text{NADPH} + \text{H}^+$. The reaction is irreversible and proceeds as follows:

Because of the central importance of glutamate in the synthesis of other amino acids and because of high proportion of glutamate formed in this manner by the plants, this reaction is of utmost importance to the nitrogen metabolism of the plant.

Ammonia nitrogen sometimes gets incorporated into other organic compounds to form different amino acids.



(2) GOGAT Pathway:

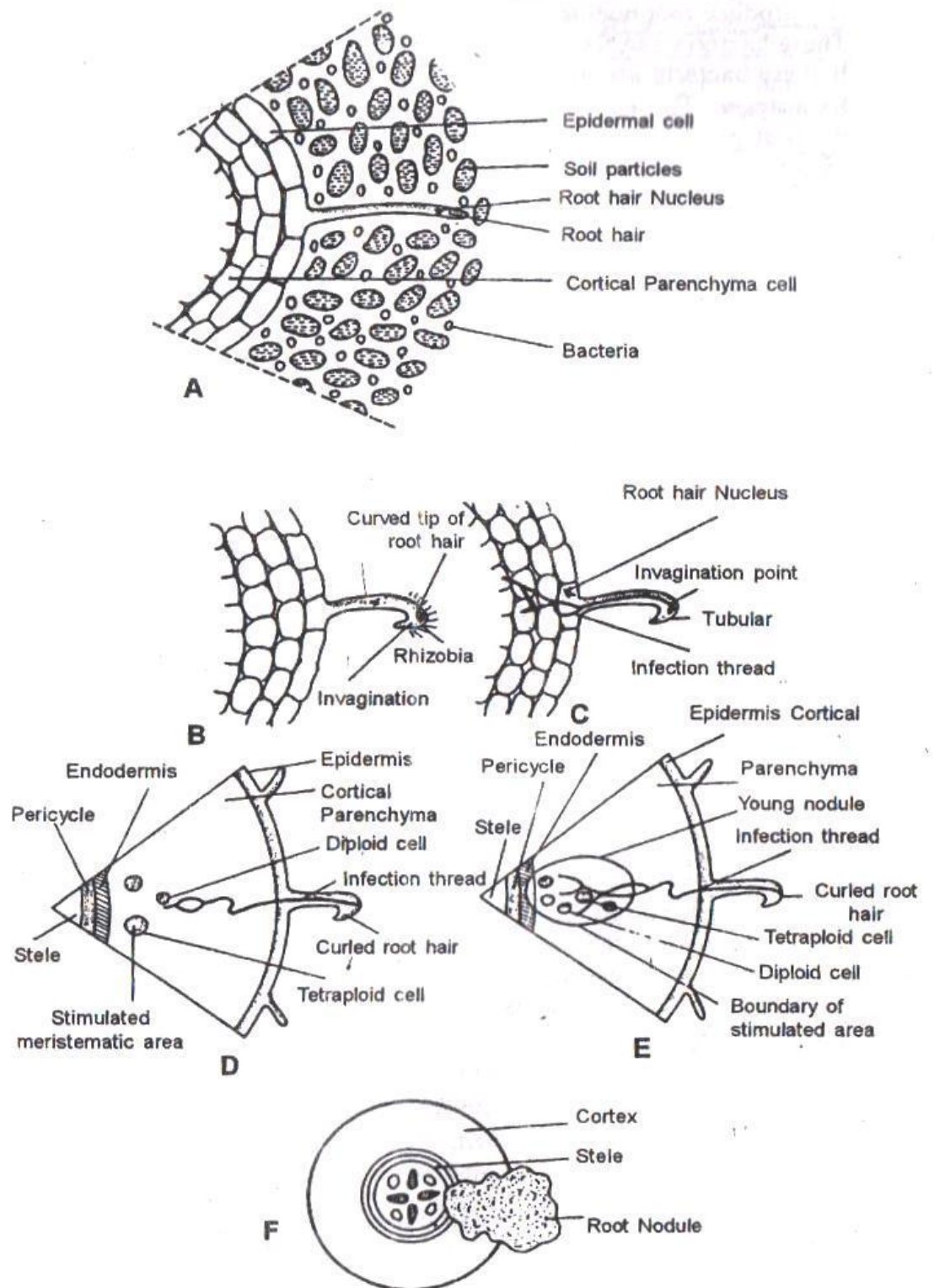
An alternative pathway for the incorporation of ammonia has also been discovered in soil bacteria and plants. The ammonia generated can be accepted by glutamate to produce glutamine. The glutamine in turn can aminate α -ketoglutarate to produce 2 molecules of glutamate.

The reaction is catalysed by two enzymes:

(a) Glutamine synthetase

(b) Glutamate synthase

These two enzymes, jointly called glutamate oxyglutarate amino transferase (GOGAT) have been detected in various systems. It is also proposed that ammonia assimilation (conversion) in symbiotic nitrogen fixing systems takes place through GOGAT pathway.



A-F. Penetration of Rhizobia into the root hair and formation of root nodule in Leguminous plant.

Basic Features of Biological Nitrogen Fixation:

- (1) Nitrogenase , the cardinal enzyme complex in the process.
- (2) A strong reductant, such as ferredoxin or flavodoxin or NADPH etc
- (3) ATP
- (4) Presence of hydrogenase enzyme.
- (5) A system for regulation of the rate of NH_3 production and one for assimilation , since biosynthesis of the nitrogenase complex ceases when ammonia accumulates.
- (6) Protection of the nitrogen fixation system from molecular oxygen, which inactivates nitrogenase and competes for reductant(in aerobic bacteria)
- (7) Coenzyme and cofactors like TPP, Co -A , inorganic phosphate Pi, and Mg^{+2}
- (8) The hydrogen releasing system or electron donor which is usually pyruvic acid but may also be glucose or sucrose in some instances.

Q4Describe in detail the transformation of fats into Carbohydrates with diagrams?

Catabolism (Transformation of fats into Carbohydrates) :

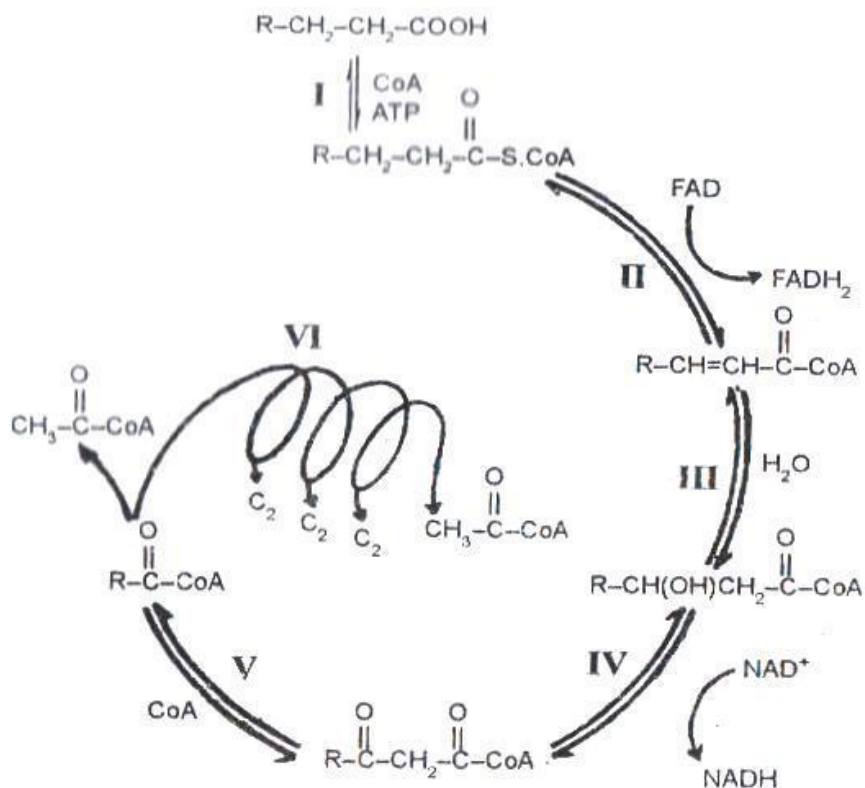
In the process of hydrolytic enzymes, fats yield back fatty acid and glycerol. This process is called **saponification**. The fatty acid and glycerol are oxidized separately.

Mazliak proposed that there can be three fatty acid catabolic pathways:

- (1) β - oxidation pathway
- (2) α -oxidation pathway
- (3) Peroxidation pathway

(1) B-oxidation pathway:

In 1904 , Franz Knoop elucidated the mechanism of fatty acid oxidation. Knoop discovered that fatty acids are degraded by oxidation at the β -carbon. The fatty acids are degraded in two-carbon units i.e. acetic acid. This finding later came to be known as **Knoop's hypothesis**.



