DEPARTMENT OF BOTANY
Guru Ghasidas Vishwavidyalaya, Bilaspur
B. Sc. VI Semester
LBC 602: Plant Metabolism & Biochemistry

SCHEME OF EVALUATION

Time : 3 hours **Maximum Marks : 30**

SECTION A

Multiple choice questions / Define the following terms **10 × 1 = 10**

1. Which one of the following DNA polymerase is essential for both the replication and repair of DNA

   (a) **DNA polymerase I** (b) DNA polymerase II

   (c) DNA polymerase III (d) DNA polymerase δ

2. The precursor for biosynthesis of fatty acids is

   (a) Acetate (b) Acetyl CoA (c) **Malonyl CoA** (d) UDP glucose

3. Nitrogen fixation is controlled by

   (a) NOD genes (b) Laghaemoglobin (c) ***Nif* genes** (d) Homoserine factors

4. Sucrose synthesis is regulated at the level of sucrose – 6 – phosphate synthetase by

   (a) Phosphorylation (b) Dephosphorylation

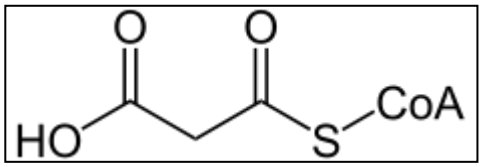
   (c) **Phosphorylation and dephosphorylation** (d) Polymerisation

5. Purine bases are

   (a) **A and G** (b) C and T (c) C, T and U (d) A, G, C, and U

6. **Translation :** Translation is the process through which cellular ribosomes manufacture proteins. It is part of the process of gene expression. In translation, mRNA produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.

7. **Malonyl CoA :** Malonyl-CoA decarboxylase is an enzyme associated with Malonyl-CoA decarboxylase deficiency. It catalyzes the conversion of malonyl-CoA into acetyl-CoA and carbon dioxide. It is involved in fatty acid biosynthesis.



8. **B form of DNA :** The vast majority of the DNA nucleotides present in the aqueous protoplasm of living cell almost exist in the Watson-Crick Model of double helix i.e. B form of DNA.

9. **Lamda phage :** Lambda phage is a bacterial virus, or bacteriophage, that infects the bacterial species Escherichia coli. This virus is temperate and may reside within the genome of its host through lysogeny. Lambda phage consists of a virus particle including a capsid, a tail, and tail fibers. The head contains the phage's double-stranded circular DNA genome.

10. **Isozymes :** Isozymes are also known as isoenzymes or more generally as Multiple forms of enzymes and they are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different KM values), or different regulatory properties.

SECTION B

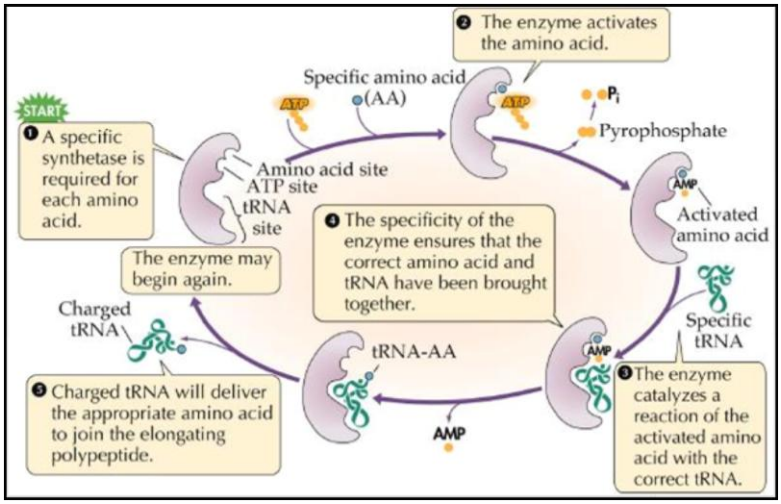
Descriptive answer type questions (attempts any four) **4 × 5 = 20**

1. Describe the activation, initiation, elongation and termination of peptides.

- The mRNA triplet codon AUG is universally the start codon used to mark the beginning of the coding sequence of a gene; thus, the tRNA with the anticodon UAC and carrying the amino acid methionine is always the first tRNA to enter the P-site during translation.
- There are three stop codons in the genetic code; none of these have a corresponding tRNA; instead, when a ribosome encounters a stop codon, a release factor binds to the stop codon, which terminates translation and allows the separation of all of its components.

