AS -2927

B.Sc. (Third Semester) Examination, 2013

Forestry

(Paper-I : Plant Biochemistry & Biotechnology)

Time : Three hours

Maximum Marks : 60

Model answer

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Note : Section-A is compulsory. Attempt any four questions from Section-B

Section : A (Compulsory)

Q1. Multiple Choice Questions **[1×1]**

(i) Father of Biochemistry

(a) Antoine Lavoisier ✓ (b) Justus Von Liebig
(c) Mendel (d) Edison

Ans : (a) Antoine Lavoisier

(ii) Term "Biochemistry" was coined by

(a) Lavoisier (b) Liebig
(c) Neuberg ✓ (d) None

Ans : (c) Neuberg

(iii) Descriptive biochemistry deals with study of what characteristics of cell components

(a) Qualitative (b) Quantitative
(c) Both a & b ✓ (d) Cell reactions

Ans : (c) Both a & b

(iv) Asymmetric organic molecules are mirror images of each other and are

(a) Super imposable (b) Non-super imposable ✓
(c) Both a & b (d) None

Ans : (b) Non-super imposable

(v) Whole genome shotgun sequencing method was developed by

(a) Venter (b) Smith
(c) Robertson (d) Both a & b ✓

Ans : (d) Both a & b

(vi) The term enzyme is derived from

(a) Greek word ✓ (b) Latin word
(c) English word (d) French word

Ans : (a) Greek word

(vii) The word enzyme was first introduced by

(a) W. Kuhne ✓ (b) Watson
(c) Schumpeter (d) None

Ans : (a) W. Kuhne

(viii) Which enzyme adds water across the bond?

(a) Oxidoreductases (b) Lyases
(c) Hydrolases ✓ (d) Ligases

Ans : (c) Hydrolases

(ix) Which enzyme removes groups from substrate?

(a) Lyases ✓ (b) Hydrolases
(c) Ligases (d) Transferase

Ans : (a) Lyases

(x) The nucleic acid was first isolated by

(a) Miescher ✓ (b) Fischer

(c) Altmann (d) None

Ans : (a) Miescher

(xi) The term nucleic acid was given by

(a) Altmann ✓ (b) Fischer

(c) Miescher (d) None

Ans : (a) Altmann

(xii) Purine base is

(a) Adenine ✓ (b) Cytosine

(c) Thymine (d) Uracil

Ans : (a) Adenine

(xiii) Nucleosides is a combination of

(a) Base (b) Sugar

(c) Phosphoric acid (d) Both a & b ✓

Ans : d) Both a & b

(xiv) Protein is derived from

(a) Greek word ✓ (b) Latin word

(c) French word (d) English word

Ans : (a) Greek word

(xv) The term protein was first proposed by

(a) Bloor (b) Elton

(c) Berzelius ✓ (d) None

Ans : (c) Berzelius

(xvi) Globular protein is

(a) Soluble in water ✓ (b) Insoluble in water

(c) Either a or b (d) None

Ans : (a) Soluble in water

(xvii) The term lipid was introduced by

(a) Bloor ✓ (b) Bernstein

(c) Southwick (d) Mathew

Ans : (a) Bloor

(xviii) When glycerol is heated with H_2SO_4, it produces

(a) Acrolein ✓ (b) Esters

(c) Ketone (d) Steroids

Ans : (a) Acrolein

(xix) Steroids are

(a) Alcohol (b) Hormones

(c) Medicines (d) Both a & b ✓

Ans : (d) Both a & b

(xx) Lecithins are

(a) Dextrorotatory ✓ (b) Levorotatory

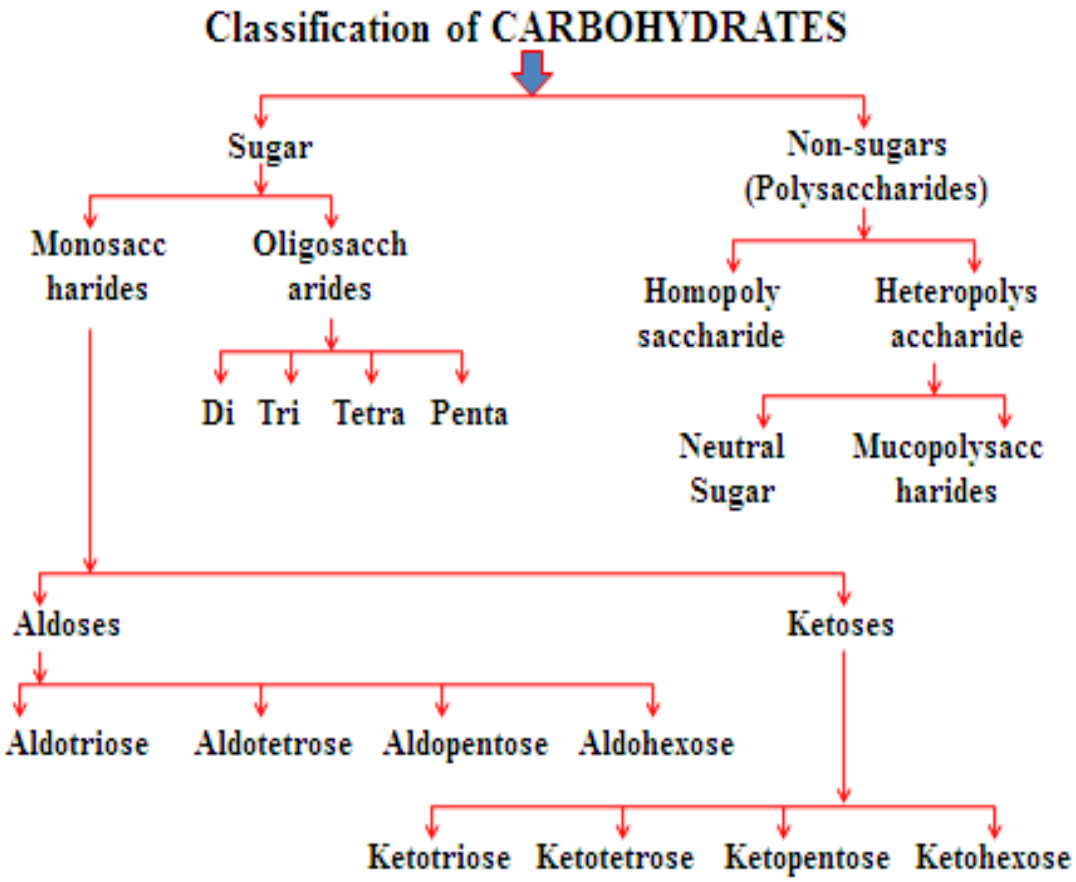
(c) Both a & b (d) None

Ans : (a) Dextrorotatory

Section : B (Attempt any four questions) **(1x10)**

Q2. Define carbohydrate. Discuss about the physical and chemical properties of carbohydrate.

Ans : The Carbohydrates, often termed as sugars, are the "staff of life" for most organisms. Carbohydrates are "hydrates of carbon". They contain carbon, hydrogen and oxygen in the ratio 1:2:1. Hydrogen and oxygen are combined in the same proportion as in water (H_2O).Carbohydrates are defined as optically active polyhydroxy aldehydes or ketones; or substances giving polyhydroxy aldehydes or ketones on hydrolysis. Carbohydrates are represented by the general formula $Cx(H_2O)y$. For example, glucose has the molecular formula $C_6H_{12}O_6$.



Physical properties of carbohydrate

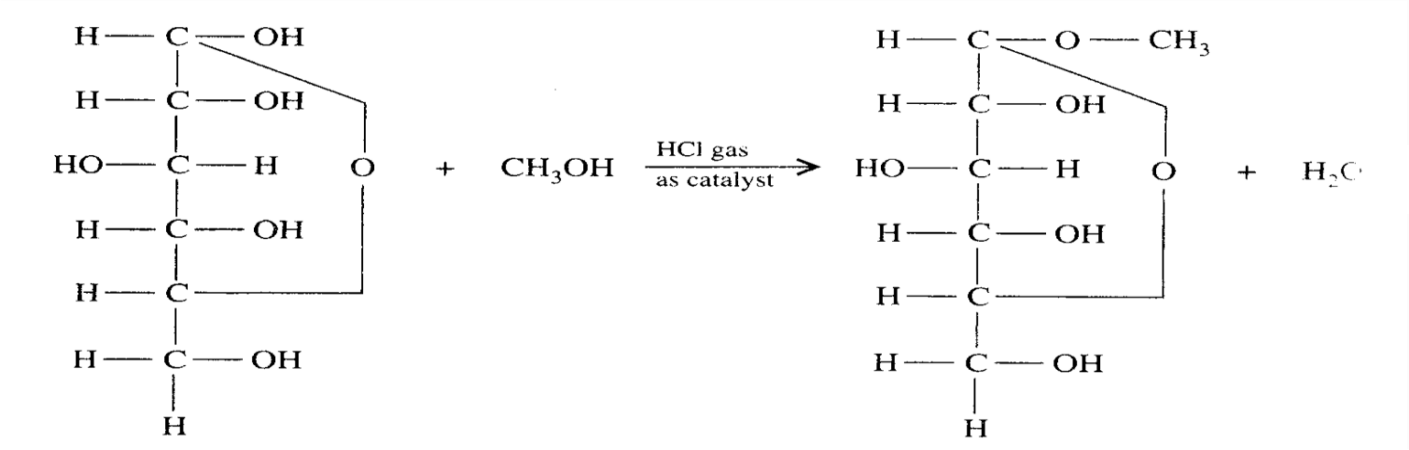
1. *Colour* - colourless
2. *Shape* - crystalline
3. *Solubility* – water soluble
4. *Taste* - sweet
5. *Optical activity* – Optically active. (a) Dextrorotatory ('d' form) and (b) Levorotatory ('l' form)
6. *Mutarotation* – The change in specific rotation of an optically active compound is called mutarotation.