β -oxidation scheme, (i) Fatty acid thiolkinase; (ii) Fatty acyl CoA dehydrogenase; (iii) Enoyl hydratase; (iv) β -hydroxyacyl dehydrogenase; (v) β -oxoacylthiolase; and (vi) β -oxidation helix.

The complete combustion of fatty acids to CO_2 and H_2O occurs in mitochondria, where the transfer of electrons from the fatty acids to oxygen can be used to generate ATP. The combustion occurs in 2 stages:

(a) the fatty acid is sequentially oxidized so as to convert all of its carbon, to the acetyl-CoA, and (β -oxidation).

(b) the acetyl-CoA is oxidized by the reaction of Krebs' cycle.

Both stages generate ATP by oxidative phosphorylation & ETC (Electron transport chain).

Activation of A Fatty Acid:

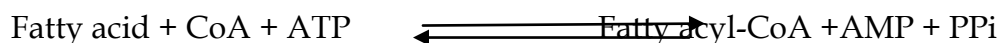
The relative stability of the C-C bonds in a fatty acid is overcome by activation of the carboxyl group at C-1 by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group at the C-3 position. This later carbon is also called the beta (β) carbon in common nomenclature, from which the oxidation of fatty acids gets its common name β -oxidation.

Reactions of Fatty Acid Oxidation:

The free fatty acid cannot pass directly through the mitochondrial membranes from cytosol. So it undergoes a series of 3 enzymatic reactions.

(1) First Reaction:

It is catalysed by an enzyme present in the outer membrane of mitochondria, **acetyl-CoA synthase** (also called **fatty acid thiokinases**)



(2) Second Reaction:

Fatty acyl-CoA formed in the outer mitochondrial membrane does not cross the inner mitochondrial membrane intact. This is done by the transfer of acyl position of fatty acyl-CoA to a -OH group of Carnitine. Acyl-carnitine is formed which is transported to the inner membrane of mitochondria.



(3) Third Reaction:

In this final step of the entry process, the fatty acyl group is enzymatically transferred from carnitine to intramitochondrial CoA by carnitine acyltransferase II. The enzyme is located on the inner face of mitochondrial membrane where it regenerates fatty acyl-CoA and releases it, along with free carnitine into the matrix.



Four steps of β -oxidation:

β -oxidation is accomplished by a four-step mechanism. The four steps of the fatty acid spiral, are described below:

First Step:

α , β dehydrogenation of acyl-CoA. In this first step, fatty acyl-CoA is oxidized by an acyl CoA dehydrogenase to produce trans- Δ^2 -enoyl (a trans bond between α and β carbons atoms (C-2 and C-3)).

Second step Hydration of α , β -unsaturated acyl-CoA:

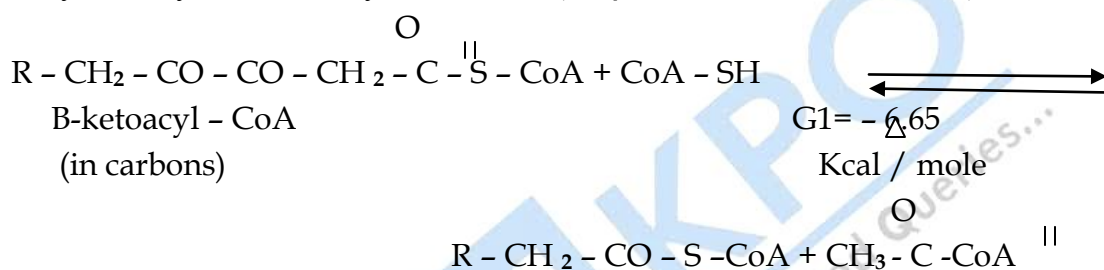
In this step, a mole of water is added to the double bond of trans- Δ^2 -enoyl-CoA to form the L-stereoisomer of β -hydroxyacyl-CoA (also called 3-hydroxyacyl-CoA). The reaction is catalysed by enoyl-CoA hydratase or crotonase.

Third Step: Oxidation of β -hydroxyacyl - CoA :

L - β - hydroxyacyl - acyl - Co A is dehydrogenated (or oxidized) to form β - ketoacyl - CoA by the action of an enzyme , β -hydroxyacyl -CoA dehydrogenase.

Fourth Step: Thiolytic or Thioclastic Scission:

Thiolytic is a splitting by thiol (- SH) group , aided by enzymatic catalysis. This is the final step and brings about the cleavage of β - ketoacyl - Co A by the thiol group of a second mole of Co A, which yields acetyl -CoA and an acyl - CoA , shortened by two carbon atoms. This thiolytic cleavage is catalysed by the enzyme , acyl - CoA acetyltransferase (or β -ketothiolase or thiolase).



The shortened acyl - CoA then undergoes another cycle of oxidation . The reaction is catalysed by acyl - CoA dehydrogenase.

Localization:

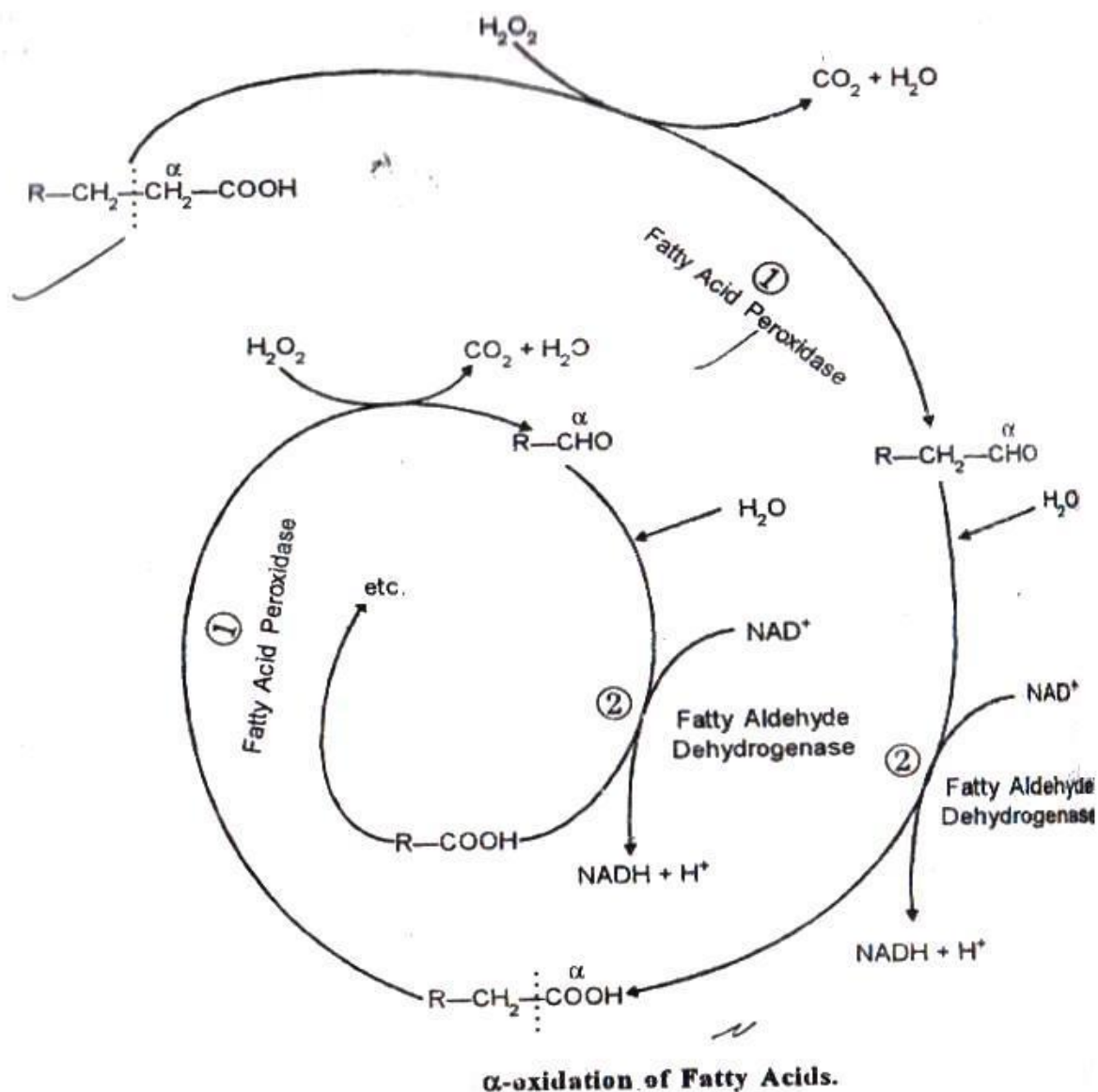
β - oxidation is found in all organisms. In Bacteria , grown in the absence of fatty acids , the β -oxidative system is practically absent but is readily induced by the presence of fatty acids in the growth medium . The bacterial β oxidation system is completely soluble and hence is not membrane bound.