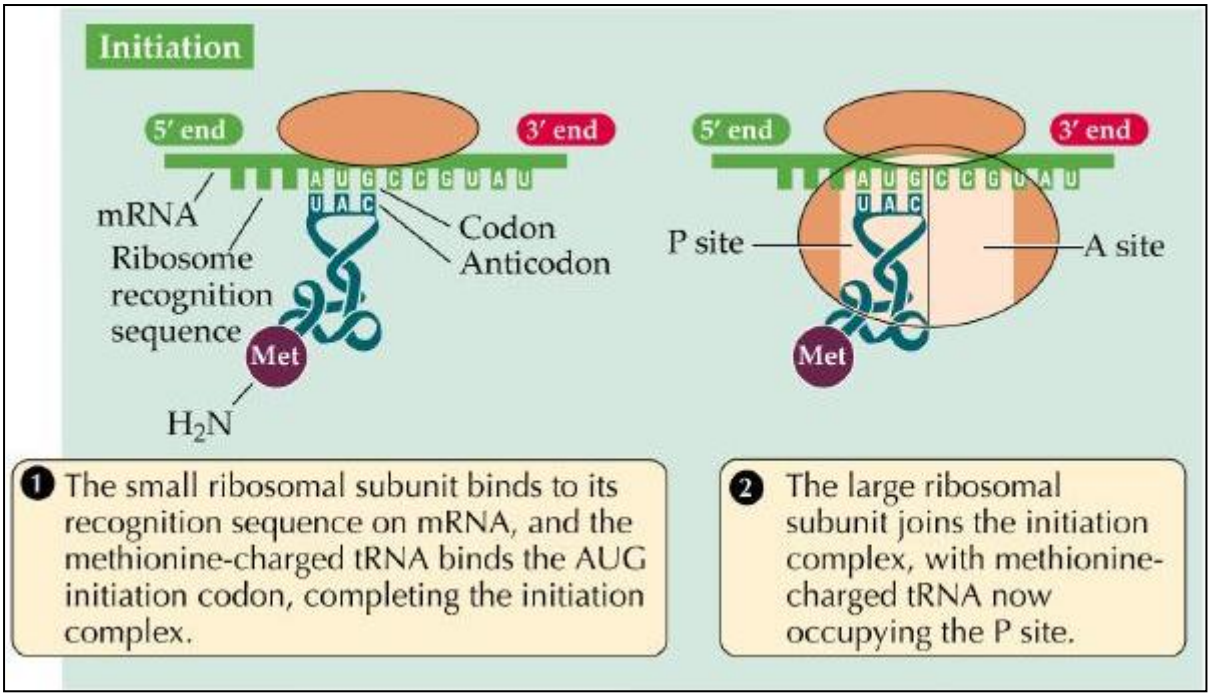
A. *Activation*

- there are many different types of tRNA in a cell
- all tRNA molecules have:
    - A triplet of bases called the anticodon, found in the anticodon loop of 7 nucleotides
    - two other loops
    - A CCA base sequence at the 3' terminal, which forms a site for attaching an amino acid
    - sections that become double-stranded by base pairing
    - These features allow tRNA to attach to binding sites on ribosomes and to mRNA
    - Variable features in each type of tRNA produce different physical and chemical properties, allowing for the correct binding of amino acids to specific tRNAs
    - tRNA activating enzyme attaches a specific amino acid to the 3' end of a tRNA
    - There are 20 different tRNA activating enzymes, one for each of the 20 amino acids
    - Each of these enzymes attaches one particular amino acid to all of the tRNA molecules that have an anticodon corresponding to that amino acid
    - ATP hydrolysis provides the energy for amino acid attachment to tRNA; this stored energy is also used later to link the amino acid to the growing polypeptide chain during translation



B. *Initiation*:

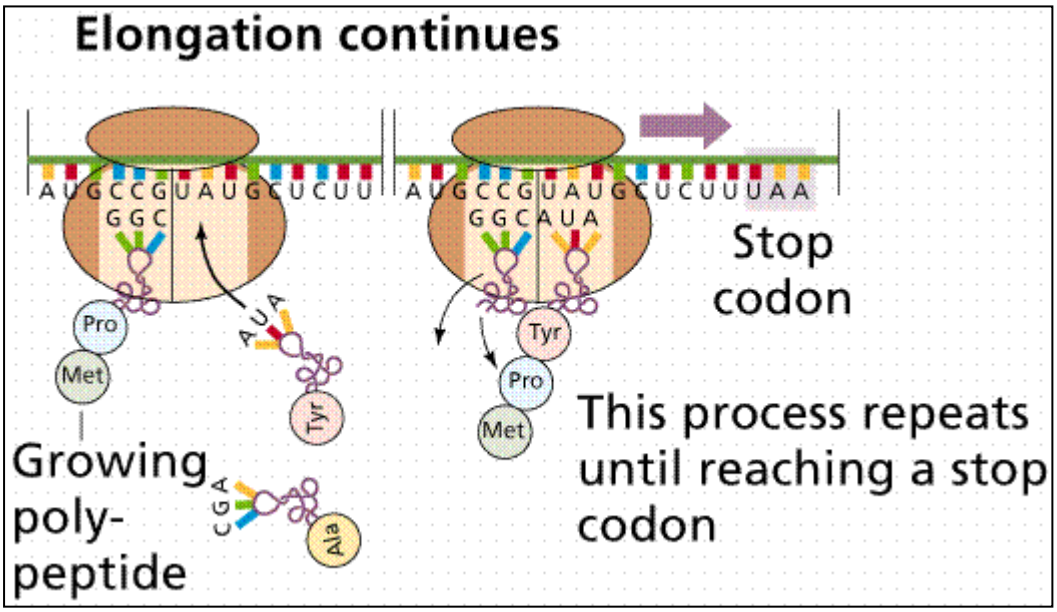
- 5’ end of mRNA binds to the small subunit of the ribosome
- Initial mRNA codon = AUG = *start codon*
- tRNA with *anticodon*: UAC binds to mRNA AUG codon by complementary base pairing, methionine attached to tRNA 3’ terminal
- Large ribosomal subunit binds, completing ribosomal structure, and producing two ribosomal binds sites: *P site* & *A site*



C. *Elongation:*

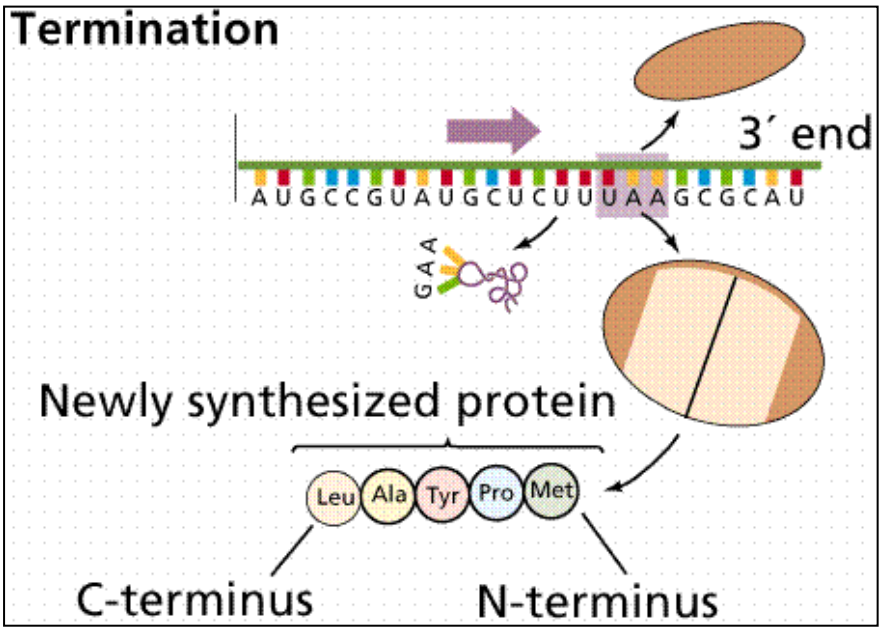
- tRNA with anticodon complementary to second mRNA codon binds to ribosomal A site, with appropriate amino acid attached to tRNA 3’ terminal
- Enzymes in ribosome catalyze formation of *peptide bond* between methionine and 2nd amino acid
- P site tRNA, now separated from methionine, exits ribosome
- Ribosome moves one codon (3 nucleotides) toward the 3’ end of mRNA, thus shifting previous A-site tRNA to P-site, and opening A-site