$+112^0 \longrightarrow +52.5^0 \longleftarrow +19^0$

α-D-glucose **β -D-glucose**

Chemical properties of carbohydrates

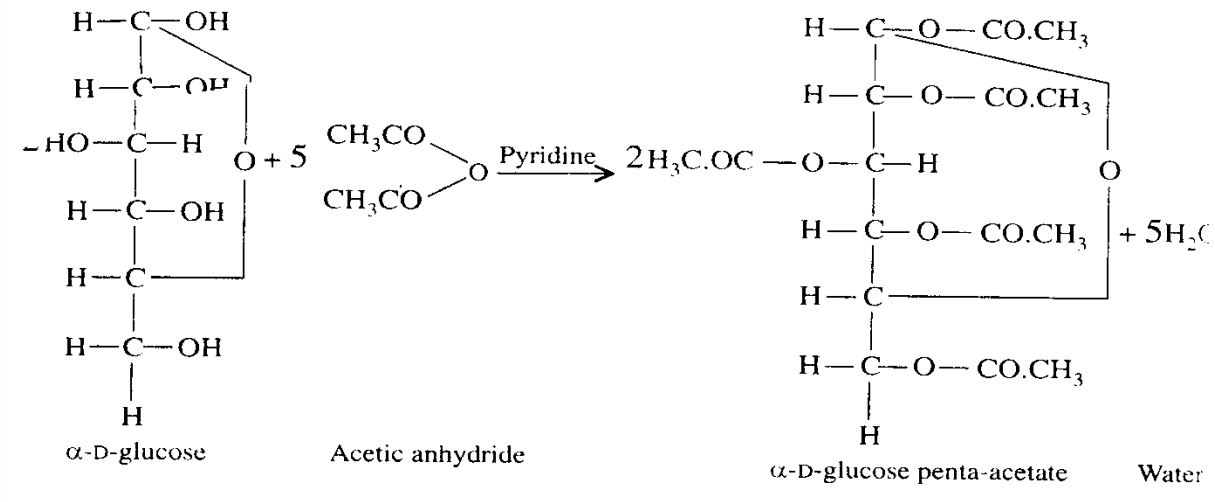
1. *Glucoside formation* -



Glucose + **Methyl alcohol =** **Methyl glucoside**

2. *Esterification*

Glucose reacts with five molecules of acetic anhydride to form ester.

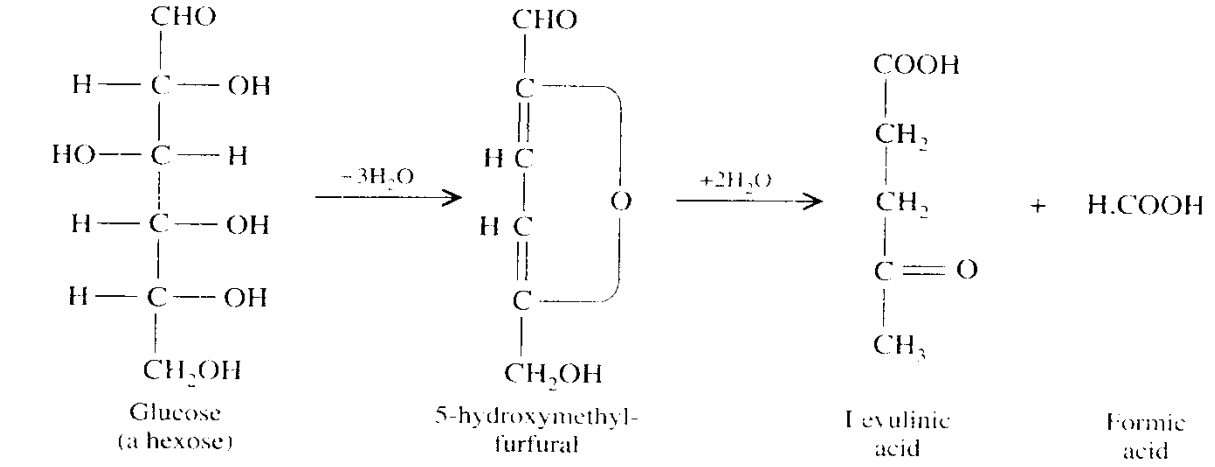


3. *Reduction –*

Monosaccharides can be reduced by various reducing agents forming alcohol.

4. *Reaction with Concentrated HCl*

When carbohydrate treated with concentrated HCl, they form 5-hydroxymethylfurfural which on further heating yields Levulinic acid and Formic acid.



5. *Reducing agents –*

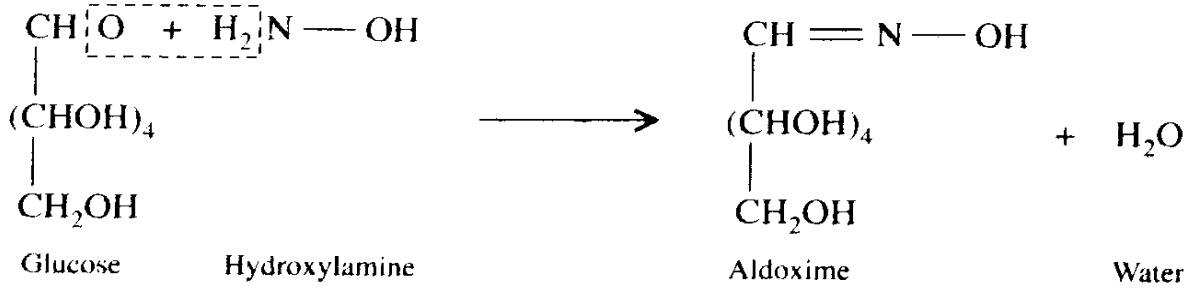
Monosaccharides reduce oxidizing agent such as hydrogen peroxide. In such reaction, sugar is oxidized at the carbonyl group and oxidizing agent becomes reduced.

$$\mathbf{C_6H_{12}O_6 + 2\ Cu(OH)_2 \longrightarrow C_6H_{12}O_7 + Cu_2O + 2H_2O}$$

Glucose Fehling's solution Gluconic acid Cuprous oxide

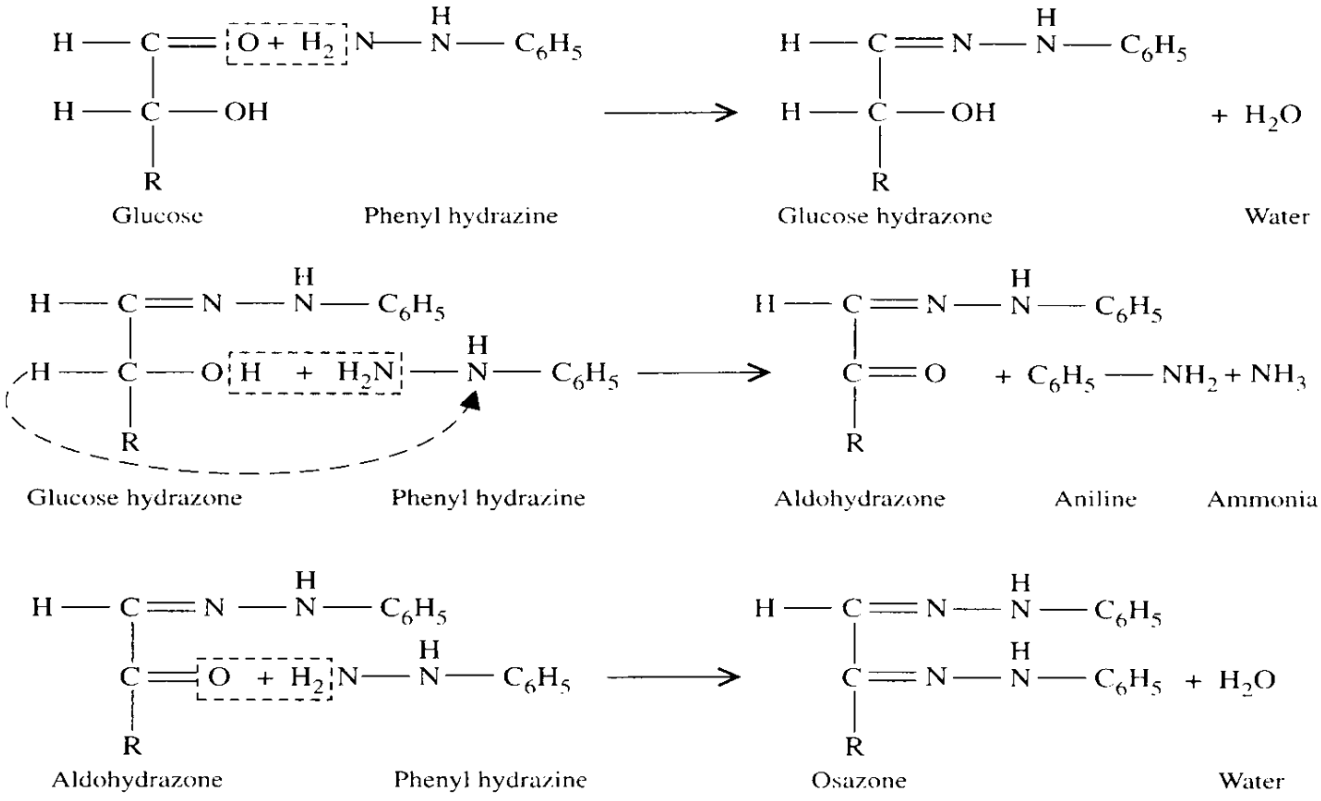
6. Formation of Oximes

Carbohydrate reacts with hydroxylamine to form Aldoxime and water.