 α - Oxidation of Fatty Acids:

α - **oxidation** is the removal of one carbon atom (i.e. α - carbon) at a time from the carboxyl end of the molecule. α - oxidation was first observed in seeds and leaf tissues of plants and also found in brain and liver cells.

Salient features:

- (1) Only free long - chain fatty acids serve as substrate.
- (2) Molecular oxygen is indirectly involved.
- (3) It does not require CoA intermediates.
- (4) It does not lead to generation of high energy phosphates i.e. ATP etc.



This mechanism explains the occurrence of α hydroxyl fatty acids and of odd-numbered fatty acids in the biomolecules.

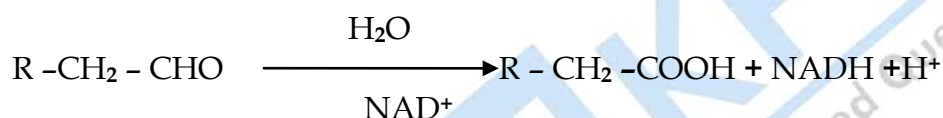
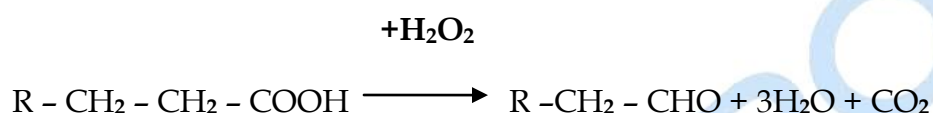
The α oxidation system plays a key role in the capacity of mammalian tissues to oxidize phytanic acid (= 3,7,11,15- tetramethyl hexadecanate) . Phytanic acid is an oxidation product of phytol and is present in animal fat , cow's milk and foods derived from milk. The phytol is a hydrolytic product of chlorophyll .

If phytanic acid is not oxidized and starts accumulating in tissues and serum of the individual, it gives rise to a rare inheritable autosomal recessive disorder which affects the nervous system, popularly known as **Rifsum's** disease.

α - oxidation was discovered by Newcomb and Stunf.

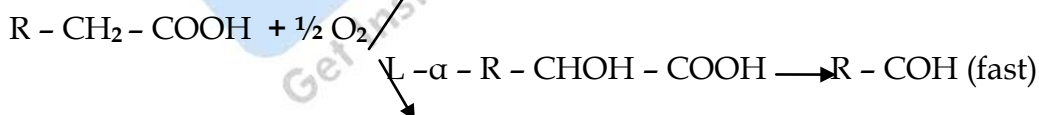
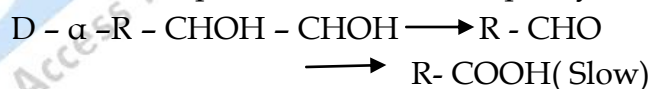
Mazliak proposed that in this oxidation two reaction sequences occur.

- (i) First step involves peroxidation of fatty acids due to which it is decarboxylated and fatty aldehyde is formed.



- (ii) The second step involves a direct oxidation by molecular oxygen and as a result 2-hydroxy acid is formed. The 2-hydroxy acid may have either D-configuration or L-configuration in its molecular arrangement.

The further oxidation of D-form proceeds slow or it shows accumulation while L-form proceeds fast; this step may be shown as:



Chapter10

Phases of growth and Development

Q1 Describe in brief the physiological effects of ethylene and the mechanism of ethylene action.

Ans

Ethylene:

Ethylene which is so well known as a product of combustion and as a common air pollutant is surprisingly a powerful plant hormone. It is a gas . $\text{CH}_2 = \text{CH}_2$ and is produced in minute quantities by plant tissues, but is active at extremely low concentrations (well below 1part in 10 million of air) . It is synthesized in the plants from the amino -acid methionine. It is not easily soluble in aqueous medium and volatilizes into the intercellular spaces from where it is released in the outer atmosphere. The history of the discovery of ethylene as a plant hormone starts with 19th century when plant growers noticed

The effect of smoke and burning gases on plants. But the first recorded effect can be credited to the Russian botanist Dimitry .N. Neljobow working in St. Petersburg , who demonstrated that ethylene affects the plant growth. He also established that ethylene was emanated from illuminating gases and it had three types of effects on pea seedling, stem elongation , stem thickening and horizontal growth. The first report that plant materials evolve ethylene came from H.H. Cousins in 1910 , who observed that when oranges and banana were stored together during shipping , some gas was emanated from oranges which caused ripening of bananas .

Physiological Effects of Ethylene:

(1) Growth :

In general , ethylene has an inhibitory effect on growth . However , retardation is mostly in the longitudinal growth. Ehtylene promotes transverse or isodiametric growth both in stems and roots. Reduction of growth may be due to inhibition of cell division , as has been observed in various tissues of etiolated pea plants. In pea seedlings , ethylene inhibits xylem vessel formation also. But no permanent damage is done to the cells , as soon as ethylene is removed , normal differentiation takes place.

(2) Tropic Movements:

Ethylene normally makes the stem ageotropic mostly due to inhibition of polar transport of auxin. However, Zobel has observed that it is required in the geotropic response of stem in tomato.

(3) Epinastic Responses:

Exposure to ethylene cause **epinastic** movement in petioles , as a result the leaves bend down. This is because of more growth on upper side than on the lower side of the petiole. Epinasty is also caused when excess of auxin is applied. This is believed to be due to endogenous ethylene production.

Stem Epinasty as in tomato, is also caused due to ethylene.

(4) Senescence and abscission :

Ethylene is an important hormone governing the senescence and abscission of plant parts , both natural and induced . It accelerates senescence of leaves , flowers and fruits. Endogenous ethylene increases during senescence. Supply of ethylene degrades chlorophyll . ACC, a precursor in ethylene biosynthesis , also accelerates chlorophyll degradation in detached leaves. Ethylene induces abscission of leaves , fruits , petals and flowers by inducing the formation of hydrolases.

(5) Flowering and Sex Expression :

Rodriguez noted the stimulation of flowering in pineapple. Similar stimulation has been noted in other bromeliads , Iris, mango . In other plants ethylene inhibits flowering and also causes deformation and fading of flowers. Ethylene also changes sex expression in unisexual plants . It increases female flowers in several members of Cucurbitaceae . It also induces male sterility in cucurbits and wheat.

(6) Fruit Ripening:

Acceleration of fruit ripening was the first discovered effect of ethylene. The hormone is now known to accelerate the ripening of mature fruits in most cases including banana , apple , tomato , avocado etc. In apples , the softening of fruits increases with the increase in ethylene concentration , reaching maximum at 50ppm ethylene. In most cases , this softening is brought about by chemical changes in the cell wall materials. Ethylene appears to play an important role in the natural ripening of fruits. During ripening of most fruits , the ethylene production increases sometimes by as much as 50 to 100 folds. The ripening process of fruits can be delayed by removing ethylene from the vicinity of developing fruits or by maintaining low O₂ levels around fruits.

(7) Water stress:

Under conditions of water stress, leaves produce ethylene. It probably stimulates abscisic acid formation for effecting stomatal closure.

(8) Germination:

Ethylene breaks the dormancy of several plant organs, though it is implicated in the inhibition of lateral bud growth. It is known to break the seed dormancy and induce germination of lettuce, groundnut, wheat, clover seeds. However, inhibition of germination by ethylene has been recorded in maize, *Potentilla*, and some weeds.

Mechanism of Ethylene Action:

Like most other hormones the action, the action of ethylene is initiated by its binding to a specific receptor site in the target cell.

This site is a membrane bound protein perhaps an enzyme containing Cu atom. Ethylene has a two fold action – rapid response and delayed response.

As usual, the rapid response is caused by release of bound enzyme. e.g. cellulase in abscission. The delayed responses may be due to the activation of gene expression and increased protein synthesis.

Involvement of Auxins:

Auxins induce ethylene production and many responses of ethylene are similar to that of auxins. These include epinasty, inhibition of stem and root elongation, flower induction etc. It is likely that mechanism of both hormones is either same or similar.