- tRNA with anticodon complementary to A-site mRNA codon binds to ribosomal A-site, with appropriate amino acid attached to tRNA 3' terminal
- Enzymes in ribosome catalyze formation of peptide bond between 2nd and 3rd amino acids
- P site tRNA, now separated from its amino acid, exits ribosome
- Ribosome moves one codon (3 nucleotides) toward the 3' end of mRNA, thus shifting previous A-site tRNA to P-site, and opening A-site
- Repetition of process until stop codon is reached



D. *Termination:*

- When ribosomal A-site reaches a *stop codon*, no tRNA has a complementary anticodon
- *Release factor protein* binds to ribosomal A-site stop codon
- Polypeptide and mRNA are released
- Large and small ribosomal subunits separate

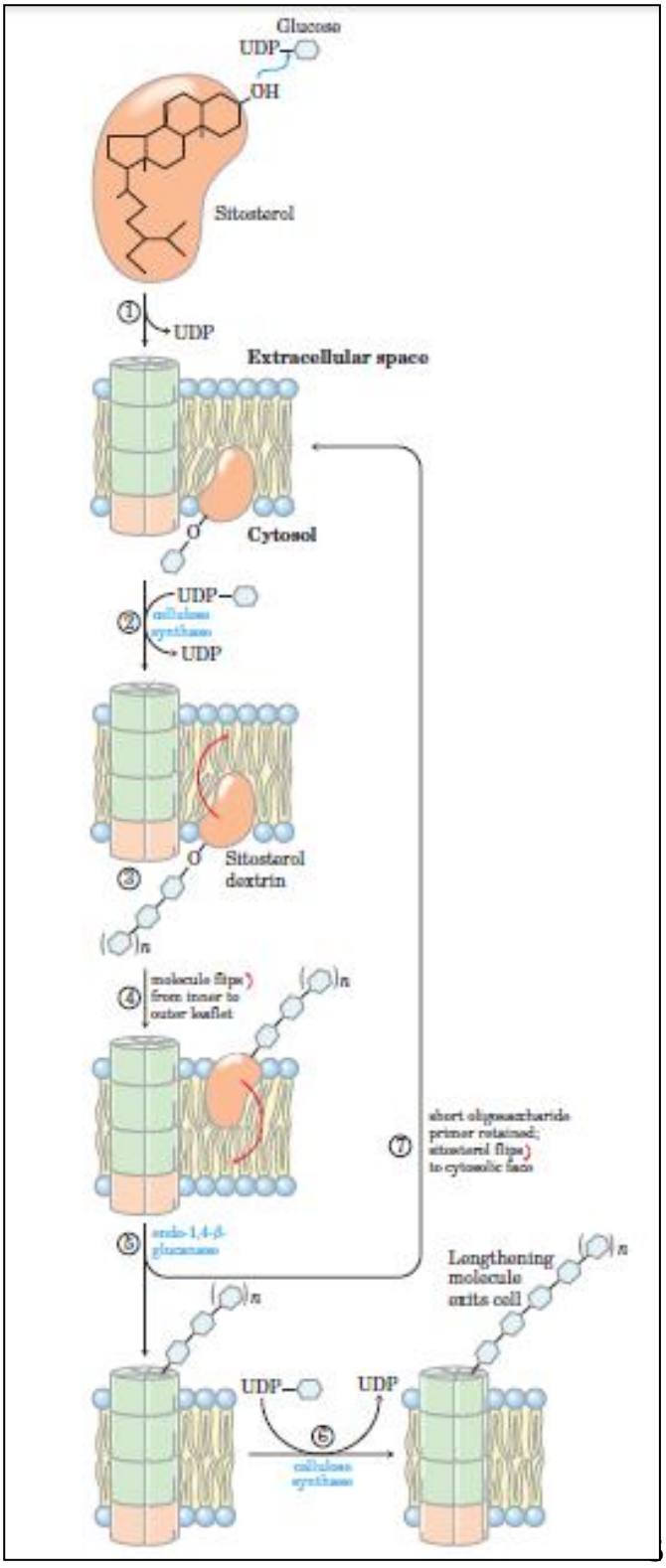


2. Write short notes on :

(i) What is precusor of cellulsoe synthesis and flip-flop movment?

Answer

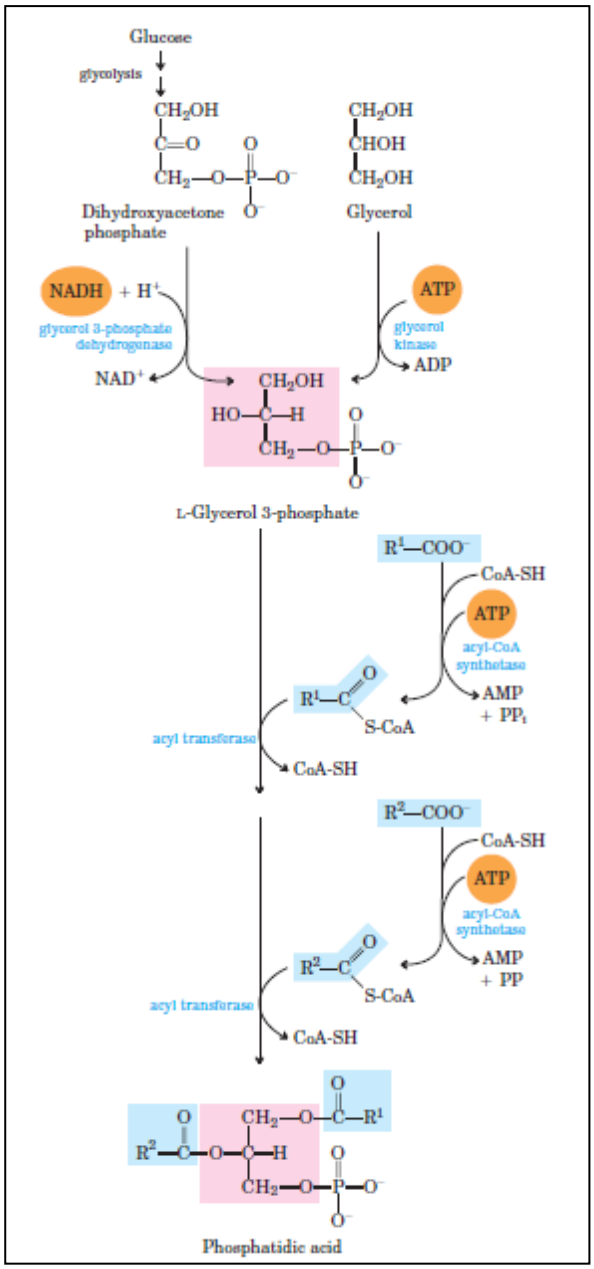
- Cellulose is a major constituent of plant cell walls, providing strength and rigidity and preventing the swelling of the cell and rupture of the plasma membrane that might result when osmotic conditions favor water entry into the cell.
- The complex enzymatic machinery that assembles cellulose chains spans the plasma membrane, with one part positioned to bind the substrate, UDP-glucose, in the cytosol and another part extending to the outside, responsible for elongating and crystallizing cellulose molecules in the extracellular space.
- Freeze-fracture electron microscopy shows these **terminal complexes**, also called **rosettes,** to be composed of six large particles arranged in a regular hexagon.
- Several proteins, including the catalytic subunit of **cellulose synthase,** make up the terminal complex.
- Glucose is transferred
- from UDP-glucose to a membrane lipid, probably the plant sterol sitosterol, on the inner face of the plasma membrane. Here, intracellular cellulose synthase adds several more glucose residues to the first one, in (β1→4) linkage, forming a short oligosaccharide chain attached to the sitosterol (sitosterol dextrin). Next, the whole sitosterol dextrin flips across to the outer face of the plasma membrane, where most of the polysaccharide chain is removed by endo-1,4-_-glucanase. The shortened sitosterol dextrin primer now associates, perhaps covalently, with another form of cellulose synthase.
- The UDP-glucose used for cellulose synthesis is generated from sucrose produced during photosynthesis, by the reaction catalyzed by sucrose synthase