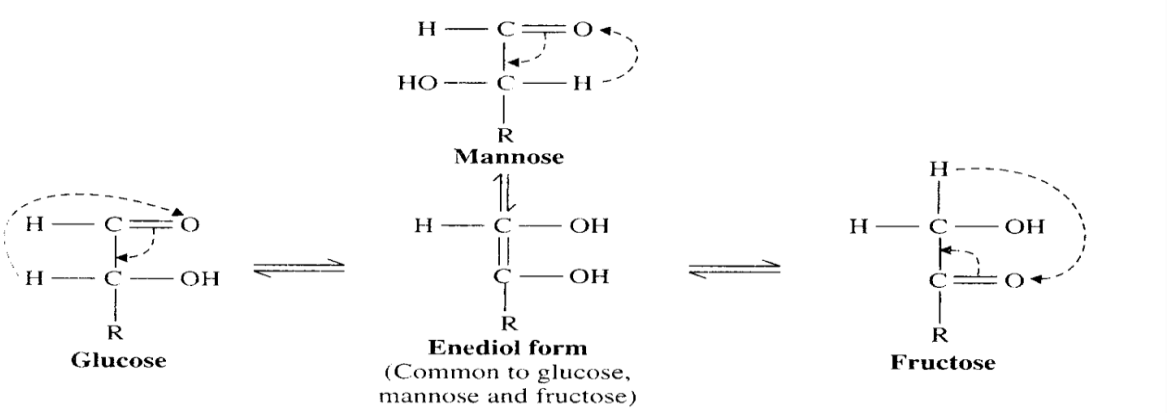
7. Formation of Osazone

Carbohydrate reacts with Phenyl hydrazine and produces Osazone, Aniline and Ammonia.



8. Enolozation

Glucose, Fructose and Mannose are interconvertible in weak alkaline solution such as calcium hydroxide at low temperature. This reaction is called Lobry de Bruyn-Alberda Ekenslein Conversion.



Interconversion of Glucose, Mannose and Fructose

9. Caramelization –

When Monosaccharides is added with concentrated alkali, it is burnt and this process is called Caramelization.

10. Phosphorylation of hexoses

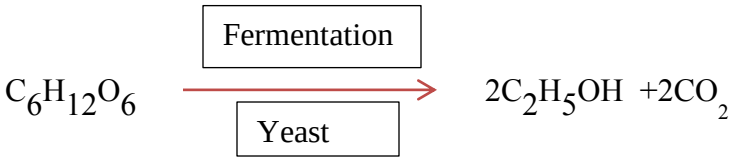
The formation of Phosphoric acid derivative of hexoses is called phosphorylation where hexoses are converted into phosphoric acid esters.

11. Kiliani Synthesis –

This reaction was proposed by Kiliani in 1886. When HCN is added to monosaccharide, it forms Cyanohydrin. The Cyanohydrin is then hydrolysed to produce Carboxylic acid which is converted into γ-lactone by lectonization. Finally lactones are reduced to aldose containing 1 carbon atom more than its parent sugar.

12. Fermentation –

Monosaccharides are readily fermented by yeast. This process of yeast fermentation is very complex.



$C_6H_{12}O_6 \xrightarrow[\text{Yeast}]{\text{Fermentation}} 2C_2H_5OH + 2CO_2$

Monosaccharides Alcohol

Q3. What do you mean by lipid? Write the characteristics features of glycerol and fatty acid.

Lipids are the esters of alcohol and fatty acid. They are water insoluble, oily or greasy organic compounds soluble in non-polar organic solvents. Lipids are made up of three fatty acid joined to an alcohol. The term lipid was first introduced by Bloor in 1943.

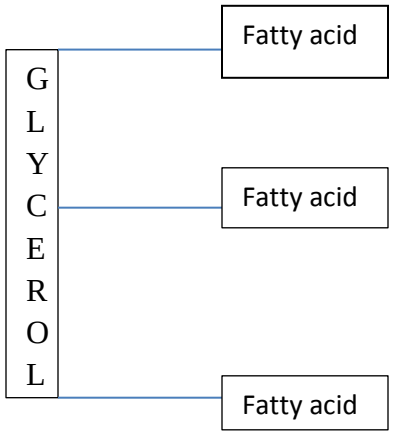
Lipids have three important properties:

(i) They are insoluble in water but soluble in non-polar organic solvents, such as acetone, alcohol, benzene etc.

(ii) They contain a large proportion of carbon and hydrogen bonds and release large amount of energy on breakdown.

(iii) On alkaline hydrolysis lipids yield alcohol and fatty acids.

Structure of Lipids



Lipids are esters of glycerol and fatty acids. They are formed by the combination of alcohol and fatty acids.

Characteristics of glycerol

Glycerol is an important component of lipids. It is an alcohol. Chemically, it is a trihydric alcohol. It contains three alcoholic groups (OH). Of these three, two are primary and the third one is called secondary. Glycerol combines with three similar fatty acids to form a simple lipid called triglyceride with the release of 3 molecules of water.

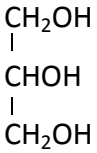


Fig-Glycerol or Glycerine

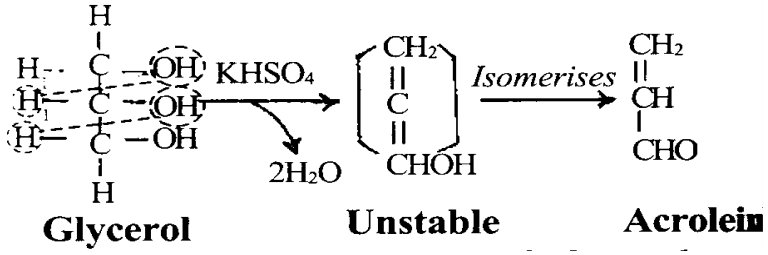
Glycerol has two important properties. They are –

(a) Formation of Esters

Glycerol reacts with acids, both organic and inorganic acids, to form esters like monoesters, diesters and trimesters. Triesters of glycerol with higher fatty acids constitute lipids.

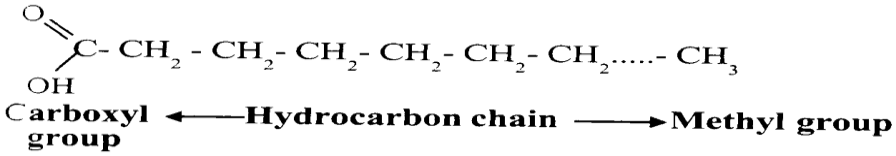
(b) Dehydration

When glycerol is heated in the presence of dehydrating agent like H_2SO_4, phosphorus pentoxide or potassium hydrogen sulphate, it produces an unsaturated aldehyde called acrylic aldehyde or acrolein. Acrolein has an unpleasant odour related to the burning of fats.



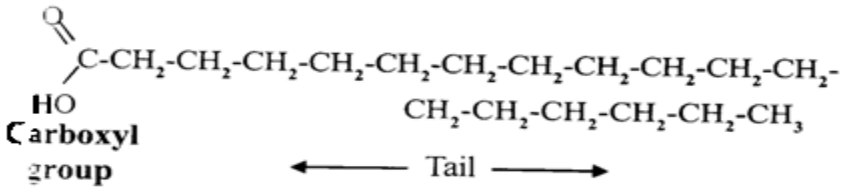
Characteristics of fatty acid

Fatty acids are aliphatic straight chain hydrocarbon compounds with a terminal carboxyl group. They have following characteristics-

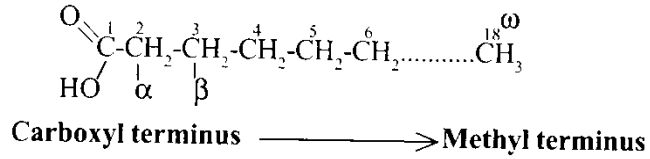


1. Fatty acids are the building block components of most lipids.

2. Fatty acids are long chain organic compounds containing even number of carbon atoms from 4 to 24.
3. They have a single carboxyl group and a long non-polar hydrocarbon tail. The non-polar tail gives most lipids their water insoluble and oily or greasy nature.



4. In fatty acids, carbon atoms are numbered starting at the carboxyl terminus.

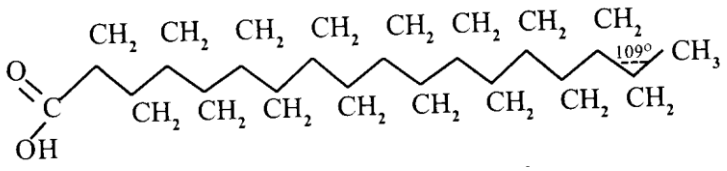


5. Carbon atoms 2 and 3 are often referred to as α and β respectively. The methyl carbon atom at the distal end of the chain is called ω carbon.
6. Fatty acids do not occur in free or unbound form. They are bound in lipids through covalent bond.
7. The hydrocarbon chain is almost unbranched; very rarely it is branched.
8. Fatty acids with 16 and 18 carbon atoms are most abundant in nature.
9. The fatty acids, containing a single bond, is called saturated fatty acid. The suffix-anoic indicates a fully saturated fatty acid.
10. The fatty acid containing one or more double bonds is called unsaturated fatty acid. The suffix enoic, dienoic and trienoic suggest the presence of one, two and three double bonds in the molecule.
11. In general, unsaturated fatty acids are twice as abundant as saturated fatty acids in both animal and plant lipids.
12. The long hydrocarbon tail may be fully saturated with only single or it may be saturated with one or more double bonds.
13. In most of the unsaturated fatty acids, there is a single double bond lying between carbon atoms 9 and 10. This is designated as Δ^9.
14. When there is more than one double bond, the additional bonds occur between the Δ^9 double bond and methyl terminal end of the chain. The symbol 18:0 denotes a C_{18} fatty acid with no double bond.