Commercial use of ethylene:

Ethylene has been used for synchronized flowering and fruit ripening for centuries. Since nineteenth century the pineapple growers lit bonfire near their plantation, knowing that smoke helped to initiate and synchronize the flowering of their crop and ripening of fruits. The feminizing effect of ethylene has been used to increase the yield in Cucurbitaceae. The growth inhibition caused by ethylene makes it useful to prevent lodging, mainly in cereals. **Ethrel** or **Ethephon** is the most commonly used chemical which is 2-chloroethyl phosphoric acid. It rapidly breaks down in water at neutral or alkaline pH to produce ethylene, Cl^- and PO_4^- .

Q2 Give a brief account of a few phytochrome mediated responses, Discuss how these responses are manifested at physiological and biochemical levels.

Ans

Phytochrome:

Among the different pigments that can promote photomorphogenetic responses in plants, the most important is **Phytochrome**. **Phytochrome** is a photoreceptor molecule which mediates several developmental and morphogenetic responses of plants to light. The existence of this molecule was indicated by a group of plant physiologists working in the United States Department of Agriculture, Beltsville, Maryland. These scientists were interested in elucidating the mechanism of light effects of flowering. In 1952, H.A. Borthwick, S.B. Hendricks and their colleagues postulated that a pigment received light and that it existed in two forms: active form and inactive form. The pigment was called **Phytochrome**. (abbreviated as P). Phytochrome red form (P_r) was inactive while phytochrome far-red (P_{fr}) was the active form. When P_r was irradiated with red light, it was converted to P_{fr} . Similarly P_{fr} could be converted to P_r form by irradiation with far-red light or by darkness. However the dark conversion of P_{fr} to P_r form appears to be restricted to the dicotyledons.

Red (660nm)

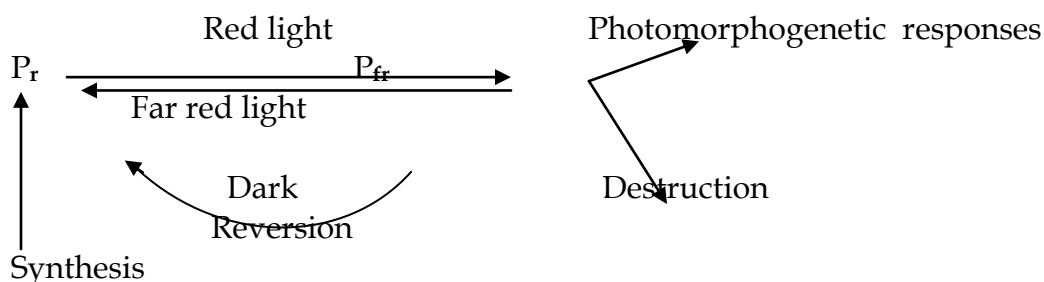


The active P_{fr} form of the phytochrome could trigger developmental and morphogenetic responses in plants. This was in line with the observations that red light (which produces P_{fr}) induced photomorphogenetic responses while far-red light reversed the red-light induced responses. Since the effects of red or far-red light were reversible and very fast, it was concluded that a single phototransforming pigment was involved in the effect of light.

The pigment phytochrome was ultimately isolated by W.L. Butter and his colleagues also from USDA, Beltsville. It was recognized as the light protein, other than enzymes to have regulatory functions in plants.

In most species, some of the P_{fr} gradually disappears even in darkness. Two processes account for this. The first is **destruction**, because after an interval of time in darkness, it is no longer possible to regenerate as much P_r in tissues by a far-red exposure; so the total amount of detectable phytochrome is less than before. This destruction probably involves protein denaturation. The second process is

dark reversion . back to P_r , which usually requires a few hours. Reversion occurs in most dicots and the gymnosperms but has not been detected in monocots. The process can be now summarized as :



Chemistry of Phytochrome:

Native phytochrome is a soluble protein with a molecular mass of about 250KDa. It occurs as a dimer made up of two equivalent subunits. Each subunit consists of two components : a light - absorbing pigment molecule called **chromophore** and a polypeptide chain called the **apoprotein**. The **apoprotein** monomer has a mass of about 125 KDa . Together , the apoprotein and its chromophore make up the **holoprotein**.

In higher plants the chromophore of phytochrome is a linear tetrapyrrole termed **phytochromobilin**. In basic structure it resembles , the open chain tetrapyrrole chromophore of the algal pigment 6-phycocyanin. There is only one chromophore per monomer of apoprotein and it is attached to the protein through a thioether linkage to a cysteine residue.

The phytochrome apoprotein alone cannot absorb red or far -red light. Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein. Phytochromobilin is synthesized inside plastids and is derived from 5-aminolevulinic acid via a pathway that branches from the chlorophyll biosynthetic pathway. It is thought to leak out of the plastid into the cytosol by a passive process.

Assembly of phytochrome apoprotein with its chromophore is **autocatalytic**; i.e. it occurs spontaneously when purified phytochrome polypeptide is mixed with purified chromophore in the test tube , with no additional proteins or cofactors. The resultant holoprotein has spectral properties similar to those observed for the holoprotein purified from the plants, and it exhibits red/ far -red reversibility. Mutant plants that lack the ability to synthesize the chromophore are defective in processes that require the action of phytochrome , even though the apoprotein polypeptides are present.

Phytochrome mediated responses:

Various physiological processes in higher plants are known to be regulated by phytochrome. Some of the important ones are discussed below:

(1) Seed germination:

Germination of many types of seeds is enhanced by light. There are seeds that can germinate only in light. Such seeds are called as **photodormant seeds**. Germination can be induced in such seeds by exposure to light. Phytochrome has been shown to be involved in this response of light as in most cases red light enhances germination and far -red light inhibits it . Experiment by Borthwick , Hendricks and their colleagues on germination of lettuce seed (*Lactuca sativa*) revealed the involvement of phytochrome. These workers allowed the seeds to imbibe water in darkness , then exposed them to different wavelength of light. They then placed the seeds back in darkness and later evaluated them for the amount of germination. They found that when the light sensitive seeds were exposed to red light (660nm) , germination resulted. They also found that far -red light (730nm) , given immediately after a red light exposure , inhibited germination. If they again treated the seeds with red light , germination was promoted. These results indicated to them that a reversible pigment system is operative in lettuce seeds after the seeds imbibe water. The last light treatment determines the response of the seeds .

(2) Seedling growth:

The effect of light on several aspects of seedling and plant growth seem to be mediated via phytochrome. The growth of coleoptile, the mesocotyl extension , unrolling of grass leaves etc. are few examples of phytochrome mediated processes.

Growth of a mature plant is affected by light through phytochrome . If a dicot plant or a conifer is grown in far -red light (as in shaded conditions) . its stem becomes considerably elongated.

(3) Flowering;

The photoperiodic flowering responses in most species has been shown to be mediated via phytochrome. If a long night of a photoinductive cycle for **Xanthium** is broken by a brief flash of light (light break) , the plant does not flower. An action spectrum for the effectiveness of different wavelengths demonstrates that the most effective wavelength for inhibition of flowering are found between 620 and 660 nm (orange -red) with a maximum at about 640nm. Therefore red light is considered to be the most efficient radiation in the light break reactions.

(4) Pigmentation (Anthocyanin synthesis) :

Anthocyanin (flavanoids) impart characteristic colour to the plant parts. Their synthesis is induced by both low and high energy radiations. For the low energy radiations , phytochrome has been shown to be the exclusive

pigment receptor. In high energy reactions, however pigments other than phytochrome, such as carotenoids, flavins and cytochromes are also involved. Plants where phytochrome mediated flavanoid biosynthesis has been observed include *Amaranthus*, barley, buckwheat, cabbage, maize, sorghum, turnip etc. Dark grown seedlings of *Sinapsis alba* do not synthesize significant amounts of anthocyanin. Five minutes of red light given 36 hours after sowing induces anthocyanin synthesis. Effect of red light can be fully reversed by 5 minutes exposure to far red following the red treatment.

(5) Chloroplast development:

When etiolated seedlings are exposed to light, rapid chlorophyll synthesis takes place from protochlorophyll present in the plants. This is followed by a lag phase and again by a rapid increase in chlorophyll synthesis. Red light has been found to eliminate the lag phase and far-red can reverse the effect of red light.

(6) Effect on later vegetative growth:

In many well established dicots and conifers, it has been noted that, if they grow under a leaf canopy where the light received is primarily far-red, their stems become considerably elongated. This light effect is therefore opposite to the retarding effect on elongation of etiolated seedlings.

Branching of stem is simultaneously retarded in many species under a canopy. So the plant uses more of its energy in raising the stem apex towards the top of the canopy than it does when unshaded. This effect of shade is mainly due to the decrease in the amount of Pfr.