Sucrose + UDP → UDP-glucose + fructose

(ii) Phosphatidic acid

Answers



- Phosphatidic acids (PAs) are the acid forms of phosphatidates, a part of common phospholipids, major constituents of cell membranes. Phosphatidic acids are the simplest diacyl-glycerophospholipids.
- Phosphatidic acid consists of a glycerol backbone, with, in general, a saturated fatty acid bonded to carbon-1, an unsaturated fatty acid bonded to carbon-2, and a phosphate group bonded to carbon-3.
- PA is a vital cell lipid that acts as a biosynthetic precursor for the formation (directly or indirectly) of all acylglycerol lipids in the cell.
- PA is, therefore, essential for lipid synthesis and cell survival, yet, under normal conditions, is maintained at very low levels in the cell.
- Two precursors (fatty acyl–CoA and L-glycerol 3-phosphate) and several biosynthetic steps. The vast majority of the glycerol 3-phosphate is derived from the glycolytic intermediate dihydroxyacetone hosphate (DHAP) by the action of the cytosolic NAD-linked **glycerol 3-phosphate dehydrogenase;** in liver and kidney, a small amount of glycerol 3-phosphate is also formed from glycerol by the action of **glycerol kinase**.
- The other precursors of triacylglycerols are fatty acyl–CoAs, formed from fatty acids by **acyl-CoA synthetases,** the same enzymes responsible for the activation of fatty acids for β – oxidation.
- The first stage in the biosynthesis of triacylglycerols is the acylation of the two free hydroxyl groups of Lglycerol 3-phosphate by two molecules of fatty acyl–CoA to yield **diacylglycerol 3-phosphate,** more commonly called **phosphatidic acid** or phosphatidate.

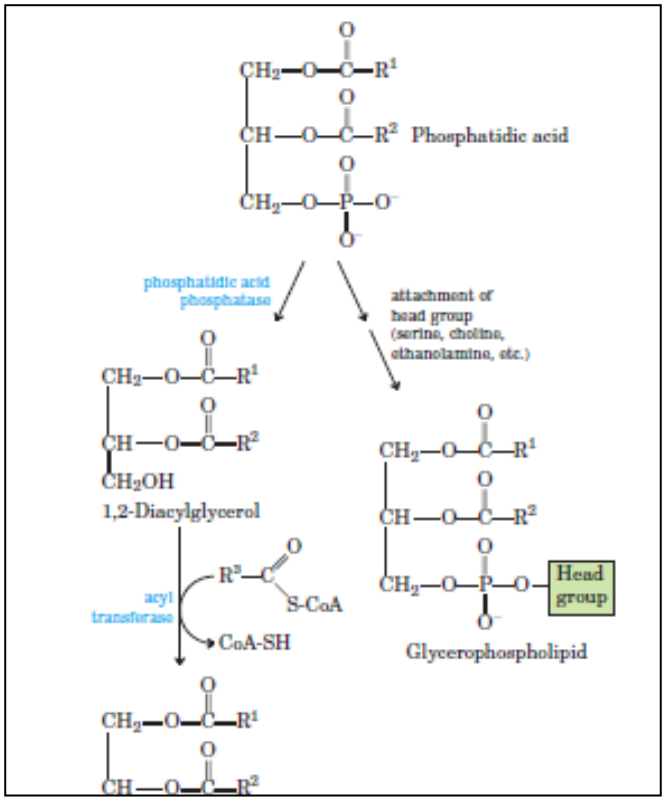
3. Write an essay on biosynthesis of triacylglycerol and glycerophospholipids.

Answer

- Two precursors (fatty acyl–CoA and L-glycerol 3-phosphate) and several biosynthetic steps. The vast majority of the glycerol 3-phosphate is derived from the glycolytic intermediate

dihydroxyacetone hosphate (DHAP) by the action of the cytosolic NAD-linked **glycerol 3-phosphate dehydrogenase;** in liver and kidney, a small amount of glycerol 3-phosphate is also formed from glycerol by the action of **glycerol kinase**.