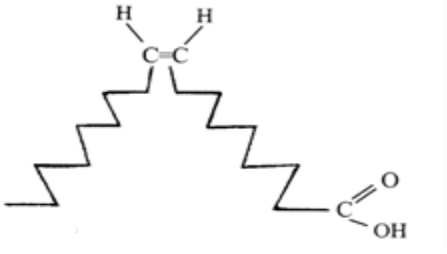
15. When two or more double bonds are present in a fatty acid, the double bonds are never conjugated. But the double bonds are separated by a methylene group.

$$-CH_2 - CH = CH - CH_2 - CH = CH - CH_2-$$

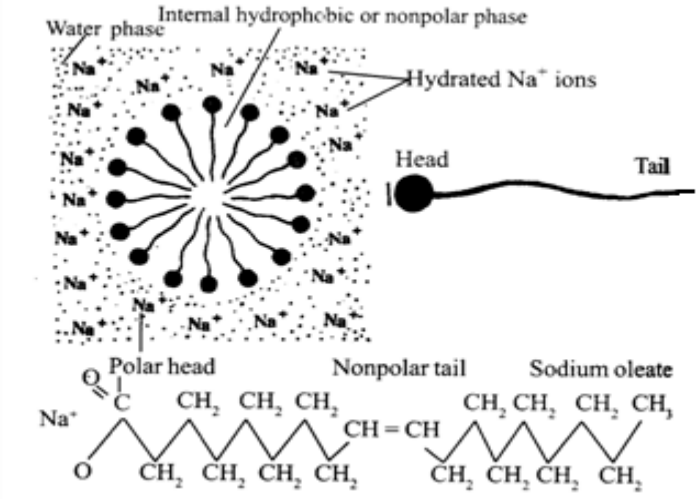
16. The hydrocarbon chain of a saturated fatty acid possesses zig-zag configuration with the bond angle between carbon-carbon being 109^0.



17. Introduction of a double bond between carbon atoms 9 and 10 causes a bend in the molecule.



18. The higher saturated fatty acids are solid having a waxy consistency.
19. The unsaturated fatty acids are oily and greasy.



20. The common higher fatty acids are insoluble in water. But they can be dispersed into micelles in dilute NaOH or KOH.
21. NaOH or KOH converts fatty acids into soaps. Soaps are the salts of fatty acids.

Q4. What is protein? Mention the different structure of protein.

Ans : **Protein :** Protein is a macromolecule composed of one or more polypeptide chains possessing a characteristics amino acid sequence. It is a polymer of amino acids. The term protein is derived from Greek word; Proteuo=primary or holding first place. The term protein was first proposed by Berzelius.

Structure of protein

Proteins are polymers of amino acid monomers. The amino acids are linked together to form a chain called peptide chain. The peptide chain has a variable length and it depends on the number of amino acids linked. The amino acids of proteins are called residues. The peptide chain is named according to the number of amino acids residue:-

(a) Dipeptide : Contains two amino acid residues.

(b) Tripeptides : Contains three amino acid residues.

(c) Oligopeptides : Contains less than 10 amino acid residues.

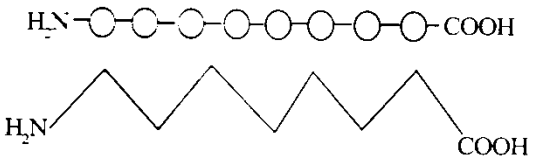
(d) Polypeptides : Contains more than 10 amino acid residues.

Proteins are Polypeptides with more than 100 amino acids residues.

A protein is formed of only one polypeptide chain or more than one polypeptide chains make up a protein.

N and C-Terminals

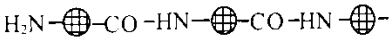
The polypeptide chain has two definite ends :– N- terminal end (having a free amino group) and C-terminal end (having a free carboxyl group).



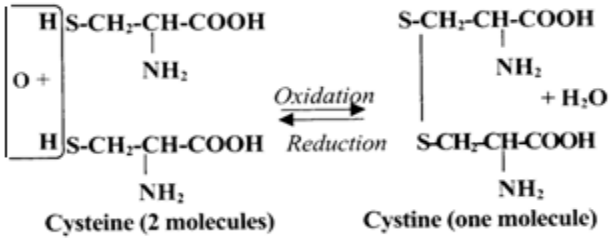
Chemical bond involved in Protein structure

Proteins are the polymers of amino acids monomers. Any two amino acid monomers are linked together by a chemical bond. There are 5 types of bonds which occur in proteins –

(a) Peptide Bond:- An amide bond where the CO group of COOH group of one amino acid is linked with the NH group of NH_2 group of the adjacent amino acid (-CO-NH-). The peptide bond produces the linear primary structure of proteins.



(b) Disulfide Bond :- It isa covalent bride formed between two polypeptide chains by a cystine residue. This bond is formed by the oxidation of thiol (-SH) groups of two cysteine molecules. This results in the formation of a molecule of cystine, an amino acid with a disulfide bridge.



(c) Hydrogen Bond :- Hydrogen bond is a week electrostatic attraction between one electronegative atom and a hydrogen atom covalently linked to a second electronegative atom.

(d) Nonpolar or Hydrophobic Bond :-The association of nonpolar groups with each other in aqueous systems because of the tendency of the surrounding water molecules to seek their most stable state, is called nonpolar or hydrophobic bond.

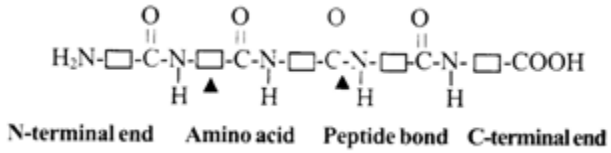
(e) Ionic or Electrostatic Bond :-Ionic bonds are formed by ionization. The bond is formed by the transfer of electrons (ions) from one atom to another. The ionic bonds maintain a folded nature of proteins and produce tertiary structure.

Configuration of protein

Linderstrom-Lang suggests four types of structural organization for proteins-

(a) Primary structure :

The primary structure of protein is defined as the linear sequence of amino acid residues making up its polypeptides chain. The protein may be formed of one or more polypeptide chains. The amino acids are arranged in specific sequence in the polypeptide chain. The amino acid residues are linked by peptide bonds. Sometimes the adjacent polypeptide chains are linked by disulfide bond.



(b) Secondary structure :

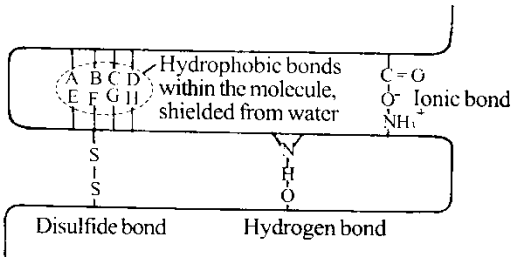
Secondary structure refers to the helical nature of the proteins. The secondary structure is derived from the primary structure by the formation of hydrogen bond interactions between amino acid residues fairly close to one another. This leads to the folding of the polypeptide chain into a helix. The intramolecular hydrogen bond occurs between amide nitrogen and the carbonyl oxygen of the 4^{th} amino acid residue of the polypeptide chain. In addition, disulfide bonds also occur either in the same chain or between polypeptide chains. The secondary structure is classified into two types-

(i) Helical structure –formed by hydrogen bond. Here the polypeptide chains are coiled as a rope

(ii) Pleated Sheet Structure of β-Structure- formed by hydrogen bond. It is of two types-(a) Parallel chain where N-terminal end of the polypeptide chains point in the same direction, and (b) Anti-parallel chain where N-Terminal end of the polypeptide chains point in the opposite direction.

(c) Tertiary structure :

The tertiary structure of protein is more complex than the secondary structure. It is exhibited by proteins having only one polypeptide chain.



The tertiary structure is attained by globular proteins. In such case, the secondary helical structure is further folded to form a globular, ellipsoidal shape. The folding is established by the appearances of more disulfide bonds as well as hydrogen bonds, ionic bonds and hydrophobic bonds.

(d) Quaternary structure

Two or more polypeptide chains associate together to produce a quaternary structure. It is exhibited by proteins containing more than one polypeptide chains. Quaternary structure is formed by the combination of primary, secondary and tertiary structures.

Q5. Define enzyme. How enzyme works?

Ans : Enzymes are biocatalysts that speed up a chemical reaction. All enzymes are protein but all proteins are not enzymes. The term enzyme is derived from Greek word means in yeast. It was first introduced by W. Kuhne in 1878. Enzymes are located in the cells, cytoplasm, mitochondria, tissues and body fluids.

Naming of enzymes

1. On the basis of substrate

Substrate is the substance on which an enzyme works.

(a) Carbohydrases: The enzyme acting on carbohydrates

(b) Proteinases : The enzyme acting on protein

(c) Lipases : The enzyme acting on lipids.

(d) Nucleases : The enzyme acting on nucleic acid.

2. Based on reaction

Some enzymes are named by adding the suffix-ase to the reaction

(a) Hydrolases : The enzymes catalyzing hydrolysis

(b) Oxidase : The enzymes catalyzing oxidation

(c) Reductase : The enzymes catalyzing reduction

(d) Dehydrogenase : The enzymes catalyzing Dehydrogenation.

(e) Phosphorylase : The enzymes catalyzing Phosphorylation.

(f) Transaminase : The enzymes catalyzing Transamination

3. *On the basis of substrate and reaction*

(a) Pyruvic decarboxylase: The enzyme removing CO_2 from pyruvic acid

(b) Isocitric dehydrogenase : The enzyme removing hydrogen from isocitric acid

4. Based on synthesis

Enzymes are named by adding the suffix ase to the substance to be synthesized

5. Based on discoverer

Eg. Pepsin, Trypsin

6. Based on Enzyme-Commission

(a) Oxidoreductases : This enzyme are involved in biological oxidation and reduction. They are of 3 types

(i) Dehydrogenases : The enzyme that catalyze the removal of hydrogen from one substrate and pass it on to second substrate. Example: Alcohol dehydrogenase enzyme

(ii) Oxidases : These are the enzymes which catalyze the removal of hydrogen from a substrate and pass it directly to oxygen. Example: Cytochrome oxidase

(iii) Oxygenases: These are enzymes which catalyze the incorporation of oxygen directly into the substrate.

(b) Transferases : These enzymes transfer a group from one substrate to another substrate. Example: Transaminase

c) Hydrolases: These are the enzyme that catalyzes hydrolysis. It is divided into three sub classes:

(i) Proteases : These are the enzymes that attack the peptide bonds on proteins and peptides. It is sub divided into

(A) Peptidases: These are the enzymes which act on peptide bonds adjacent to free amino or carboxyl group. It is known as exopeptidases.

(B) Proteinases: These enzymes act on the interior peptide bonds of protein. It is known as endopeptidases.

(ii) Esterases: These enzymes catalyze the hydrolysis of ester linkages. Example: Lipase

(iii) Carbohydrases: These enzymes hydrolyze the glycosidic linkage of simple glycosides, oligosaccharides and polysaccharides.

(d) Lyases : These are the enzymes which catalyze either the removal of group of atoms from their substrate leaving double bonds or add groups to double bonds without hydrolysis, oxidation or reduction. Example: Fumarase

(e) Isomerases: These catalyze the interconversion of a compound to one of its isomers. Example: Phosphohexose isomerase

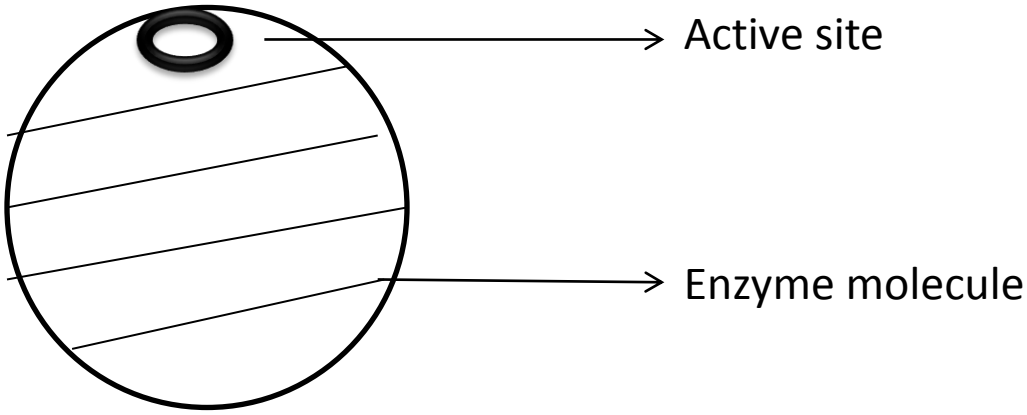
(f) Ligases : These enzymes catalyze synthesis reactions by joining two molecules coupled with the breakdown of a pyrophosphate bond of ATP. Examples: DNA ligase, RNA synthetase

How enzymes work

The breaking of substrate into end products by an enzyme is called enzyme action. Michaelis and Menton proposed a hypothesis for enzyme action. It occurs in the following steps

1. The enzyme molecule (E) combines with a substrate molecule (S) to form an enzyme-substrate complex. It is also called as Michaelis complex.

E + S ⟶ ES



2. The enzyme contains specific sites for attachment of substrate called as active site. They are made up of amino acid residue.

3. The active sites loosen the chemical bonds in the substrate and this leads to the breaking of substrates into end products.

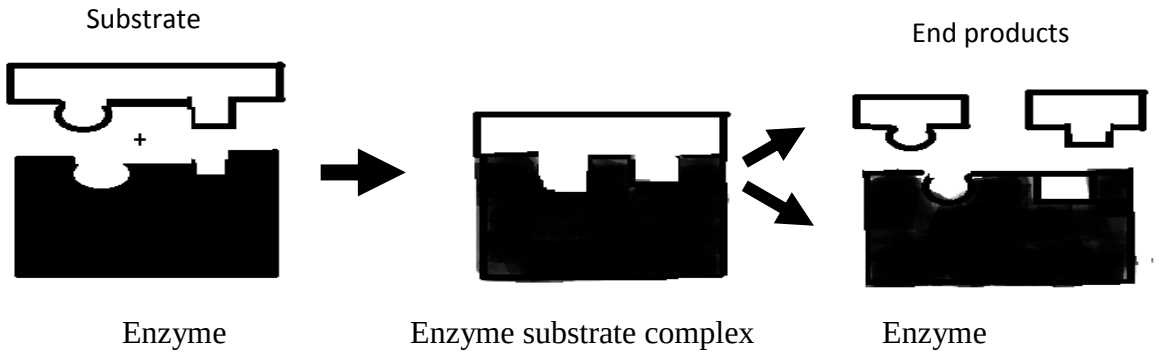
4. Finally the enzyme dissociates from end products

5. The enzyme is now free to combine with another molecule of substrate.

Hypothesis

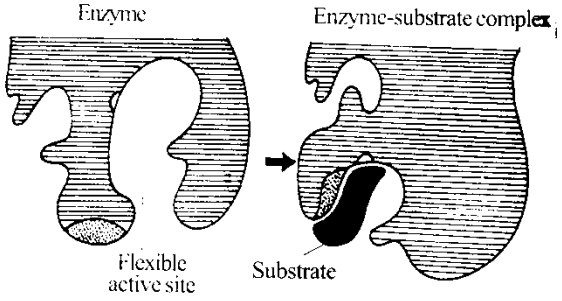
There are two hypotheses to explain the mechanism of formation of enzyme-substrate complex-

(a) Lock and Key hypothesis : This is proposed by Emil Fisher (1914). According to this hypothesis, the enzyme molecule has one or more specific points called active sites. The active sites exist in the enzyme in a rigit and proper conformation even in the absence of substrate. During enzyme action, the substrate fits into the active site of the enzyme as a key fits into the lock.



(b) Induced Fit hypothesis :

This was proposed by Koshland (1963). This theory says that the active site does not possess a rigid and performed structure. The region of the active site is flexible. When the enzyme reacts with the substrate, the substrate induces is conformational change in the active site of the enzyme. This change results in the development of attraction between enzyme and substrate so that an enzyme-substrate complex is formed. It leads to the loosening of the chemical bond linking the components of the substrate. As the reaction is completed, the substrate is split into an end product and the enzyme is released.

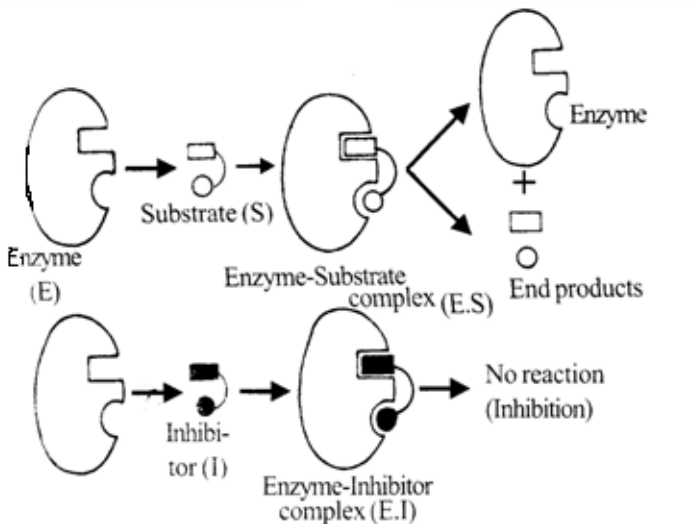


Enzyme inhibition

There are three types of enzyme inhibition-

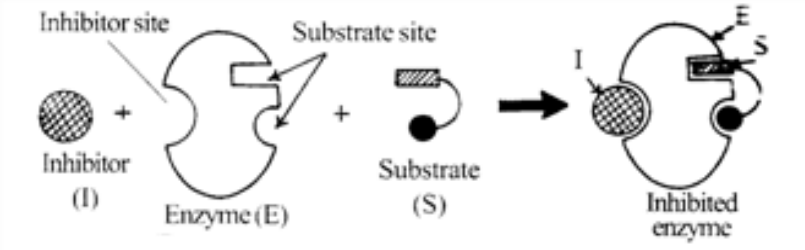
(a) Competitive inhibition-

It is a reversible enzyme inactivation. The substance bringing about reversible in activation is called Competitive inhibitor. The Competitive inhibitor closely resembles the substrate. So it can compete with the substrate to attach with the active site of the enzyme. It combines with the active site of the enzyme to form enzyme-inhibitor complex.



(b) Non-competitive inhibition-

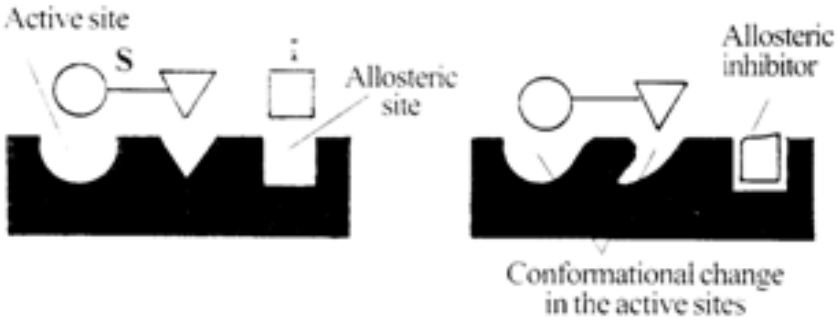
Here the inhibitor does not bind at the active site of the enzyme. It binds at a different site other than the active site and deforms the enzyme. This is an irreversible enzyme in activation. The inhibitor combines strongly with a site on the enzyme surface that it cannot be replace by increasing the substrate concentration.



(c) Allosteric inhibition-

Allosteric means other sites. The inhibitors binding to the other site is called allosteric inhibitors. It is a non-competitive reversible inactivation of the enzyme. Here the inhibitor is not competing with the substrate. The allosteric enzyme has two types of binding sites namely- active site and allosteric site. When a inhibitor binds at the allosteric site, it affects the conformation of the active

site. As a result, the substrate cannot bind with the enzyme. Here the inhibitor is the end product of the reaction. When more end products are produced, the whole reaction slows down and in course of time, the reaction comes to a halt. As the end product is consumed, synthesis resumes because there will not be any end product to inhibit enzyme.



Q6. Explain the term tissue culture. Mention the different techniques of tissue culture.

Ans :

Tissue culture : The culture of plant cells or plant tissues in a synthetic culture medium under controlled aseptic conditions is known as tissue culture. It is also called in vitro culture.

Different techniques of tissue culture

The culture of plant tissues in vitro involves eight main steps which are given below :

(a) Formulation of culture medium

Plant cells and tissues require a proper nutrient medium for their growth and development. The medium must contain the following components :-

i) A carbon source-Sucrose.

ii) Macronutrients – Nitrogen, phosphorous, potassium, magnesium etc.

iii) Micronutrients-Iron, manganese, zinc, boron, copper, molybdenum and chlorine.

iv) Organic supplements-Coconut milk, tomato juice, potato extract or yeast extract.

v) Vitamins

vi) Hormones-IAA, NAA, 2-4-D and kinetin.

The pH between 5.5 and 5.8 is suitable for cell growth. So pH of the medium should be adjusted to 5.5-5.8.

(b) Collection of explants materials :

Explant materials are taken from standing crops in green houses or from wild plants. They are cut just below the desired portion with a sharp knife and put in a screw-cap bottle to bring them to the laboratory. The following portions may be used as explants materials :-

i) Tap root (Eg. Carrot)

ii) Stem (Eg. Teak)

iii) Leaf (Eg. Tobacco)

iv) Endosperm (Eg. Corn)

v) Basal plate (Eg. Onion)

(c) Surface sterilization of explants materials

In this stage, the explant materials are cleaned well with running water. It is then transferred to a vial containing detergents like tween-80 and the vial is shaken two or three times. Thereafter, it is kept dipped in 70% ethyl alcohol for 5 minutes. It is again washed with distilled water to remove the ethanol. It is then submerged in 1% mercuric chloride solution for 10 minutes. After this, the plant material is washed well with distilled water. Now the explants material is free from microbial contaminants and growth inhibiting chemicals.

Seeds are surface sterilized by submerging them in 95% ethyl alcohol for 10 minutes and then in sodium hypochlorite (20%) for 20 minutes. Finally, they are washed with distilled water.

(d) Inoculation of explants :

The explants material is cut into small pieces of desirable size with the help of a sterile forceps and knife.

Mouth of the culture flask is opened near flame of a spirit lamp, the explants are transferred to the semi-solid medium in the flask by holding in forceps and the explants are presses gently in the medium.

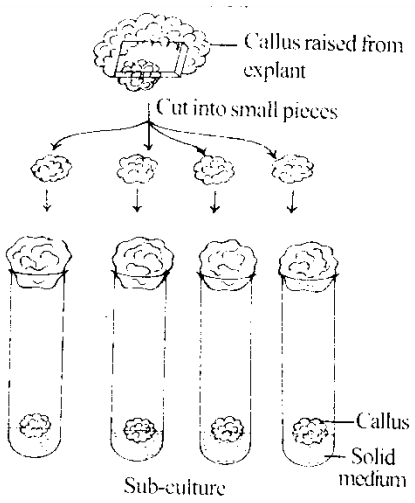
(e) Incubation of culture flasks

The explants-inoculated flasks are kept in a constant temperature room or incubator for a considerable time. This process is called incubation. The temperature inside the incubator is adjusted to 25 ± 2^0C which provides a constant temperature around 25^0C. A small illumination is given to the culture to ensure better growth of callus.

Cells of the explants divide repeatedly and grow into a mass of parenchyma cells. The mass of actively divided parenchymatous cells is called callus.

(f) Sub-culture of callus

Callus grows till enough nutrients are available in the culture medium. When the nutrient level comes down, the callus growth also decreases simultaneously. So the callus is cut into small pieces and each piece is transferred to a fresh medium. The maintenance of fragmented callus in fresh medium is called sub-cultures. Generally sub-cultures are practiced at regular intervals of 4 weeks. Plants may be regenerated from the sub-cultures.

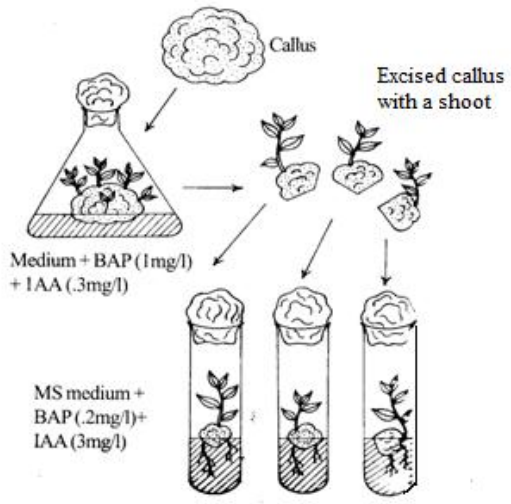


(g) Regeneration of plants from callus

Callus can be regenerated into plantlets in two ways. They are-

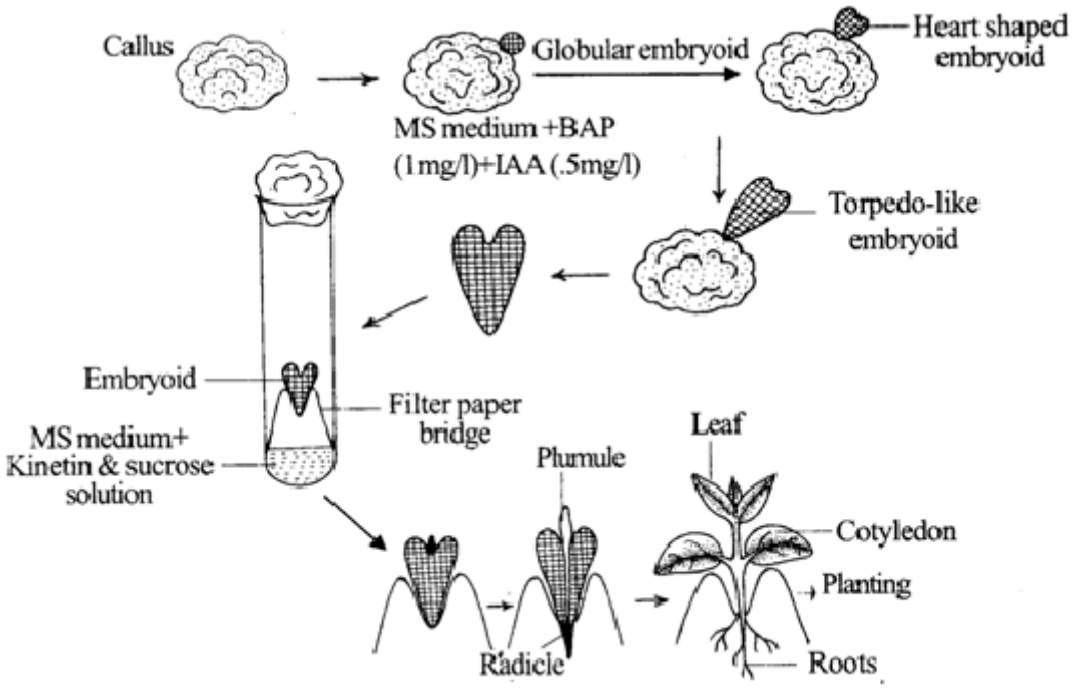
(i) ***Organogenetic method*** :-Organogenesis refers to development of adventives roots and shoots directly from the callus.

For plant regeneration, a callus is first grown in a shooting medium for 4-8 weeks. Many small meristems develop at the periphery of the callus. They are called meristemoids. Some meristemoids grow into shoots. After shooting, individual shoots are cut along with a portion of the callus and grown in the rooting medium. A few adventives roots develop from the shooted callus. As a result, a plantlet is formed.



(ii) ***Embryogenesis method*** **:-** Production of embryo-like structures from callus is known as embryogenesis or somatic embryogeny. The embryoid initial cell contains dense cytoplasm and a large nucleus. It divides repeatedly to form a globular mass of cells called globular embryo. The letter becomes heart shaped and then torpedo-like.

The torpedo-like embryoid is transferred to top of a filter paper bridge that is contact with a liquid medium in a tube. The embryoid develops into a plantlet within a few weeks. Well grown plantlets are then transferred to pots in a green house.



(h) Hardening

Gradual exposure of plantlets to outdoor environment for acclimatization is called hardening.

The regenerated plantlets are transferred to large bottles containing nutrient medium and kept in constant temperature room with illumination. It helps for growth of roots and shoots within a week. This step is called first stage hardening.

The grown up plantlets are planted in pots or polythene bags containing soil;, nutrients and spores of VAM fungus. During this period, the plant produce well developed root systems to absorb enough nutrients and water. VAM fungi help the roots to tolerate soil conditions. This stage is called second stage hardening.

Finally, polythene bags covers are removed from the plants and the plants are kept either in green house or in the outdoor environment for 5-10 days. This is called third stage hardening. After this hardening, plants are transplanted in the main field as usual.

Advantages of tissue culture :

The tissue culture technology is advantageous over the conventional breeding in the following ways :-

- Tissue culture produces identical clones.
- It helps to produce haploids and polyploids in a much desired direction.
- It overcomes the limitation of pollination barriers in conventional breeding.
- It can produce variability within a strain by in vitro mutations.
- It is less time consuming and less labour intensive.

Q7. Write short notes on

(i) Micropropagation

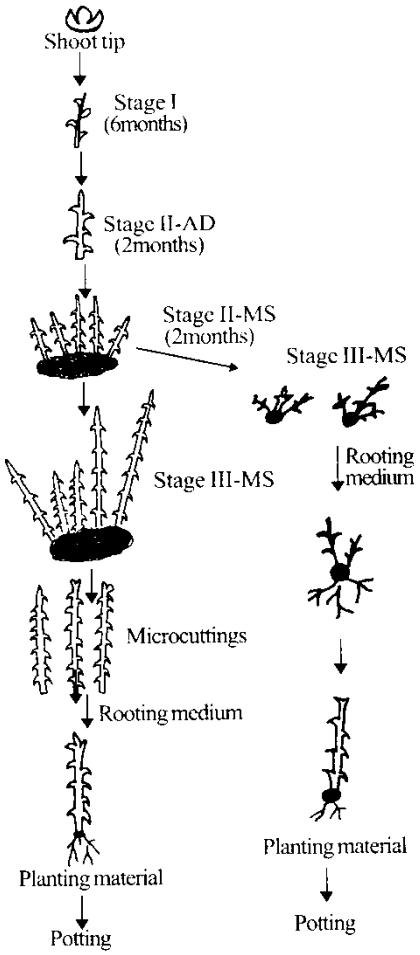
Ans : Micropropagation refers to the production of a large number of plants invitro within a limited duration and space for transplantation. Here, a single propagule is cultured to produce so many identical individuals for planting purpose. So the original genetic make-up is maintained in these plants.

Various stages of micropropagation

Fossard explained a detailed procedure of micropropagation in 1987. This procedure involves four stages-

(a) Stage-I

In the first stage, suitable explants (budsor nodal segments) are chosen, surface sterilized and inoculated into a suitable nutrient medium. This stage is extended upto 6 months. Generally no light is provided to the culture, but some antioxidants are added to it to oxidize the phenolic compounds in plant materials.



(b) Stage-II

The second stage is the multiplication stage that involves subcultures of propagules taken from stage-I. This stage is extended for 4 months. It include two sub-stages

i) Stage II-AD : The culture shoot is cut into small pieces, each of which is then inoculated into a fresh medium and cultured for two months to produce apically dominant shoots.
ii) Stage II-MS : Here the apically dominant shoots are sub-cultured to form multishooted cultures without roots. This callus with many shoots is obtained. This stage is last for 2 months.

(c) Stage-III

Here plantlets are obtained by changing the cultural conditions and media components. About 3,000-10,000 lux light intensity is provided to the culture. It includes three sub-stages-
i) Stage III-MC- Here the multishooted callus is transferred to a multishooted inducing medium to get long shoots.
ii) Stage III-MS Culture-The individual shoots are cultured in suitable medium to form strong shoot with dominant apical growth and roots.
iii) Stage III-MS-In this stage, small clumps of multirooted callus is transferred to root and shoot inducing media to form strong plants.

(d) Stage-IV

In this stage, the plants are transferred from the test tube environment to outdoor environment. For this, the plants are planted in pots containing potting-mix (peat or vermiculite etc.) and VAM fungal spores. The plants start to grow independently in two months by producing well developed roots and become ready for issuing to farmers.

(ii) Somaclonal variation

While most of the clone retain uniformity and identity to parent, genetic changes occur in some tissues and these changes are transmitted to regenerated plants. Such plants that differ from their parents in one or few traits are called somatic variants. The formation of somatic variants among the tissues in cultures is called somaclonal variation. It may occur spontaneously during repeated subcultures or due to induced mutations. Now a day, production of somaclonal variants is one of the objectives of tissue culture. The name somaclonal variation was coined by Larkin and Scoweroft in 1981.

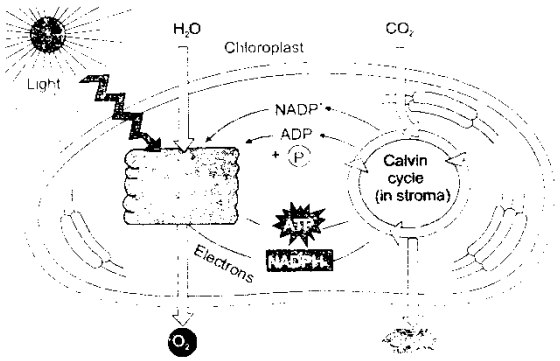
In no case, somaclonal variation appears to be a species or organic specific, but genetic variability is generated for many traits of agronomic value. Wide range of genetic variations has been so far created in plants through culture of protoplast, cells and tissues in vitro. Since somaclonal variation provides plants for new breeding programmes, it is considered to be adjunct of modern plant breeding. It provides the maximum utilizable germplasm for crop improvement.

Somaclonal variants are obtained from the cultures of embryos, meristem, anther, leaf callus, tip of inflorescence, microscopes, ovaries and protoplasts. Before regenerating plantlets, the calli are tested for the desired trait (variation) expected in them. Somaclonal variants have been selected for the following traits:

(i) Rice : Number of tillers per plant, fertile tillers per plant, panicle length, plant height, early maturity, seed fertility, disease resistance, drought tolerance and colour tolerance.
(ii) Maize : Plant height, node number, ear arrangement, stalk number, toxin resistance, mitochondrial pattern etc.
(iii) Tomato : Disease resistant and early maturity.
(iv) Cotton : Insect resistance.

Q8. Describe the different stages of photosynthesis.

Photosynthesis is the process by which chloroplast bearing organisms transform solar energy into chemical bond energy. It provides food and oxygen to all living organisms. Plants need only light energy, CO_2, and H_2O to make sugar. The process of photosynthesis takes place in the chloroplasts, specifically using chlorophyll, the green pigment involved in photosynthesis.



Photosynthesis takes place primarily in plant leaves, and little to none occurs in stems, etc. The parts of a typical leaf include the upper and lower epidermis, the mesophyll, the vascular

bundle(s) (veins), and the stomata. The upper and lower epidermal cells do not have chloroplasts, thus photosynthesis does not occur there. They serve primarily as protection for the rest of the leaf. The stomata are holes which occur primarily in the lower epidermis and are for air exchange, they let CO_2 in and O_2 out. The vascular bundles or veins in a leaf are part of the plant's transportation system, moving water and nutrients around the plant as needed. The mesophyll cells have chloroplasts and this is where photosynthesis occurs.

The parts of a chloroplast include the outer and inner membranes, intermembrane space, stomata, and thylakoids stacked in grana. The chlorophyll is built into the membranes of the thylakoids.

Chlorophyll looks green because it absorbs red and blue light, making these colors unavailable to be seen by our eyes. It is the green light which is not absorbed that finally reaches our eyes, making chlorophyll appear green. However, it is the energy from the red and blue light that are absorbed that is, thereby, able to be used to do photosynthesis. The green light we can see is not/cannot be absorbed by the plant, and thus cannot be used to do photosynthesis.

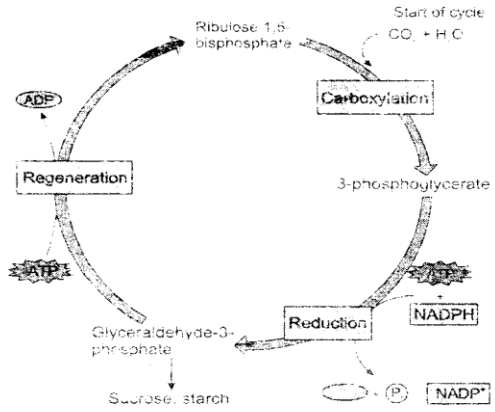
The overall chemical reaction involved in photosynthesis is: $6CO_2 + 6H_2O$ (+ light energy) $\rightarrow$ $C_6H_{12}O_6 + 6O_2$. This is the source of the O_2 we breathe, and thus, a significant factor in the concerns about deforestation.

There are two parts to photosynthesis:

(a) Light reaction : Light dependent reaction capture the energy of light and use it to make high energy molecules. It happens in the thylakoid membrane and converts light energy to chemical energy. This chemical reaction must, therefore, take place in the light. Chlorophyll and several other pigments such as beta-carotene are organized in clusters in the thylakoid membrane and are involved in the light reaction. Each of these differently-colored pigments can absorb a slightly different color of light and pass its energy to the central chlorophyll molecule to do photosynthesis. The central part of the chemical structure of a chlorophyll molecule is a porphyrin ring, which consists of several fused rings of carbon and nitrogen with a magnesium ion in the center.

The energy harvested via the light reaction is stored by forming a chemical called ATP (adenosine triphosphate), a compound used by cells for energy storage. This chemical is made of the nucleotide adenine bonded to a ribose sugar, and that is bonded to three phosphate groups. This molecule is very similar to the building blocks for our DNA.

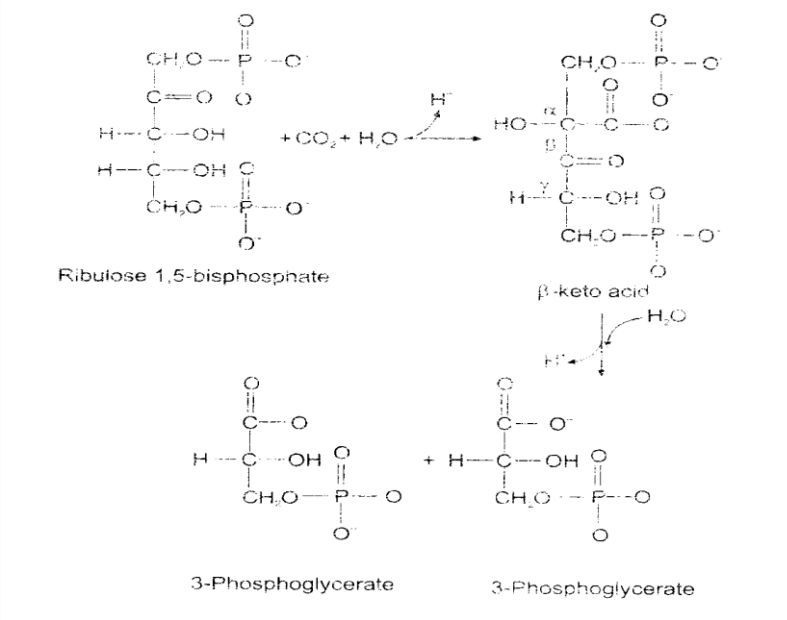
(b) Dark reaction : Light independent reactions or dark reactions are also called as Calvin-Benson Cycle. Now this reaction is also called as Carbon reaction as they are also regulated by light. The dark reactions do not require darkness, but because they are several steps removed from the light gathering process, they can continue for sometime after the light has been cut off.



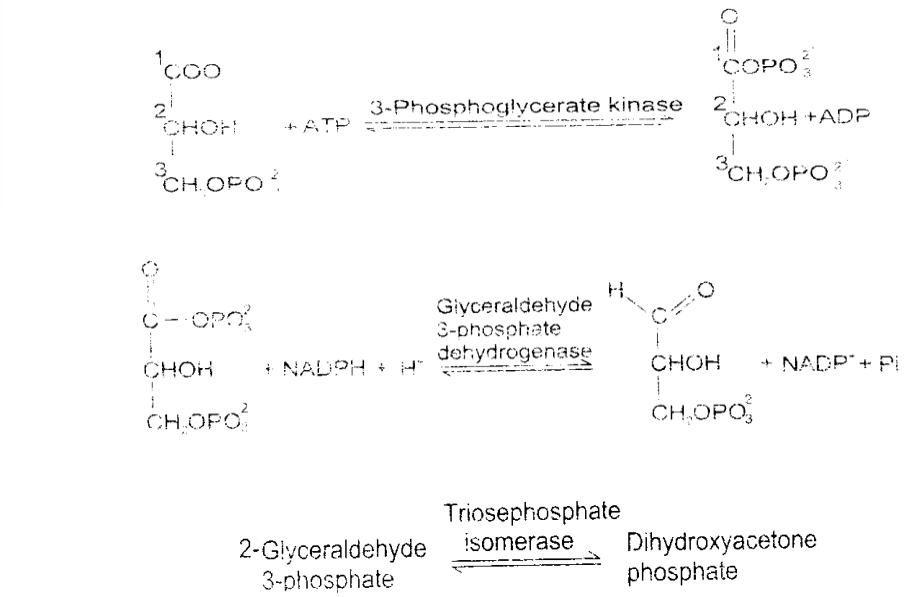
The dark reaction takes place in the stroma within the chloroplast, and converts CO_2 to sugar. The dark reaction involves a cycle called the Calvin cycle in which CO_2 and energy from ATP are used to form sugar. The first product of photosynthesis is a three-carbon compound called glyceraldehyde 3-phosphate. Almost immediately, two of these join to form a glucose molecule.

The Calvin cycle occurs in three phases-

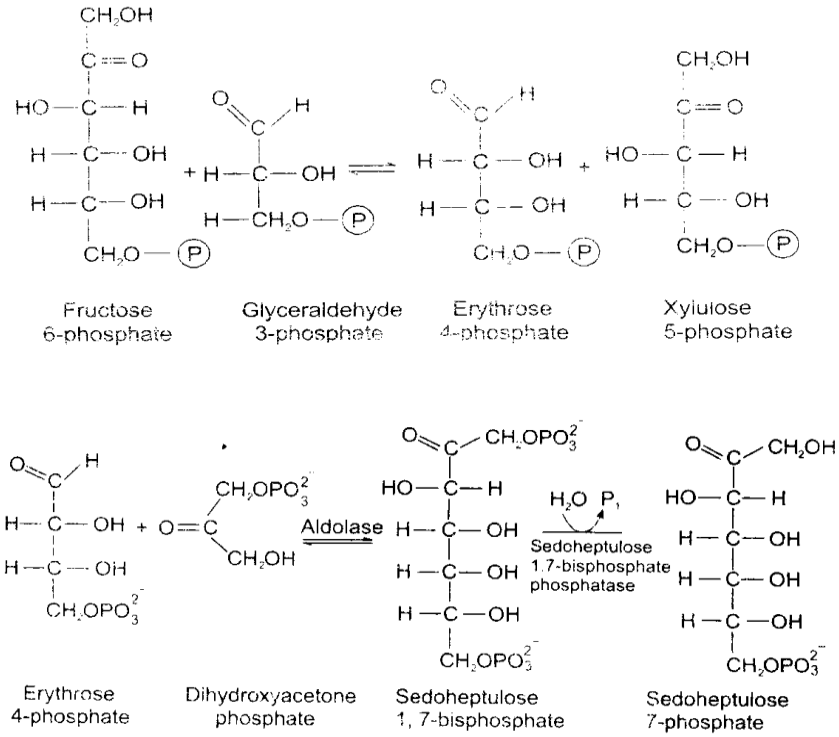
(i) Carboxylation – This reaction is given by CO_2+Rubulose 1,5-bisphosphate+ H_2O $\rightarrow$ 2,3-Phosphoglycerate (PGA).



(ii) Reduction – The three-carbon molecules thus generated are phophorylated by ATP to form 1,3-bisphophoglycerate (1,3-BPG). BPG receives electron from NADPH and gets reduced to two glyceraldehydes 3-phosphate. Glyceraldehydes 3-phosphate and dihydroxyacetone phosphate (DHAP), are in equilibrium with each other catalysed by the action of triose phosphate isomerize. The triose phosphate isomerize are either converted to starch or converted to sucrose.



(iii) Regeneration of the starting product- Here G3P molecules react to regenerate ribulose 1,5 bisphosphate and hexosphosphate molecules.



DHAP + Erythrose 4-Pi → Sedoheptulose 1,7-bisPi

Most plants put CO_2 directly into the Calvin cycle. Thus the first stable organic compound formed is the glyceraldehyde 3-phosphate. Since that molecule contains three carbon atoms, these plants are called C_3 plants. For all plants, hot summer weather increases the amount of water that evaporates from the plant. Plants lessen the amount of water that evaporates by keeping their stomata closed during hot, dry weather. Unfortunately, this means that once the CO_2 in their leaves reaches a low level, they must stop doing photosynthesis. Even if there is a tiny bit of CO_2 left, the enzymes used to grab it and put it into the Calvin cycle just don't have enough CO_2 to use. Some plants like crabgrass, corn, and sugar cane have a special modification to conserve water. These plants capture CO_2 in a different way: they do an extra step first, before doing the Calvin cycle. These plants have a special enzyme that can work better, even at very low CO_2 levels, to grab CO_2 and turn it first into oxaloacetate, which contains four carbons. Thus, these plants are called C_4 plants. The CO_2 is then released from the oxaloacetate and put into the Calvin cycle. This is why crabgrass can stay green and keep growing when all the rest of the grass is dried up and brown.

Some plants (for example, cacti and pineapple) that live in extremely hot, dry areas like deserts, can only safely open their stomata at night when the weather is cool. Thus, there is no chance for them to get the CO_2 needed for the dark reaction during the daytime. At night when they can open their stomata and take in CO_2, these plants incorporate the CO_2 into various organic compounds

to store it. In the daytime, when the light reaction is occurring and ATP is available (but the stomata must remain closed), they take the CO_2 from these organic compounds and put it into the Calvin cycle. These plants are called CAM plants, which stands for crassulacean acid metabolism after the plant family, Crassulaceae (which includes the garden plant *Sedum*) where this process was first discovered.

The overall process of photosynthesis is as follows-