(7) Arrangement of chloroplasts:

Light influences the arrangement of chloroplasts in the mesophyll cells of the leaves. The phytochrome seems to be responsible for the movement of chloroplasts. Under strong illumination they move towards the longitudinal wall (parallel to sunlight) of chlorenchyma cells and arrange themselves one behind the other with their edges towards the light. This position is known as **parastrophe**. In moderate or weak light, the chloroplast arrange themselves towards the best illuminated side with their flat side facing the light. Such an arrangement is known as **epistrophe**. In dark, the chloroplasts are arranged variously, the position being described as **apostrophe**.

(8) Physiological and biochemical responses:

Besides morphological and morphogenetic responses the phytochrome induces many physiological and biochemical changes, as well. Some of the physiological changes are :

Change in the rate of cellular respiration . membrane permeability , change in auxin catabolism and in cell turgidity. Some biochemical changes include increase in RNA and protein synthesis and changes in some enzyme activities viz – amylases, lyases, nitrate reductases etc.

Mechanism of Phytochrome Action:

The phytochrome mediated responses appear to be of two types:

- (i) **Fast responses** : which are seen within a few minutes of desired irradiation such as changes in membrane permeability and in cell turgor , and
- (ii) **Slow responses**: which are seen after hours, days or weeks after irradiation such as seed germination or flowering. Two hypothesis have been proposed to explain these mechanisms.

(1) Membrane permeability:

In both types of responses it appears that the **primary target of phytochrome action is the membrane**. It is however not clear , whether the plasma membrane or the organellar membrane is affected when phytochrome is converted to its active form(Pfr) . However , it is clear that permeability of cells to water and ions such as H^+ , K^+ , Ca^{2+} is changed. Perhaps Pfr promotes movement of Ca^{2+} from outside to the cytoplasm. This Ca^{2+} after complexing with calmodulin changes the permeability of membranes for ions such as H^+ , K^+ , Cl^- etc.

The change in membrane permeability can be manifested in a variety of physiological and biochemical responses. Changes in the membrane permeability may be associated with the appearance of a specific charge on the plant surfaces , as is observed in the root -tip. During $Pr \rightleftharpoons Pfr$ interconversions , some cation such as H^+ are preferentially held at the outer surface of the root. It results in a positive charge on the root surface. This can be demonstrated in attraction and attachment of these root tips on a negatively charged glass surface. This phenomenon has been termed as **Tanada Effect** , as it was first discovered by Takuna Tanada at Beltsville, USA. If phytochrome is converted from Pfr to Pr , the charge on the root surface is neutralized and ultimately the specific enzymes could be synthesized.

(2) Gene mediated enzyme synthesis:

According to this theory, the phytochrome interconversion (Pr $\xrightarrow{\text{enzymes}}$ Pfr) is due to the enzymes produced as a result of specific gene expression. This hypothesis was supported by the fact that in many cases inhibitors of transcription and translation, such as actinomycin-D and puromycin prevent phytochrome mediated increase in enzymes. But this hypothesis can explain only the slow responses of phytochrome.



Transpiration

Q1 The metal ion involved in stomatal regulation is :

- (a) iron
- (b) magnesium
- (c) zinc
- (d) **potassium**

Q2 In which of the following plants would the metabolism be hindered if the leaves are coated with wax on their upper surface:

- (a) Hydrilla
- (b) **Lotus**
- (c) Pistia
- (d) Vallisneria

Q3 The active K^+ exchange mechanism for opening and closing of stomata was given by :

- (a) Von Mohl
- (b) Linsbauer
- (c) **Levitt**
- (c) Mansfield

Q4 Root pressure is maximum when:

- (a) transpiration is high and absorption low
- (b) **transpiration is low and absorption high**
- (c) transpiration and absorption both are high
- (d) transpiration and absorption both are low

Q5 In many thin leaved mesophytes , stomata open during the day and close during the night , this comes under:

- (a) Barley type
- (b) Potato type
- (c) **Alfalfa type**
- (d) Bean type

Q6 Which one of the following factors is the most important in regulation of transpiration:

- (a) **Humidity**
- (b) Temperature
- (c) Light
- (d) Wind

Q7 In old trees or woody plants transpiration occurs through :

- (a) Stomata
- (b) Bark
- (c) **Lenticels**
- (d) Root

Q8 The instrument used to measure transpiration is known as :

- (a) Porometer
- (b) Photometer
- (c) Osmometer
- (d) **Potometer**

Answer Key Q1 (d) Q2 (b) Q3(c) Q4 (b) Q5(c) Q6 (a) Q7 (c) Q8(d)

Mineral Nutrition

Q1 An essential element is one which :

- (a) is available in soil
- (b) improves plant growth
- (c) **is indispensable & irreplaceable for growth**
- (d) is present in plant ash

Q2 Which of the following is not an essential element for plants?

- (a) Iron
- (b) Zinc
- (c) Potassium
- (d) **iodine**

Q3 Which of the following elements is responsible for maintaining turgor?

- (a) Na
- (b) **K**
- (c) Ca
- (d) Mg

Q4 The elements which is required in largest quantities by plant is ?

- (a) **Nitrogen**
- (b) Calcium
- (c) Sulphur
- (d) Phosphorus

Q5 When chlorophyll is burnt , which one is obtained?

- (a) Fe
- (b) **Mg**
- (c) Both (Fe & Mg)
- (d) Ca and Mn

Q6 Which element facilitates the translocation of carbohydrates in plants?

- (a) Fe
- (b) Mo
- (c) Zn
- (d) **B**

Answer Key : Q1 (c) Q2(d) Q3(b) Q4(a) Q5(b) Q6 (d)

Enzymes

Q1 An enzyme can be synthesized by chemically bonding together the molecules of:

- (a) Carbohydrates
- (b) **Amino acids**
- (c) Lipase
- (d) CO₂

Q2 Which energy is not proteinaceous :

- (a) Isozyme
- (b) **Ribozyme**
- (c) Holozyme
- (d) Trypsin

Q3 The non- protein part of the enzyme is called:

- (a) Apoenzyme
- (b) Holoenzyme
- (c) **Prosthetic group**
- (d) Isoenzyme

Q4 Which among the following was the highest catalytic efficiency?

- (a) Catalase
- (b) Urease
- (c) **Carbonic anhydrase**
- (d) Pepsin

Q5 K_m value refers to :

- (a) Maximum velocity reaction
- (b) Threshold value
- (c) Near maximum velocity
- (d) **One half of the maximum reaction velocity.**

Q6 Which of the following is the best evidence for template theory of enzyme reaction:

- (a) **Compounds similar in structure to the substrate inhibit the reaction.**
- (b) Enzymes speed up reaction by definite amount
- (c) Enzymes determine the direction of a reaction
- (d) Enzymes are found in living organisms and increase the rate of certain reactions.

Answer key: Q1 (b) Q2(b) Q3(c) Q4 (c) Q5 (d) Q6 (a)

Photosynthesis and Respiration

Q1 Photosynthesis is a process in which :

- (a) **CO₂ is reduced to carbohydrates**
- (b) NADH is reduced to NAD
- (c) ATP is generated
- (d) Oxidative phosphorylation occurs

Q2 The unit of photosynthesis:

- (a) **Quantasome**
- (b) Angstrom
- (c) Geotropism
- (d) None of the above

Q3 Quantasome contains:

- (a) 200 chl molecules
- (b) **230 chl molecules**
- (c) 250 chl molecules
- (d) 300 chl molecules

Q4 The oxygen evolved in photosynthesis comes from water was confirmed by using:

- (a) C¹⁴
- (b) p²²
- (c) **O¹⁸**
- (d) Chlorine ions

Q5 Thylakoids of grana possess the :

- (a) Enzymes for Calvin Cycle
- (b) **Enzymes for photophosphorylation**
- (c) Enzymes for C₃ – cycle
- (d) Enzymes for C₄ – Cycle

Q6 CO₂ acceptor in C₃ plants is :

- (a) Phosphoglyceric acid (PGA)
- (b) Ribulose monophosphate (RMP)
- (c) **Ribulose -1,5- biphosphate (RUBP)**
- (d) Phosphoenol pyruvate

Q7 Respiration is :

- (a) **Exothermic process**
- (b) Endothermic process
- (c) Endergonic process
- (d) Anabolic process

Q8 Common immediate source of (highest) energy in cellular activity is:

- (a) NAD
- (b) **ATP**
- (c) DNA
- (d) RNA

Q9 Enzymes taking part in glycolysis are present in :

- (a) Mitochondria
- (b) **Cytoplasm**
- (c) Vacuole
- (d) Both mitochondria & Cytoplasm

Q10 Which of the following is important in carbohydrate and fat metabolism?

- (a) CO₂
- (b) **Acetyl – CoA**
- (c) Pyruvic acid
- (d) Citric acid

Q11 Which component of ATPase is embedded in mitochondrial membrane?

- (a) **F₀**
- (b) F₁
- (c) Both F₁ and F₀
- (d) None

Q12 The value of R.Q. at compensation point is :

- (a) **Unity** (b) infinity
(c) >1 (d) Zero

Q13 Wounding of plant organs results in :

- (a) Decrease in the rate of respiration (b) **Increase in the rate of respiration**
(c) Stopping of respiration (d) No effect on respiration

Answer key : Q1 (a) Q2 (a) Q3(b) Q4 (c) Q5 (b) Q6 (c) Q7(a) Q8(b) Q9(b) Q10(b) Q11(a) Q12 (a) Q13 (b)

Nitrogen Metabolism

Q1 Nitrifying bacteria are those which can convert :

- (a) Atmospheric nitrogen to NH_3 (b) **Ammonia into nitrate**
(c) Nitrites into nitrates (d) Nitrates into ammonia

Q2 Most plants obtain their nitrogen from the soil in the form of :

- (a) NO_2 (b) NO_3
(c) Free N_2 gas (d) NH_3

Q3 The enzyme responsible for the reduction of molecular nitrogen to the level of ammonia in leguminous root nodule is:

- (a) **Nitrogenase** (b) Nitrate reductase
(c) Nitrite reductase (d) None of the above

Q4 Which is a symbiotic nitrogen fixing bacteria?

- (a) **Rhizobium** (b) Azotobacter
(c) Clostridium (d) Nitrosomonas

Q5 Winogradsky discovered one of the nitrogen fixing bacterium which is :

- (a) Rhizobium (b) Azotobacter
(c) **Clostridium** (d) Bacillus radiculicola

Answer key : Q 1(b) Q2 (b) Q3 (a) Q 4(a) Q5 (c)

Growth and Development:

Q1 Growth involves :

- (a) Cell division
- (b) Cell elongation
- (c) Cell maturation
- (d) **All the above**

Q2 What do the growth of a single cell , organ , organism and population have in common:

- (a) They are influenced by the environment
- (b) They require energy and acquire mass
- (c) **They result in S- shaped growth curve**
- (d) All the above

Q3 Auxanometer is an instrument to measure:

- (a) Auxins
- (b) **Growth**
- (c) Plant Movements
- (d) Respiration

Q4 Which of the following is the motivative force for growth?

- (a) **Turgor pressure**
- (b) Root pressure
- (c) Osmotic pressure
- (d) Atmospheric pressure

Q5 Which of the following induces cell division?

- (a) Auxin
- (b) Gibberellin
- (c) **Cytokinin**
- (d) Ethylene

Q6 Primary precursor of IAA is:

- (a) Phenylalanine
- (b) Tyrosine
- (c) **Tryptophan**
- (d) Methionine

Q7 During adverse conditions , plants develop a Stress hormone:

- (a) **ABA**
- (b) IAA
- (c) Ethylene
- (d) 2,4,- D

Q8 IAA was first isolated from:

- (a) **Human urine**
- (b) Fusarium fungus
- (c) Corn germ oil
- (d) Herring sperm DNA

Q9 Hormone used in the early ripening of fruits:

- (a) Auxin
- (b) Cytokinin
- (c) ABA
- (d) **Ethylene**

Q10 Bio –essay for gibberellins is:

- (a) Avena curvature test
- (b) Soybean callus test
- (c) **Amylase activity test**
- (d) Barley leaf disc test for chlorophyll

Q11 Genetically dwarf plants can be made taller by:

- | | |
|--------------------------|-------------|
| (a) Gibberellin | (b) Auxin |
| (c) Cytokinin | (d) ABA |

Q12 Which of the following does not occur naturally in the plants?

- | | |
|---------------------|---------------------|
| (a) IAA | (b) GA ₃ |
| (c) 2, 4-D | (d) Zeatin |

Answer key: Q1 (d) Q2 (c) Q3 (b) Q4 (a) Q5 (c) Q6 (c) Q7(a) Q8 (a) Q9 (d) Q10 (c)
Q11 (a) Q12 (c)



Seed Germination and Dormancy

Q1 The micropyle in the seed helps in the entry of :

- (a) **Water**
- (b) pollen tube
- (c) male gamete
- (d) none

Q2 Vivipary in plants means:

- (a) germination of seeds in crevices of stem where some soil is deposited
- (b) Organisation of several shoots
- (c) development of fruit without fertilization
- (d) **germination of seeds inside the fruit while it is still on the parent tree**

Q3 Removal of seed coat in dormant seeds by mechanical method is called:

- (a) emasculation
- (b) separation
- (c) **scarification**
- (d) stratification

Q4 When seeds show hypogeal germination , the part that pushes the cotyledons into the soil is:

- (a) **epicotyl**
- (b) hypocotyl
- (c) plumule
- (d) radicle

Q5 Epigeal germination is found in :

- (a) maize
- (b) wheat
- (c) pea
- (d) **castor**

Q6 Which of the following is most essential for seed germination?

- (a) light
- (b) minerals
- (c) high temperature
- (d) **Water**

Answer key : Q1 (a) Q2(d) Q3 (c) Q 4(a) Q5 (d) Q6 (d)

Plant Movements

Q1 Twining of tendrils around a support is an example of :

- (a) Chemotropism (b) nastic movement
(c) phototropism (d) **thigmotropism**

Q2 An apparatus commonly used to demonstrate phototropism is :

- (a) **heliotropic chamber** (b) clinostat
(c) arc auxanometer (d) potometer

Q3 The movements where organism or its organs move from one place to another in response to external stimuli are called:

- (a) tropic (b) nastic
(c) **tactic** (d) nutational

Q4 Most of the leaves and flower buds open because of differential growth of their surface. This is known as :

- (a) hyponasty (b) **epinasty**
(c) thigmonasty (d) seismonasty

Q5 Which of the following is an example of chemotropic movements:

- (a) Movement of moss antherozoid in response to sugar secreted by archegonia
(b) Movement of fern antherozoid in response to malic acid
(c) **Growth of pollen tube on stigma**
(d) Growth of pollen inside the chamber

Q6 Leaves of clover close during night and open during the day, such a movement is known as:

- (a) **nyctinasty** (b) thermonasty
(c) phototropism (d) phototaxis

Q7 Clinostat is employed in the studies of :

- (a) Osmosis (b) **growth movements**
(c) photosynthesis (d) respiration

Q8 Movement of hairs in Drosera is :

- (a) Photonastic (b) Thermonastic
(c) **Thigmonastic** (d) Seismonastic

Q9 The period between the application of stimulus and its visible response is called:

- (a) Presentation time (b) Relaxation time
(c) Stimulation period (d) **Reaction time**

Q10 Closure of lid of pitcher plant is :

- (a) **paratonic movement** (b) a tropic movement
(c) a turgor movement (d) an autonomic movement

Answer key: Q 1(d) Q2 (a) Q3(c) Q4(b) Q5(c) Q6(a) Q7(b) Q8 (c) Q9 (d) Q10 (a)

Physiology of flowering

Q1 The response of plants to environmental rhythms of light and darkness is called:

- (a) Vernalization
- (b) Phototropism
- (c) **Photoperiodism**
- (d) Phototaxis

Q2 Induction of flowering by low temperature in plants is:

- (a) Cryobiology
- (b) **Vernalization**
- (c) Pruning
- (d) Photoperiodism

Q3 Hormone responsible for Vernalization is:

- (a) Abscisin
- (b) **Vernalin**
- (c) Florigen
- (d) Colchicine

Q4 If dark period of a short day plant is interrupted by a flash of light, then it:

- (a) flowers immediately
- (b) turn into a long day plant
- (c) **will not flower at all**
- (d) produce profuse flowers

Q5 Flowering in short day plants does not take place when:

- (a) **critical period is interrupted by white / red light**
- (b) dark period is interrupted by red light followed by far red light
- (c) light period is interrupted by darkness
- (d) auxins are not supplied to it

Q6 A plant which needs not less than 10 hours of light to flower is :

- (a) **long day plant**
- (b) short day plant
- (c) intermediate plant
- (d) day neutral plant

Q7 Which of the following is not a short day plant:

- (a) *Saccharum officinarum*
- (b) ***Solanum tuberosum***
- (c) *Glycine max*
- (d) *Brassica campestris*

Q8 In short day plants flowering is induced by :

- (a) long night
- (b) photoperiod less than 12 hours
- (c) **photoperiod less than critical and uninterrupted long night**
- (d) darkness less than critical and long uninterrupted daylight

Q9 Hormone which substitutes for long photoperiod

- (a) auxin
- (b) cytokinin
- (c) Florigen
- (d) **gibberellin**

Q10 The name florigen was given by:

- (a) **Cajalchjan**
- (b) Melchers
- (c) Stanley
- (d) Garner and Allard

Answer key : Q1 (c) Q2(b) Q3 (b) Q4 (c) Q5 (a) Q6 (a) Q7 (b) Q8 (c) Q9 (d) Q10 (a)



Fruit Ripening , Senescence and Biological Clock

Q1 Ripening hormone is :

- (a) auxin
- (b) **ethylene**
- (c) gibberellin
- (d) Cytokinin

Q2 Daily movements of leaves is an example of:

- (a) Lunar rhythm
- (b) **circadian rhythm**
- (c) Annual rhythm
- (d) tidal rhythm

Q3 Rhythms that occur in organism with a periodicity of 24 hours is:

- (a) **Circadian rhythm**
- (b) Lunar rhythm
- (c) Tidal rhythm
- (d) Annual rhythm

Q4 Hormone which delays senescence is:

- (a) auxin
- (b) ethylene
- (c) **cytokinins**
- (d) gibberellins

Q5 The final phase of development is called:

- (a) juvenile
- (b) maturity
- (c) seedling
- (d) **senescence**

Q6 The deciduous trees show:

- (a) sequential leaf senescence
- (b) **synchronous leaf senescence**
- (c) whole plant senescence
- (d) organ senescence

Q7 The enzymes which increase during fruit ripening are:

- (a) **hydrolytic enzymes**
- (b) oxido reductases
- (c) synthases
- (d) ligases

Q8 Fruit ripening is a :

- (a) reversible process
- (b) **irreversible process**
- (c) light controlled phenomenon
- (d) response to light stimulus

Answer key: Q1 (b) Q2 (b) Q3 (a) Q4 (c) Q5 (d) Q6 (b) Q7 (a) Q8 (b)

Photo Morphogenesis and Phytochrome

Q1 Lettuce seeds germinate only when:

- (a) Placed in dark
- (b) Any colour of light is available
- (c) **Red light is available**
- (d) Blue light is available

Q2 Pfr → Pr conversion is caused by :

- (a) blue light
- (b) red light
- (c) **far → red light**
- (d) green light

Q3 The active form of phytochrome has the colour:

- (a) **blue**
- (b) green
- (c) red
- (d) yellow

Q4 Pfr shows maximum absorption at:

- (a) 660nm
- (b) **730nm**
- (c) 466nm
- (d) 650nm

Q5 Phytochrome is involved in:

- (a) **Photoperiodism**
- (b) Phototropism
- (c) Photosynthesis
- (d) Photorespiration

Q6 In the red and far → red responses of plants , it is the :

- (a) first treatment that counts
- (b) **last treatment that counts**
- (c) middle treatment that counts
- (d) sum total of all treatment that counts

Q7 Phytochrome is found in :

- (a) Thallophytes
- (b) seed plants
- (c) vascular plants
- (d) **whole plant kingdom**

Answer key : Q1(c) Q2 (c) Q3 (a) Q4 (b) Q5 (a) Q6 (b) Q7 (d)

Plant – Water Relations

Q1 The process of taking gaseous carbondioxide or oxygen by leaves is termed as :

- (a) exosmosis
- (b) endosmosis
- (c) transpiration
- (d) **diffusion**

Q2 if osmotic potential of a cell is -10 bars and its pressure potential is 5 bars , its water potential would be:

- (a) **-5 bars**
- (b) 5 bars
- (c) -10 bars
- (d) 10 bars

Q3If a cell is fully turgid , which of the following would be zero :

- (a) Turgor pressure
- (b) Wall pressure
- (c) **Diffusion pressure deficit**
- (d) Osmotic pressure

Q4 DPD may be defined as the amount by which diffusion pressure of :

- (a) **a solution is lower than that of its solvent**
- (b) a solution is higher than that of its solvent
- (c) a solvent is higher than that of its solutes
- (d) a solvent is lower than that of its solutes

Q5 When a cell is kept in 0.5 M solution of sucrose , its volume does not alter. If the same cell is placed in 0.5 M solution of NaCl , the volume of cell will:

- (a) remain same
- (b) be plasmolysed
- (c) **decrease**
- (d) increase

Q6 The magnitude of diffusion pressure deficit in a non turgid cell is equal to :

- (a) **osmotic pressure – wall pressure**
- (b) osmotic pressure + wall pressure
- (c) turgor pressure
- (d) osmotic pressure

Answer key : Q1 (d) Q 2 (a) Q3 (c) Q4 (a) Q5 (c) Q6 (a)

Absorption and Transport of Water : Ascent of sap

Q1 Root cap has no role in absorption because it has :

- (a) no direct connection with vascular system
- (b) loosely arranged cells
- (c) no cells containing chloroplasts
- (d) **no root hairs**

Q2 Wilting of plant occurs when :

- (a) phloem is blocked
- (b) **xylem is blocked**
- (c) both xylem and phloem are blocked
- (d) a few old roots are removed

Q3 Which of the following is a rapid type of absorption?

- (a) **passive absorption**
- (b) active osmotic absorption
- (c) active non osmotic absorption
- (d) salt absorption

Q4 Water available to plants is :

- (a) run off water
- (b) hygroscopic water
- (c) gravitational water
- (d) **capillary water**

Q5 Root pressure is maximum when:

- (a) transpiration is very high and absorption is very low
- (b) **transpiration is very low and absorption is very high**
- (c) both absorption and transpiration are very high
- (d) both absorption and transpiration are very low

Q6 The pulsatile theory is given by :

- (a) **J.C. Bose**
- (b) Dixon
- (c) Strassburger
- (d) Sachs

Q7 Flowers cut under water remain fresh for a longer time because :

- (a) it has sufficient supply of water
- (b) **water column is not blocked by air bubbles**
- (c) transpiration is lowered
- (d) there is no vascular supply

Q8 Exudation is associated with :

- (a) capillarity
- (b) imbibition
- (c) **Root pressure**
- (d) pulsation in innermost layer of cortex

Q9 Girdling experiment cannot be performed in sugarcane because:

- (a) it cannot stand injury
- (b) **Vascular bundles are scattered in it**

- (c) in it the phloem lies inner to xylem
- (d) sugarcane plants are too delicate

Answer key: Q1(d) Q2 (b) Q3 (a) Q4 (d) Q5 (b) Q6 (a) Q7 (b) Q8 (c) Q9 (b)



Key Terms

Abscission: The dropping off of leaves, flowers, fruits, or other plant parts, usually following the formation of an abscission zone.

Aerobic respiration: respiration which occurs in presence of oxygen

Aleurone: a kind of protein found in plants , particularly in the peripheral region of seeds.

Day Neutral Plants: Flower without respect to length of the day.`

Anthesis: - the opening of flower

Assimilation- uptake and formation of simple food stuffs.

Bio-assay- the quantitative estimation of biologically active substances in standardized conditions by the amount of the growth of micro –organisms.

Bioluminescence- production of light by living organisms

Fermentation:- A process containing decomposition of organic substances by micro- organisms

Florigen – a kind of plants hormone (hypothetical) stimulating flowering in plants.

FMN – Flavin mononucleotide

Fucoxanthin: -a kind of carotenoid pigment present with chlorophyll in brown algae.

Galactose – a hexose sugar , common constituent of many gums , mucilage and pectins.

Gelation- colloid transformation from the sol to the gel condition

Gibberellins- a group of hormones showing stimulating effect on stem elongation.

Globulins:- group of proteins

Glucosides- a group of enzymes catalyzing the hydrolysis of glucosides

Glycolysis- The course of stepwise degradation from glucose to pyruvic acid **Golgi apparatus :** name of a cell organelle discovered by Camillo Golgi.

Growth – a permanent increase in size

apositional growth- growth due to deposition on the surface of tissue.

determinant growth - growth of a plant stem that is terminated early by the formation of a bud or naturally self-limited growth, resulting in a plant of a definite maximum size

Guttation- loss of water in form of water drops through hydathodes.

Heredity – transmission of character from one generation to next generation (parent to offspring).

Hormone- hormones are organic substances which are produced in exceedingly minute quantities in one part of plant and transported to other parts where they exert a profound effect on growth.

Hydathode- water excreting gland occurring on the edges or tips of leaves.

Heliotropism: (Gk. helios, the sun) Ability of the leaves and flowers of many plants to move diurnally, orienting themselves either perpendicular or parallel to the sun's direct rays; also called heliotropism.

Inhibitor – a substance that inhibits the process

Insulin: a kind of hormone and active in the control of glucose metabolism

Isoenzyme (isozyme) – an enzyme that occurs in different structural forms but has the same function.

Isomer – two compounds identical but different in structure are called isomers

Ligase - a group of enzymes which catalyses the combination of two molecules using energy derived from ATP molecules.

Lipoid – It is used for three different senses –

- (i) a substance which resembles fats in solubility , but not containing fatty acid e.g. carotene , terpene
- (ii) a substance which resembles fat in solubility , but may contain fatty acid e.g. phospholipid (excluding material)
- (iii) Occasionally fat

Long Day Plants: Flower in the summer, when the light periods are longer than the critical length.

Maltose : a disaccharide sugar formed during the breakdown of starch

Mesosome- invagination of plasma membrane in prokaryotic cells such as bacteria

Metabolism- the chemical processes occurring within the organism

Metabolite- substance that takes part in the process of metabolism

Mutase – a term used for enzymes which catalyse intramolecular transfers.

Short Day Plants: Flower in early spring or fall when days are shorter with less intense light.

Starch - the principle reserve food materials of the green plants , frequently found in colourless plastids

Stoma – pore in the epidermis of plants , frequently found in the green region of the plants. It is helpful in gaseous exchange.

Symbiosis – association of two dissimilar organism to their mutual advantage e.g. root nodules of leguminous plants (Bacteria are present in root nodules.

Transpiration - loss of water in the form of vapour by the aerial parts of terrestrial plants.

Thigmotropism: (Gk. thigma, to touch) Response to contact with a solid object as seen in tendrils. They wrap around any object with which they come in contact, and so enable the plant to cling and climb.

Vacuole – a fluid filled (cell sap) space bounded by a membrane (tonoplast) found in a cell.

Vernalin – a hormone produced by vernalization

Vernalization - treatment of seeds with low temperature to shorten the maturation period.

Zymase – an enzyme system which breaks down hexose sugars into alcohol and carbon dioxide, found in yeast. Zymase is the first cell free enzyme preparation .

B.Sc. (Part-II) Examination, 2011

Faculty of Science

[Also common with subsidiary Paper of B.Sc. (Hons.)Part-II]

(Three Year Scheme of 10+2+3 Pattern)

Botany**Third Paper****(Plant Physiology and Biochemistry)****Time : 3 hours****Max. Marks : 34****All questions are compulsory.****Question No. 1 Sub point (i) to (xx) each carry equal half marks.****Question No. 2 to 5 each carry equal 6 marks.**

1. Give short answer the following:-

- (i) What is wilting coefficient?
- (ii) Guard cells help in.....
- (iii) Name any two diseases caused by mineral deficiency.
- (iv) Write a chemical structure of protein.
- (v) Write botanical names of two plants having C₄ cycle.
- (vi) What is the full form of CAM?
- (vii) What is R.Q.?
- (viii) Name the mineral elements involved in the opening and closing of stomata.
- (ix) Sunken stomata are seen in
- (x) What is pH?
- (xi) Who discovered tri-carboxylic acid cycle?
- (xii) How many molecules of NADH and ATP are synthesized in one turn of Krebs' cycle?
- (xiii) Name the phenomenon of formation of pyruvic acid from glucose.
- (xiv) Lock and key method of enzyme substrate complexing was given by.....
- (xv) NADH, NADPH act as an electron.....
- (xvi) Amino acids are building blocks of

- (xvii) The phenomenon of photoperiodism was characterized and named by.....
- (xviii) Auxin shows accumulation.....from light.
- (xix) What is Gibberella fujikori?
- (xx) Which plant hormone is primarily involved in inducing cell division?

2. Write short notes on the following :-

- (i) Transpiration and Guttation
- (ii) Role of K^+ in stomatal movement.

Or

What is root pressure? How will you demonstrate it?

3. Write short notes on the following :-

- (i) What is the difference between aerobic and anaerobic respiration?
- (ii) Pentose Phosphate Pathway of respiration.

Or

Describe Krebs's cycle (TCA) in detail.

4. Describe any two of the following:-

- (i) Explain the lock and key hypothesis of enzyme action.'
- (ii) Alkaloids
- (iii) Oxidation of fatty acids.

Or

Give an account of the structure and biological significance of at least two polysaccharides.

5. Write short notes on any two-

- (i) Sigmoid curve
- (ii) Apical dominance
- (iii) Auxins.

Or

Differentiate between Vernalization and Photoperiodism.

B.Sc. (Part-II) Examination, 2010**Botany****Third Paper****(Plant Physiology and Biochemistry)****Time : 3 hours****Max. Marks : 34****All questions are compulsory.****Question No. 1 Sub point (i) to (xx) each carry equal half marks.****Question No. 2 to 5 each carry equal 6 marks.****1. Give short answer the following:-**

- (i) Stomatal index.
- (ii) Pressure working against turgor pressure.
- (iii) Name element essential for activity of nitrate reductase enzyme.
- (iv) Define channel proteins.
- (v) Write botanical names of two plants having C-4 cycle.
- (vi) Write chemical formula of chlorophyll a and chlorophyll b.
- (vii) have more than unit RQ.
- (viii) Function of Leg haemoglobin is
- (ix) Write glycosidic linkage present in starch.
- (x) Write inhibition and its importance in plants.
- (xi) Which biological process produces oxygen?
- (xii) Write full names of ATP and PGAL.
- (xiii) GTP is produced in Krebs cycle in which step?
- (xiv) Genetic code for termination of polypeptide chain.
- (xv) Define prosthetic group with one example.
- (xvi) In oxidation of lipids more energy is released in α / β oxidation.
- (xvii) Name two examples of secondary metabolites.
- (xviii) Draw sigmoid curve and name its steps.
- (xix) Which hormone is responsible for tropic movement?
- (xx) Name precursor of indole-3-acetic acid.

2. What is transpiration? Describe mechanism and controlling factors of transpiration.

Or

Write short notes on the following:-

- (i) Water potential
- (ii) Hydroponics.

3. Describe Calvin-Malvin cycle in detail.

Or

Illustrate the following:-

- (i) Electron transport system
- (ii) Photorespiration
- (iii) Biochemistry of Nitrogen fixation.

4. Describe properties, classification and biosynthesis of Amino Acids.

Or

Describe the following:-

- (i) Enzyme kinetics
- (ii) Polysaccharides
- (iii) Flavanoids.

5. Write in detail about photoperiodism.

Or

Write short notes on the following:-

- (i) Phototropism
- (ii) Phytochrome
- (iii) Morphectin.

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1. Gives short answers of the following:-

- (i) Write the water potential of pure water.
- (ii) Why does water act as a temperature buffer?
- (iii) Which property of water makes water an excellent solvent?
- (iv) Define osmotic potential.
- (v) What is scotoactive movement of stomata?
- (vi) What is the unit of photosynthesis?
- (vii) What is the first stable product during C_4 cycle?
- (viii) Is photorespiration associated with ATP formation?
- (ix) Name any two algae associated with nitrogen fixation.
- (x) In what form do plants obtain their nitrogen from the soil?
- (xi) Nitrogen containing heterocyclic compounds are known as alkaloids/steroids.
- (xii) Flavanoids which protect plants from compounds are known as alkaloids/steroids.
- (xiii) Flavanoids which protect plants from parasitic diseases are called.....
- (xiv) Name the alkaloid obtained from Ephedra.
- (xv) What is the central dogma?
- (xvi) Which category of enzymes catalyze the transfer of a specific group from one substance to the other?
- (xvii) Name the enzyme which is responsible for degradation of auxin.
- (xviii) What is the primary precursor of IAA?

- (xix) What is bolting?
- (xx) Synthesis of which hormone increase during seed germination?
- (xxi) Name the hormone which delays senescence.

2. Explain the transpiration pull-cohesion theory with its merits and demerits.

Or

Write short notes on the following:-

- (i) Mass flow hypothesis
- (ii) Deficiency symptoms of potassium and magnesium on plants.

3. What is photophosphorylation ? Describe non-cyclic photophosphorylation.

Or

Write short notes on the following:-

- (i) Nitrogen fixation by symbiotic bacteria
- (ii) Glycolysis.

4. What are enzymes? Describe the mechanism of action of enzyme.

Or

Write short notes on any two:-

- (i) B-oxidation
- (ii) Classification of carbohydrates
- (iii) Structure and importance of amino acids.

5. Write in detail about biosynthesis and physiological effects of ethylene.

Or

Write short notes on the following:-

- (i) Vernalisation
- (ii) Senescence.

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