- The other precursors of triacylglycerols are fatty acyl–CoAs, formed from fatty acids by **acyl-CoA synthetases,** the same enzymes responsible for the activation of fatty acids for β – oxidation.
- The first stage in the biosynthesis of triacylglycerols is the acylation of the two free hydroxyl groups of Lglycerol 3-phosphate by two molecules of fatty acyl–CoA to yield **diacylglycerol 3-phosphate,** more commonly called **phosphatidic acid** or phosphatidate.
- The first stage in the biosynthesis of triacylglycerols is the acylation of the two free hydroxyl groups of Lglycerol 3-phosphate by two molecules of fatty acyl–CoA to yield diacylglycerol 3-phosphate, more commonly called phosphatidic acid or phosphatidate.
- Phosphatidic acid is present in only trace amounts in cells but is a central intermediate in lipid biosynthesis; it can be converted either to a triacylglycerol or to a glycerophospholipid. In the pathway to triacylglycerols, phosphatidic acid is hydrolyzed by phosphatidic acid phosphatase to form a 1,2-diacylglycerol.
- Diacylglycerols are then converted to triacylglycerols by transesterification with a third fatty acyl–CoA.

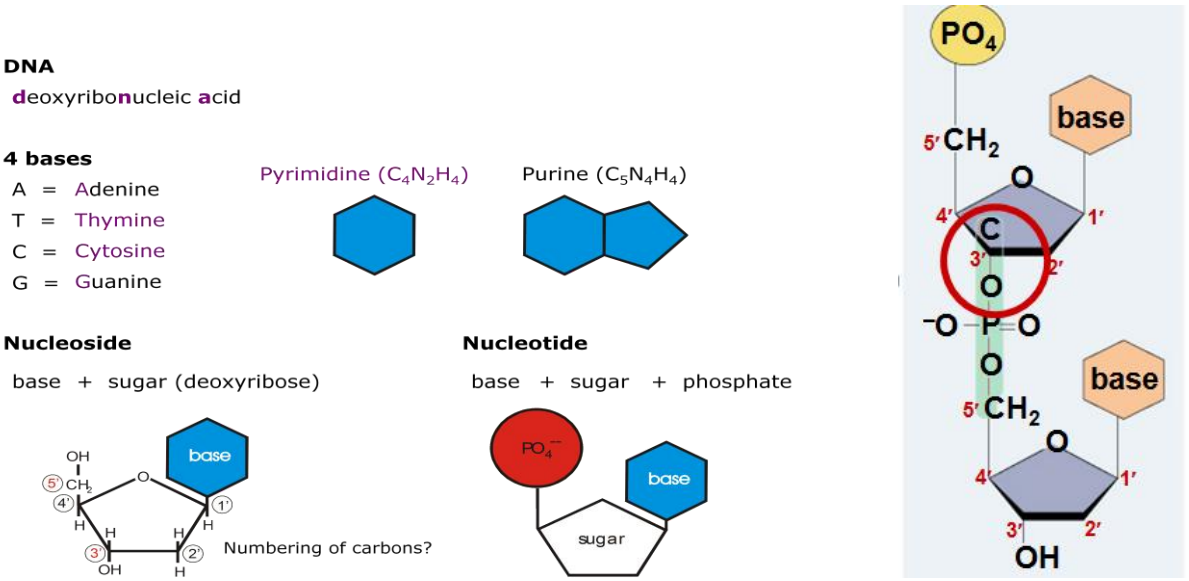


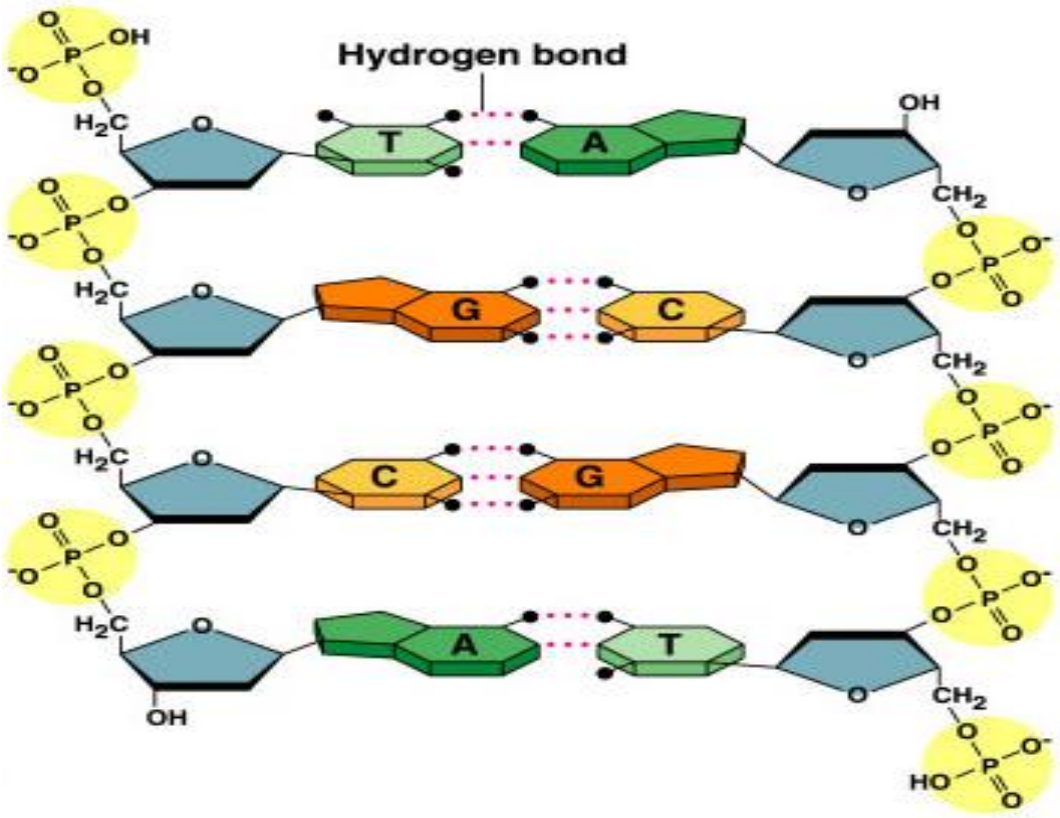
4. Explain the followings :

(i) Phosphodiester bonds and chemical structure of DNA double helix

Answer

- A phosphodiester bond is a group of strong covalent bonds between a phosphate group and two 5-carbon ring carbohydrates (pentoses) over two ester bonds.
- Phosphodiester bonds are essential to all known life, as they make up the backbone of each helical strand of DNA.
- In DNA and RNA, the phosphodiester bond is the linkage between the 3' carbon atom of one sugar molecule and the 5' carbon atom of another; the sugar molecules being deoxyribose in DNA and ribose in RNA.
- The phosphate groups in the phosphodiester bond are negatively charged. Because the phosphate groups have a pKa near 0, they are negatively charged at pH 7.
- This repulsion forces the phosphates to take opposite sides of the DNA strands and is neutralized by proteins (histones), metal ions such as magnesium, and polyamines.



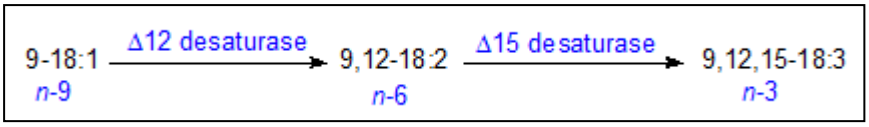


(ii) Biosynthesis of polyunsaturated fatty acids.

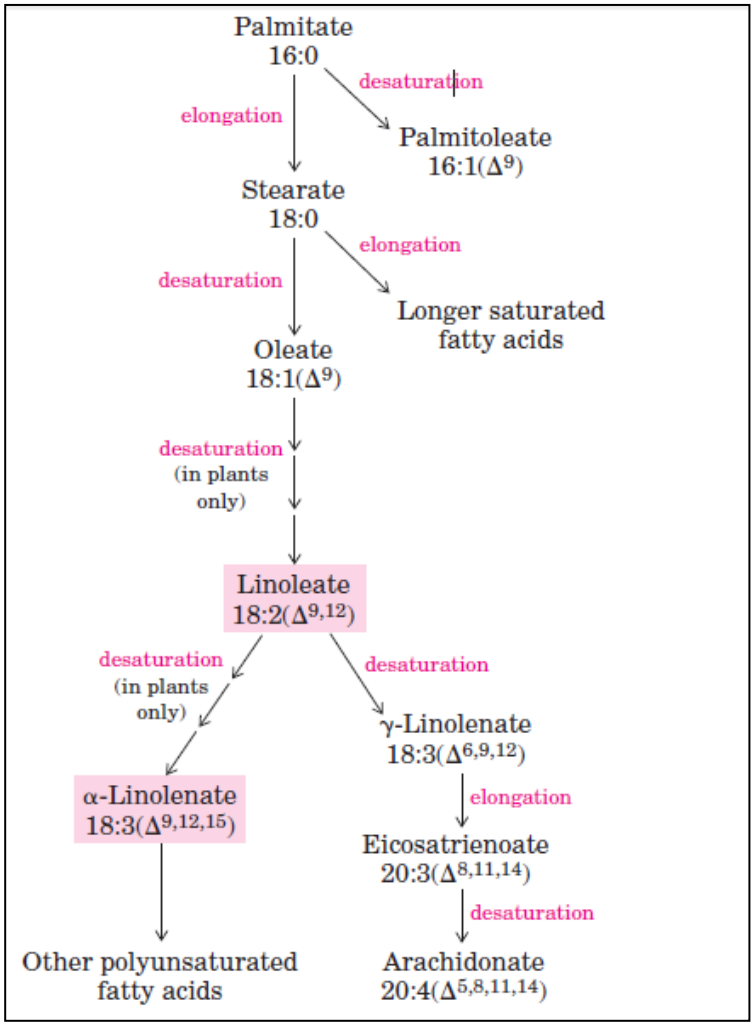
Answers

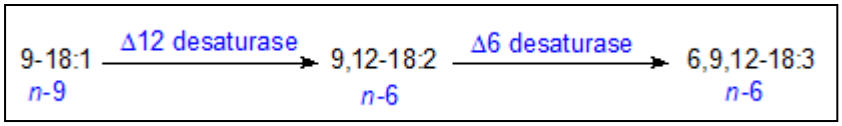
Biosynthesis of Polyunsaturated Fatty Acids

- Linoleic and α-linolenic acids are synthesised in plant tissues from oleic acid by the introduction of double bonds between the existing double bond and the terminal methyl group by the sequential action of Δ12 and Δ15 desaturases.



- Palmitate, the principal product of the fatty acid synthase system in animal cells, is the precursor of other long-chain fatty acids.
- It may be lengthened to form stearate (18:0) or even longer saturated fatty acids by further additions of acetyl groups, through the action of **fatty acid elongation systems** present in the smooth endoplasmic reticulum and in mitochondria.
- The more active elongation system of the ER extends the 16-carbon chain of palmitoyl-CoA by two carbons, forming stearoyl-CoA. Although different enzyme systems are involved, and coenzyme A rather than ACP is the acyl carrier in the reaction, the mechanism of elongation in the ER is otherwise identical to that in palmitate synthesis: donation of two carbons by malonyl-CoA, followed by reduction, dehydration, and reduction to the saturated 18-carbon product, stearoyl-CoA.
- Palmitate is the precursor of stearate and longer-chain saturated fatty acids, as well as the monounsaturated acids palmitoleate and oleate.
- Mammals cannot convert oleate to linoleate or linolenate which are therefore required in the diet as essential fatty acids.
- Infrequently in plants, a double bond is inserted between an existing double bond and the carboxyl group as in the biosynthesis of γ-linolenic acid in evening primrose and borage seed oils.





- In this instance, the double in position 6 is inserted after those in positions 9 and 12. These processes are discussed in much more detail in a web page in the plant biochemistry section of this site.

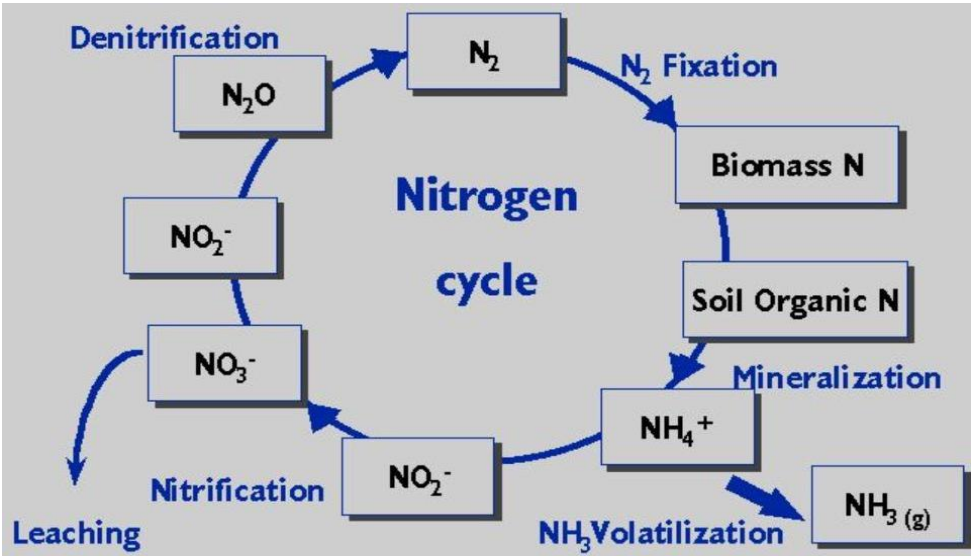
5. Write an essay on nitrogen metabolism in plants.

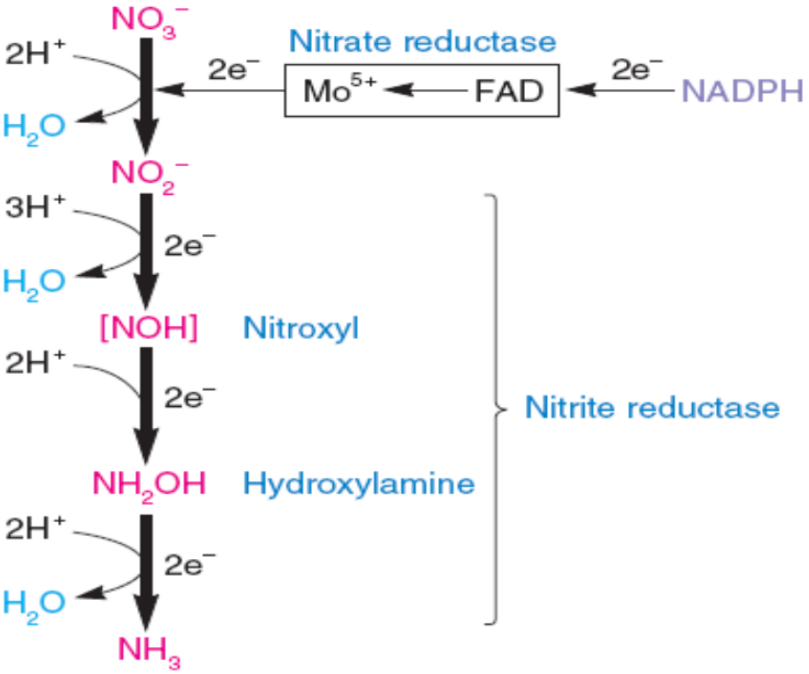
Nitrogen Fixation

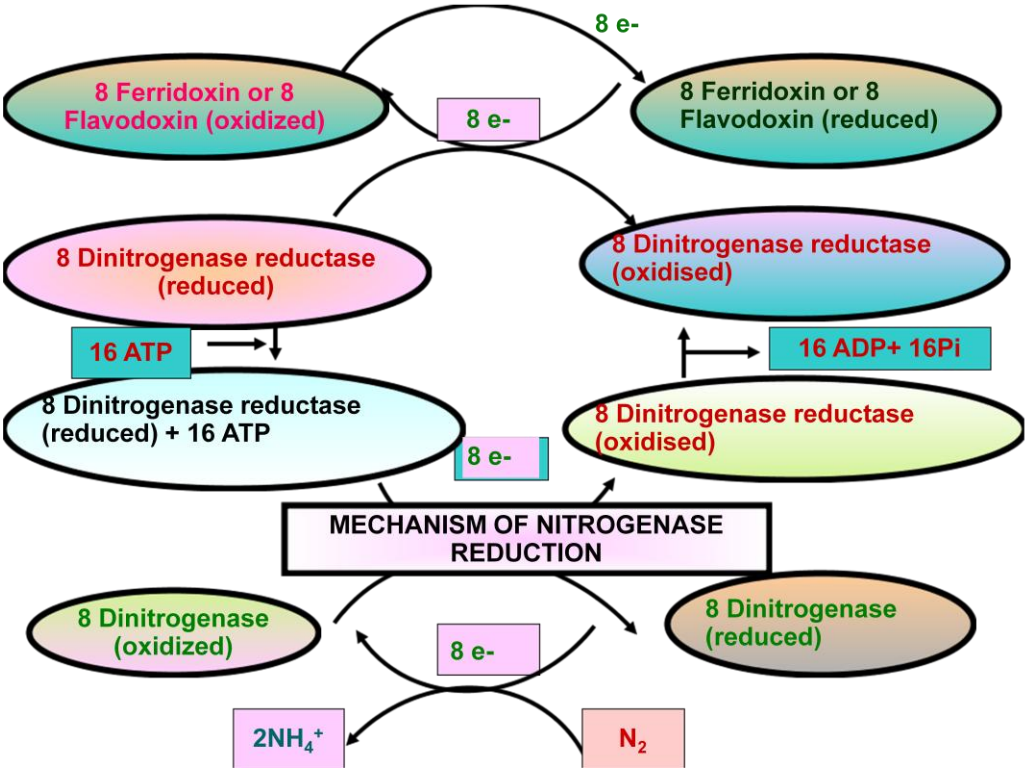
- Nitrogen fixation is the process by which atmospheric nitrogen gas is converted into ammonia. The ammonia is subsequently available for many important biological molecules such as amino acids, proteins, vitamins, and nucleic acids. The reaction can be presented as follows:

$$N2 + 16\ ATP + 8e^- + 2H^+ \rightarrow NH3 + 16\ ADP + 16\ Pi + H2$$

- Until the invention of the Haber-Bosch process for industrial fixation of N, three groups of microorganisms were the primary source of fixed nitrogen. In this course, we'll discuss N fixation due to the symbiotic relationship between Rhizobium bacteria and Legume plants (such as soybean, peanut, beans, and peas). The legume provides the bacteria with carbohydrates and the Rhizobium fixes inert N2 into $NH4^+$.
- **Nitrification:** Most Nitrogen is absorbed by plants in the form of Nitrate ($NO3^-$). Ammonium is converted to Nitrate by the following equation with the help of two types of soil bacteria: *Nitrosomonas, Nitrobacter*
- **Ammonification:** Nitrogen is a very mobile element and is easily lost from the soil. Ammonium $NH4^+$ can be converted to Ammonia ($NH3^+$). The gaseous ammonia can be lost to the atmosphere. This occurs when water availability is low and the pH is high.
- **Denitrification :** Nitrate can be lost through denitrification. Nitrate nitrogen is reduced to nitrous oxide (N2O), elemental nitrogen (N2) and nitric oxide (NO). All are volatile. Denitrification occurs under conditions of high water availability and temperatures greater than 50^o F.




NO_3^-
$2H^+$
H_2O
2e⁻
Nitrate reductase
Mo^{5+}
FAD
2e⁻
NADPH
NO_2^-
$3H^+$
H_2O
2e⁻
[NOH]
Nitroxyl
$2H^+$
2e⁻
NH_2OH
Hydroxylamine
$2H^+$
H_2O
2e⁻
NH_3
Nitrite reductase


8 e-
8 Ferridoxin or 8 Flavodoxin (oxidized)
8 e-
8 Ferridoxin or 8 Flavodoxin (reduced)
8 Dinitrogenase reductase (reduced)
8 Dinitrogenase reductase (oxidised)
16 ATP
16 ADP+ 16Pi
8 Dinitrogenase reductase (reduced) + 16 ATP
8 e-
8 Dinitrogenase reductase (oxidised)
MECHANISM OF NITROGENASE REDUCTION
8 Dinitrogenase (oxidized)
8 e-
8 Dinitrogenase (reduced)
$2NH_4^+$
N_2

6. Give a brief account of mechanism action of coenzymes and isozymes.

Answer

❖ **Mechanism of action of Coenzymes**

- The coenzyme usually occurs in living cells in low concentrations and it is of highest importance that reactions have been studied in vitro. The coenzyme is as essential reactant as the substrate that is activated by the enzyme-

(Enzyme A)

Substrate (A) + Coenzyme ⟶ Substrate (A) derivative + Coenzyme derivative

- The coenzyme is regenerated and again become available as an essential component for the reaction.

(Enzyme B)

Substrate (B) + Coenzyme ⟶ Substrate (B) derivative + Coenzyme

- The function of coenzyme in the enzymatic reaction is thus to assist in the cleavage of the substrate by acting as an acceptor for one of the cleavage product.
- Here the substrate and apoenzyme form a complex in the presence of coenzyme.
- When the bond in the substrate becomes activated, one of the cleavage products is transferred directly to the coenzyme.
- The coenzyme here acts as a receptor because it has an appropriate receptor site in its structure.
- Left of the substrate now dissociates from the apoenzyme.
- The attached fragment of the substrate in the coenzyme is either liberated as such or is passed on to other enzyme system for additional changes; in either case the coenzyme is regenerated.
- Both apoenzyme and coenzyme are then able to repeat the same cycle of events, hence it can be said that both act catalytically.
- Thus the coenzyme becomes bound with apoenzyme during the reaction.
- For example dehydrogenases utilize either NAD^+ OR NADP. Their function is to transfer the hydrogen nuclei with two electrons from the substrate thus oxidizing it.

Substrate + NAD^+ + enzyme ⟶ Oxidized substrate +NADH + H^+

❖ **ISOZYMES**

- Isozymes are also known as isoenzymes or more generally as Multiple forms of enzymes and they are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.
- Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual.
- These enzymes usually display different kinetic parameters (e.g. different KM values), or different regulatory properties.
- The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage (for example lactate dehydrogenase (LDH)).

In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes.

- Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identicalto the original, then it is likely that one or the other will be lost as mutation accumulate, resulting in a pseudogene.
- Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the DNA coding sequence of the gene. As with any other new mutations, there are three things that may happen to a new allozyme:
    a. It is most likely that the new allele will be non-functional — in which case it will probably result in low fitness and be removed from the population by natural selection.
    b. Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may be selectively neutraland subject to genetic drift.
    c. In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the mutation may cause an increase in fitness, and be favoured by natural selection.

7. Write short notes on followings :

(i) Plant breeding

Answer

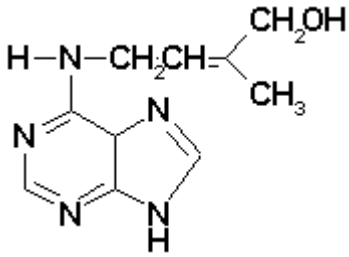
- Plant breeding is the most important technology developed by man. It allowed civilization to form and its continual success is critical to maintaining our way of life.
- Goals of Plant breeding
    - Increase the frequency of favorable alleles within a line
        - Additive effects
    - Increase the frequency of favourable genotypes within a line
        - Dominance and interaction effects
    - Better adapt crops to specific environments
        - Region-specific cultivars (high location G x E)
        - Stability across years within a region (low yearto-year G x E)
- Objectives
    - Development of pure (i.e. highly inbred) lines with high per se performance
    - Development of pure lines with high hybrid performance (either with each other or with a testcross)
    - Less emphasis on developing outbred (random-mating) populations with improved performance
    - Development of lines with high regional G x E, low year G x E
- Modern tools
    - Molecular markers
        - Initially low density for QTL mapping, introgression of major genes into elite germplasm

        - With high-density markers, association mapping and MAS/genomic selection
    - New statistical tools
        - Mixed model methods
        - Bayesian approaches to handle high-dimensional data sets
        - New methods to deal with G x E
    - Other technologies
        - Better standardization of field sites (laser-tilled fields, GPS, better micro- and macro-environmental measurements)
        - High throughput phenotypic scoring
        - DH lines
- Plant breeders face the conundrum of using inbred lines to concentrate elite genotypes, but requiring a very large collection of such lines to store variation for further selection
- Landraces or local cultivars may be highly adapted to specific evironments, but otherwise not elite
- Issue with keeping germplasm elite while introgressing genes/regions of interest.

(ii) Functions of cytokinins

Answer

- Cytokinins are compounds with a structure resembling adenine which promote cell division and have other similar functions to kinetin. Kinetin was the first cytokinin discovered and so named because of the compounds ability to promote cytokinesis (cell division). Though it is a natural compound, It is not made in plants, and is therefore usually considered a "synthetic" cytokinin (meaning that the hormone is synthesized somewhere other than in a plant). The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (Zea mays).



Cytokinin Functions

- Stimulates cell division.
- Stimulates morphogenesis (shoot initiation/bud formation) in tissue culture.
- Stimulates the growth of lateral buds-release of apical dominance.
- Stimulates leaf expansion resulting from cell enlargement.
- May enhance stomatal opening in some species.
- Promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis.