Introduction

Living organisms need nitrogen because it is a part of the amino acids that make up proteins, and the nucleic acids that make up DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).Nitrogen(dinitrogen, N2) is highly inter gas. Because the nitrogen atoms in dinitrogen are bound by a very strong triple bond, this gas is very stable and cannot be utilized as a source of nutrition by any but a few highly specialized microorganisms. Nitrogen within living organisms is eventually decomposed and converted to atmospheric nitrogen (N2). Molecular nitrogen (N2) is the major component (approximately 80%) of the earth's atmosphere but most organisms cannot use free nitrogen, to build the chemicals required for growth and reproduction.But it has to be combined with C,H,N,O to form compounds. Before its incorporation into plants, N2 must first be "fixed" (combined) in the form of ammonium (NH4) or nitrate (NO3) ions. This process of reduction of N2 , commonly known as "nitrogen fixation" (N-fixation) .

Nitrogen fixation is the process by which atmospheric nitrogen gas is converted into salts of nitrogen such as, ammonia, nitrate and nitrogen dioxide .

$$N_2 + 16\ ATP + 8e^- + 8H^+ \longrightarrow 2NH_3 + 16\ ADP + 16\ Pi + H_2$$

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Although ammonia (NH3) is the direct product of this reaction, it is quickly ionized to ammonium (NH4+). The reaction is mediated by an oxygen-sensitive enzyme nitrogenase and requires energy, as indicated by the consumption of adenosine triphosphate (ATP).This complex process is carried out by nitrogen-fixing bacteria present in the soil.

Nitrogen fixation is of two types :

1. Non Biological Fixation
    - Atmospheric nitrogen fixation (or Natural) by lightning:- It contributes about 10% of the total nitrogen fixation.Usually atmospheric nitrogen fixation (or Natural) by lightning occurs in rainy season during lightning or thunder storms.

During lightning the the free nitrogen combines with the oxygen to form nitric oxide.

$$N_2 + O_2 \xrightarrow[\text{Thunder}]{\text{lightning}} 2\ NO \text{ (nitric oxide)}$$

Nitric oxide oxidises into nitrogen dioxide in presence of excess oxygen.

$$2NO + O_2 \xrightarrow{\text{Oxidation}} 2\ NO_2 \text{ (Nitrogen dioxide)}$$

Nitrogen dioxide may react with only water to produce nitric and nitrous acids.

$$2\ NO_2 + H_2O \longrightarrow HNO_3 + HNO_2$$

Or may react with the atmospheric oxygen and rain water to form nitric acid

$$4\ NO_2 + 2\ H_2O + O_2 \longrightarrow 4\ HNO_3 \text{ (Nitric acid)}$$

These acids reaches the soil with rain water and combines with alkaline substances readily release the hydrogen, forming nitrate and nitrite ions.

$$HNO_3 \longrightarrow H^+ + NO_3^- \text{ (nitrate ions)}$$

$$HNO_2 \longrightarrow H^+ + NO_2^- \text{ (nitrite ions)}$$

The nitrate can be readily utilized by plants and micro-organisms.

- Industrial fixation through the Haber-Bosch process, and combustion.Some ammonia is also produced industrially by the Haber-Bosch process. When nitrogen (dinitrogen) combines with hydrogen in the presence of an iron-based catalyst, at a pressures of 35-100 MPa and fairly high temperature. Usually fossil fuels are used both as a source of energy and hydrogen.Most nitrogenous fertilizers are now derived from atmospheric nitrogen through this type of fixation process.

2. Biological fixation by certain microbes — alone or in a symbiotic relationship with some plants and animals:-Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck.It contributes 60% of total nitrogen fixation.But the major conversion of atmospheric N2 into salts of nitrogen , and then into proteins, is achieved by microorganisms (prokaryotes) such as bacteria ,fungi and algae in the process called biological nitrogen fixation (or dinitrogen fixation).Microorganisms that fix nitrogen are called diazotrophs.Biological nitrogen fixation are of two types :-
   - Free living or non-symbiotic nitrogen fixation :- The fixation of free nitrogen of the soil by all the microorganisms living freely or outside the plant cell is called non-symbiotic biological N2 fixation.It is performed by the aerobic and anaerobic bacteria and blue green algae.
     i. By bacteria Nitrogen fixing bacteria :- which are present in the soil convert free nitrogen into soluble compound which are absorbed from the soil by plants. The nitrogen fixing bacteria are of four types:-
        - Free living non-photosynthetic aerobic nitrogen fixing bacteria e.g., Azotobacter , Beijerinckia and Derxia.
        - Free living non-photosynthetic anaerobic nitrogen fixing bacteria e.g.,Clostridium .
        - Free living photosynthetic nitrogen fixing bacteria e.g,Chromatium, Rhodopseudomonas, Rhodospirillum,
        - Free living chemosynthetic nitrogen fixing bacteria e.g., Desulfovibrio
     ii. By free living nitrogen fixing Blue-Green algae:- About 15 genera of photosynthetic cyanobacteria (blue-green algae) are found freely in the soil where they fix free N2 into nitrogenous and ammonium compound. Mostly they are heterocysts e.g,Nostoc, Anabaena, Aulosira, Cylindrospernum, Calothrix. Nitrogen fixation occurs in special thick walled cells termed heterocysts or heterocytes (H) which occur at intervals along the cyanobacterial filaments. This separation of cellular functions is necessary because cyanobacteria have oxygen-evolving photosynthesis but the nitrogen-fixing enzyme, nitrogenase, is unstable in the presence of oxygen. This problem is overcome because the heterocysts contain only part of the photosynthetic apparatus, termed photosystem I, which can be used to generate energy (as ATP). But the heterocysts do not contain photosystem II, which is used to split water into hydrogen (for combination with CO2 to produce organic products) and oxygen.Non heterocystous nitrogen fixing blue-green algae are less in number e.g., Oscillatoria, Phormidium, Gleocapsa.

Free-living, non-photosynthetic bacteria depend on soil organic matter as a food source whereas the photosynthetic microorganisms may derive their food from the products of photosynthesis.

The nitrogen fixing activity of free-living, non-photosynthetic, aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen, and an organic food source. Anaerobic representatives (Clostridium) predominate in grassland and waterlogged soils and soil aggregates where moisture conditions and organic substrates are available but oxygen supply to the micro-environment of the bacteria is severely restricted.

   - Symbiotic:- Some N2-fixing organisms developes loose (associative) symbiosis (DeBary in 1879) with plants or animals (Acetobacter and sugarcane), or establish longer-term relationships within specialized structures provided by their host (Rhizobium and the legume nodule).To provide them with sugars, supplying both a source of energy and a source of carbon for the bacterium's own synthetic reactions.(Symbiosis is a close ecological relationship between the individuals of two (or more) different species. Sometimes a symbiotic relationship benefits both species, sometimes one species benefits at the other's expense, and in other cases neither species benefits.)
   - The fixation of free nitrogen of the soil by N2-fixing organisms living symbiotically inside plants is known as symbiotic biological nitrogen fixation.
   - The symbiotic biological nitrogen fixation are of three types :-
     i. Nitrogen fixation through nodule formation in Leguminous plants :- The bacteria responsible for the formation of root nodules in leguminous plants belong to the genus Rhizobium. Rhizobium also lives free in soil but only fixes N2 when inside plant The symbiotic Bacteria Rhizobia (from the Greek

words Riza = Root and Bios = Life) are soil bacteria that fix nitrogen (diazotrophy) after becoming established inside root nodules of legumes.According to host specificity and growth of bacteria have been divided into three groups:-

- Rizobium :-

Rizobium.

Genus	Species	Plant host
Rizobium	leguminosarum	Peas
Rizobium	meliloti	Lucerne
Rizobium	trifolii	Clover
Rizobium	phaseoli	Beans
Rizobium	lupini	Lupins

- Bradyrhizobium japonicum is the group of slow growing symbiont s of Soybeans (plant host).
- Azorhizobium caulinodans is a bacterium that forms stem nodules in Sesbania (plant host).

The bacteria "invade" the plant and cause the formation of a nodule by inducing localised proliferation of the plant host cells.Root nodules acts as a site of Nitrogen fixation. The root nodules contain a pigment called leghaemoglobin(serving the same function as the oxygen-carrying haemoglobin in blood).The heme (oxygen-binding) portion is produced by the bacterium, while the globin (protein) portion is produced by the host plant, again showing the closeness of the symbiotic relationship.The function of this molecule in nodules is to reduce the amount of free oxygen, and thereby to protect the nitrogen-fixing enzyme nitrogenase, which functions only under anaerobic conditions. Nitrogenase is the only enzyme that can split nitrogen molecule for nitrogen fixation.

b. Nitrogen fixation through nodule formation in None-Leguminous plants :- There are many plants belonging non- Leguminosae families, specially shrubs and plants which produces root nodules.Example:-
    - Frankia is a genus of the bacterial group termed actinomycetes - filamentous bacteria.
    - Frankia form nitrogen-fixing root nodules (sometimes called actinorhizae) with numerous genera of non-leguminous angiosperms , such as alder (Alnus species), sea buckthorn (Hippophae rhamnoides, which is common in sand-dune environments) and Casuarina (a Mediterranean tree genus).
    - Alder and the other woody hosts of Frankia are typical species that invade nutrient-poor soils. These plants probably benefit from the nitrogen-fixing association, while supplying the bacterial symbiont with photosynthetic products.
    - Rhizobium also form nitrogen-fixing root nodules with genus Parasponia.
    - Sometimes nodules are also formed in the roots of certain gymnosperms e.g., Podocarpus and in the leaves of Pavetta zinumermanniana and Chomelia

c. Nitrogen fixation through non-nodulation :- In some plants root nodules are not formed but symbiotic nitrogen fixation takes place.Examples :-
    - Lichens live as symbionts with photosynthetic cyanobacteria (blue green algae or Green chlorophyllous and with fungi.
    - Anthroceros (Bryophte):- It contains Blue green alga Nostoc inside mucilage cavities present on ventral side.
    - Azolla:- The water fern, Azolla lives symbiotically with the nitrogen-fixing cyanobacteria (Anabaena azollae) Azolla is grown in rice paddies early in the season. As the rice grows above the water surface, it shades out the fern, which dies, releasing the stored nitrogen. In this way, the paddy is fertilized without application of chemical fertilizers.
    - Cycas (gymnosperms) It contains cyanobacteria (blue-green algae) Anabaena or Nostoc. Aerial roots contain a nitrogen-fixing cyanobacterial symbiont.
    - Gunnera macrophylla (angiosperms):- Its stem contains Nostoc

E. Associative Symbiotic Nitrogen Fixation :- When bacteria form a close association with the roots of cereals and grasses and fix nitrogen, the association is of loose mutualism type and known as loose (associative) symbiosis and this type of nitrogen fixation is known as associative symbiotic nitrogen fixation.The bacteria grow in the rhizosphere in close contact with the roots, sometimes invade the outer cortical regions of the roots, and fix nitrogen. Azospirillum bransilense (= Spirillum lipoferum) a bacterium discovered J. Dobereiner (Edomonds, 1978), is the bacterium forming associate-symbiosis with the cereal roots. Others are Pseudomonas azotogensis, Enterobacter, Bacillus, Klebsiella etc.

Rates of symbiotic N2 fixation in legumes vary with plant species and cultivator, growing season, and soil fertility. Some forage legumes can fix 600 kilograms per hectare per year but more common values are 100 to 300 kilograms per hectare per year. Rates for grain legumes are often lower. Inclusion of legumes in crop rotations is generally thought to improve soil nitrogen levels, but benefits depend on the level of N2 fixed and the amount of nitrogen removed in grain or forage. A good soybean crop might fix 180 kilograms per hectare but remove 210 kilograms per hectare in the grain.Nonsymbiotic bacteria fix only upto 5 kg of nitrogen per hectare in one year <p**Formation of Nodule**

6. **Rhizobia is the group of genera of alpha-proteobacteria (family Rhizobiaceae) which includes all of the nitrogen-fixing species that produce nodules with legumes such as clover and soybean, Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Phyllobacterium, Rhizobium, and Sinorhizobium, as well as the plant pathogen Agrobacterium (Sprent, 2001).**
7. **Rhizobia produce stem or root nodules on their host(s), and within these nodules receive protection from external stresses and energy for growth and N2 fixation. The host receives most of the nitrogen it needs for growth.**
8. **Only infection via root hairs is considered here.**
9. **Plants of legume family secrete flavonoids which are recognized by bacterial NodD protein When NodD binds flavonoid it activates other nodulation genes.**

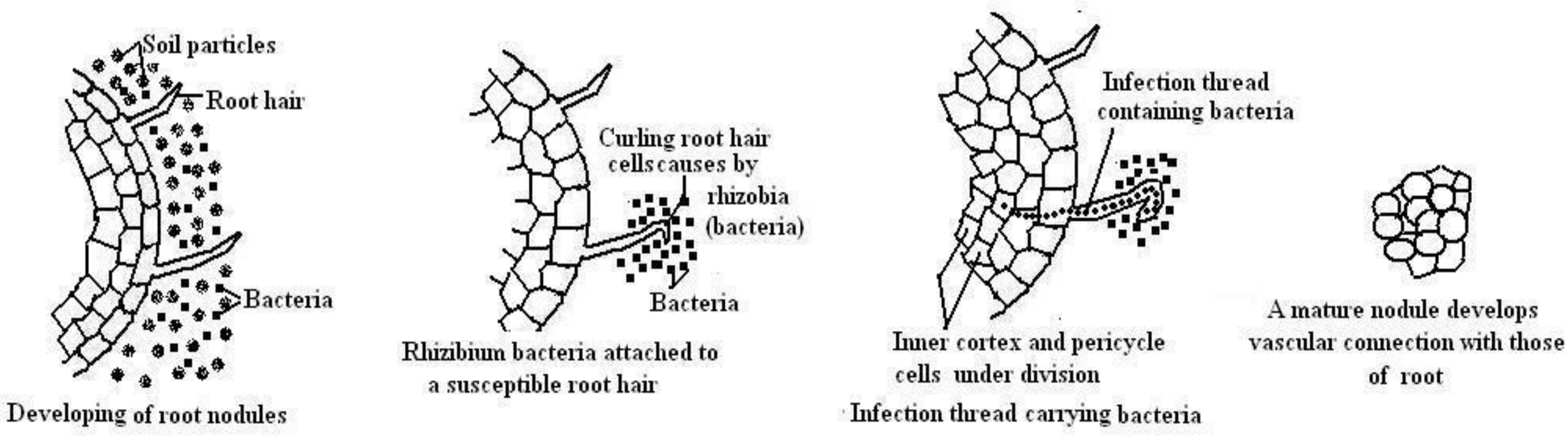


Fig 4. Development of root nodule

10. **Rhizobium secretes Nod factors (Some nod genes encode enzymes that make Nod factors) the leguminous plant recognizes Nod factors.**
11. **Then growing root hair of a leguminous plant comes in contact with the bacterium -Rhizobium, the growing root hairs curl and form a pocket for the rhizobia. the bacteria invade the plant by a newly formed infection thread growing through it. the root hair cell wall.**
12. **Simultaneously, cortical cells are mitotically activated, giving rise to the nodule primordium.**
13. **lnfection threads grow toward the primordium, and the bacteria are then released into the cytoplasm of the host cells, surrounded by a plant derived peribacteroid membrane (PBM). This separation is usually seen as a mechanism to suppress plant defense responses likely to harm the bacteria.**
14. **With the production of the infection threads, bacteria produce cytokinins (type of plant hormone). Cytokinins promote division of plant cells to form nodules and nodules begin to form in the root hairs.**
15. **The nodule primordium thereupon develops into a mature nodule, while the bacteria differentiate into their endosymbiotic form, which is known as the bacteroid . Bacteroids, together with the surrounding PBMs, are called symbiosomes.**
16. **Cell division now sets in, in the infected tissue leading to nodule formation. The area of active N2 fixation is either pink or red in color due to the presence of leghaemoglobin needed for oxygen transport. The nodule thus formed establishes a direct vascular connection with the host for the exchange of nutrients.**
17. **All of the steps of nodule development involve the expression of nodule-specific plant genes, the so called nodulin genes (van Kammen, 1984). The early nodulin genes encode products that are expressed before the onset of nitrogen fixation and are involved in infection and nodule development. The products of the late nodulin genes are involved in the interaction with the endosymbiont and in the metabolic specialization of the nodule (Nap and Bisseling, 1990).**

Mechanism of nitrogen fixation

The bacteroids within the nodules formed on legume roots fix nitrogen".

Structure and Operation of Nitrogenase The nitrogenase is an enzymatic complex , which converts atmospheric nitrogen (N2) to ammonia.The nitrogenase complex exists in both free living nitrogen fixing organisms as well as in symbiotic nitrogen fixing bacteria.Nitrogenase is a complex of two separately isolated proteins- an iron protein or dinitrogen reductase and a molybdenum-iron protein or dinitrogenase.The proteins have a negative redox potential. The MoFe protein (Iron-Molybdenum protein),is a heterotetramer composed of two alpha subunits and two beta subunits . The protein contains two copies of each of two types of clusters: P clusters and FeMo cofactors. Each P cluster contains 8 iron atoms and 7 sulfides linked to the protein by 6 cysteinate residues. Each FeMo cofactor contains one molybdenum atom, 7 iron atoms, 9 sulfides. This protein is responsible for reducing atmospheric nitrogen to ammonia via a series of electron transfers within the protein to the substrate molecule. The reaction requires the addition of six electrons for each nitrogen molecule that is split into two ammonia molecules. The Fe protein (Nitrogenase Reductase -NR) is a dimer and formed by 2 subunits of polypeptide chains linked by a 4Fe-4S cluster. Each monomer contains an ATP binding site.

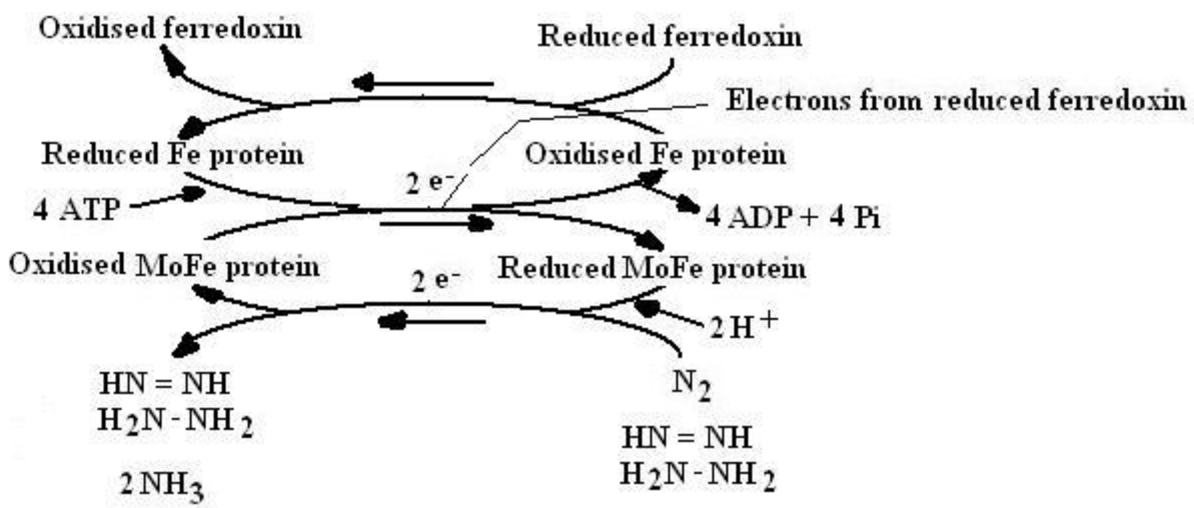


Fig 2 working of enzyme nitrogenase

The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to H_2N-NH_2, and this in turn is reduced to $2NH_3$.The reactions occur while N_2 is bound to the nitrogenase enzyme complex.

The favorable condition for nitrogen fixation are :-

18. **Presence of enzymes nitrogenase and hydrogenase in the nitrogen fixing cells or organisms.**
19. **Presence of leghaemoglobin which protect the enzyme nitrogenase from oxygen.**
20. **Ferredoxin which supplies electrons for this process.**
21. **A source of hydrogen (strong reducing agent) like NADPH or $FMNH_2$**
22. **Constant supply of ATP to transfer hydrogen atoms to dinitrogen.ATP is provided by aerobic respiration of sugars, ulitmately produced by photosynthesis. Phosphorous is an important component of the biochemical energy source, ATP (adenosine triphosphate). Thus, for legumes to fix N, there must be adequate available soil P.**
23. **Presence of coenzymes and cofactors .**
24. **Compounds for trapping ammonia formed by the reduction of dinitrogen (N_2)**

Nitrogen fixation is controlled by plant nod genes and bacterial nod , nif and fix gene cluster.Biological nitrogen fixation by free living and symbiotic bacteria is carried out by step by step progressive reduction of dinitrogen (N_2) molecules by the addition of of a pair of hydrogen atoms. Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation. In the heterocystous bacteria the primary electron donor to nitrogenase is also a ferrredoxin ,but it receives electrons produced by the action of light on the photosynthetic apparatus.The electrons are supplied via ferredoxin to nitrogenase reductase and then nitrogenase. The reductase donates 8 electrons in succession to the nitrogenase cofactor, a molybdenum-iron containing active center which catalyses the actual reduction of dinitrogen.Iron (Fe^{+3}) and molybdenum (Mo^{+4}) of enzyme nitrogenase takes part in attachment of a dinitrogen molecule (N_2) and weaken the bonds between the two atoms of the nitrogen.The weakened molecule of nitrogen is reduced by the reducing agent (NADPH ,$FMNH_2$).

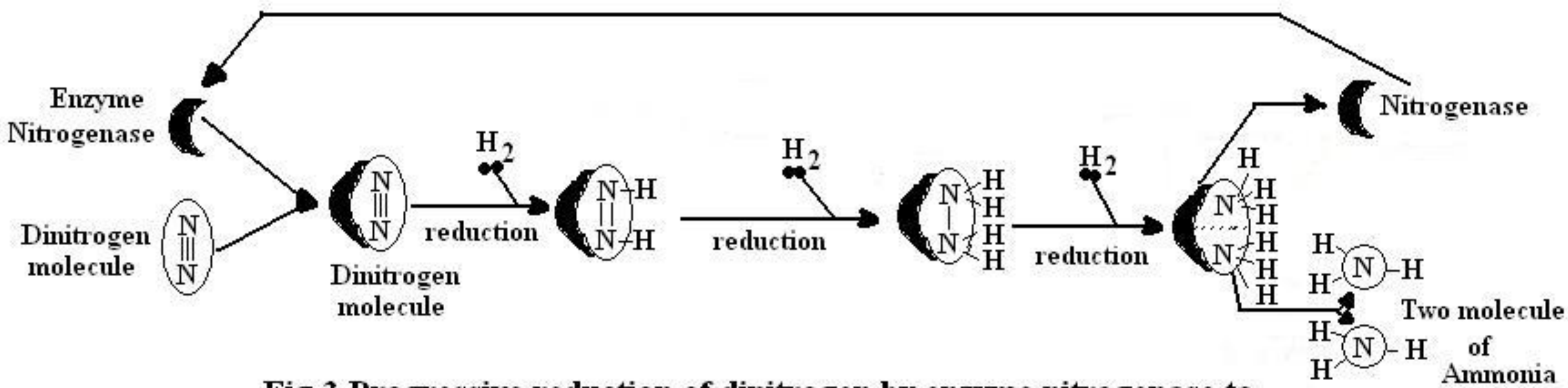


Fig 3.Progressive reduction of dinitrogen by enzyme nitrogenase to form ammonia

It produces dimide (N_2H_2), hydrazine (N_2H_4) and then ammonia ($2NH_3$)Where one molecule of N_2 is reduced in the presence of protons to 2 NH_3, and H_2 as a byproduct.Semi-activated nitrogenase can reduce easy substrates such as acetylene. In the typical reaction, two molecules of ATP are consumed for each electron transferred.16 ATP's are needed to fix a single nitrogen molecule in nitrogen fixation, the plant regulates the nitrogenase's activity and expression according to reduced nitrogen availability and oxygen presence.(7,8). Molecular oxygen is a strong inhibitor of the nitrogenase Mo-Fe cofactor and is removed by the plant oxygen binding protein leghemoglobin in the root nodules.Some of the cyanobacteria have yet another mechanism for protecting nitrogenase: nitrogen fixation occurs in special cells (heterocysts) which possess only photosystem I (used to generate ATP by light-mediated reactions) whereas the other cells have both photosystem I and photosystem II (which generates oxygen when light energy is used to split water to supply H_2 for synthesis of organic compounds). Nitrogenase also converts hydrogen ions to hydrogen gas at the same time thus consuming even more ATP in the process.

Nitrogen metabolism All of the nitrogen in a plant, whether derived initially from nitrate, nitrogen fixation, or ammonium ions, is converted to ammonia, which is rapidly incorporated into organic compounds through a number of metabolic pathways is known as nitrogen metabolism ,consists of :-

- **Nitrogen fixation**
- **Nitrogen reduction or Nitrification :- Ammonia formed as a result of nitrogen fixation is used for the synthesis of amino acids. Amino acids are transported through phloem to other parts for the synthesis of proteins .Ammonium ions can be taken by higher plants but plants are more adapted to absorb nitrate (NO_3^-) than ammonium ions (NH_4^+) from soil.Nitrification is an aerobic microbial process by which specialized bacteria oxidize ammonium to nitrite and then to nitrate.It is accomplished by nitrifying bacteria like nitrosomonas, nitrosococcus and nitrobacter.Nitrification is a two-step process. The first stage is the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-), a function carried out by bacteria in the genus Nitrosomonas. The nitrite formed is rapidly oxidized to nitrate (NO_3^-) by bacteria in the genus Nitrobacter.**

$$NH_4^+ + 2O_2 \xrightarrow[\text{Nitrosococcus}]{\text{Nitrosomonas}} NO_2^- + 2H_2O + \text{Energy}$$

(Ammonia) (Nitrite ion)

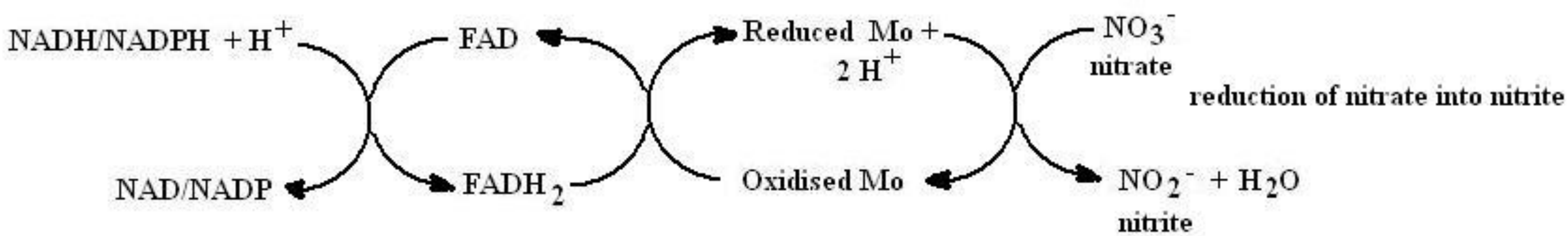
$$NO_2^- + O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- + \text{Energy}$$

(Nitrite ion) (Nitrate ion)

The nitrifying bacteria nitrosomonas, nitrosococcus and nitrobacter are chemoautotrophs :-they gain their energy by chemical oxidations (chemo-) and they are autotrophs (self-feeders) because they do not depend on pre-formed organic matter. As they derive energy for synthesis of organic food by oxidising inorganic materials ammonia. Nitrification is an autotrophic process during which energy is liberated from the oxidation of ammonium with the biosynthesis of simple inorganic molecules such as carbon dioxide and water into organic compounds and oxygen is an electron acceptor.nitrifying bacteria,gain their energy by oxidising ammonium, while using CO2 as their source of carbon to synthesise organic compounds

- **Nitrogen assimilation :-Nitrogen assimilation is the conversion of inorganic nitrogen (such as nitrate) into an organic form of nitrogen like, for example, an amino acid. Nitrate is reduced for this purpose by enzymes first to nitrite (by nitrate reductase), then to ammonia (by nitrite reductase). Ammonia is incorporated into amino acids.The process of nitrate reduction to ammonia is accomplished in two steps, each mediated by a specific enzymes:-**

  . **Reduction of nitrate to nitrite :- The nitrate serves as a terminal electron acceptor for anaerobic respiration. The nitrate is reduced to nitrite by enzyme nitrate reductase.It is co-enzyme NADH/NADPH-dependent according to organism.**



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The enzyme is a flavoprotein that contains iron and molybdenum serves as an electron carrier. FAD receives hydrogen from reduced co-enzyme NADH/NADPH +H+ (serves as hydrogen donor) for the reduction of nitrate

  i. **Reduction of nitrite to ammonia :- Nitrite reductase reduces the nitrite ions to ammonium ions.Nitrite reductase does not require molybdenum and may contain copper and iron.Ferredoxin is the direct source of electrons for nitrite reduction, which occurs in higher plants mostly in the leaves.**

$$2\,NO_2 + 7NADPH + 7H^+ \xrightarrow[\text{Ferredoxin}]{\text{Nitrite reductase}} 2\,NH_3 + 4\,H_2O + 7\,NADP^+$$

The nitrite ions formed in other parts of the plant are also transported to leaves and reduced to ammonia.The reduced coenzyme NADPH +H+ or NAD + H+ serves as hydrogen donor for the reduction of nitrite.

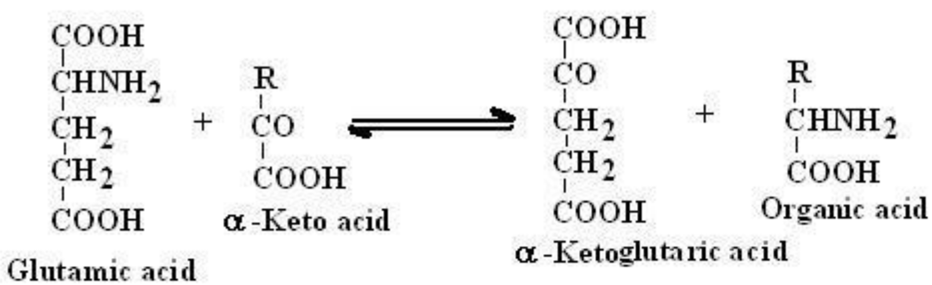
Ammonia thus formed as a result of nitrogen fixation is not given out .It is highly toxic and used for the synthesis of amino acids. Amino acids are the building blocks for the synthesis of proteins.The amino acids are transported through phloem to other parts of the plant for the synthesis of proteins.

Amino acids are the initial products of nitrogen assimilation. An amino acid molecule consists of at least one carboxyl (-COOH) groups and one or several amino (-NH2) groups.Majority of amino acids are synthesised in plants by two main processes :- :-

28. **Reductive animation :- In this process, ammonia reacts with alpha-ketoglutaric acid to form glutamic acid in the presence of enzyme glutamate dehydrogenase.A reduced coenzyme NADPH in leaves ,NADH in roots is required.**

$$NH4^{+} + \alpha\text{ - Ketoglutaric acid} + NADPH \xrightarrow[\text{dehydrogenase}]{\text{Enzyme: Glutamate}} \text{Glutamate} + H_2O + NADP$$

29. **Transamination :-Glutamic acid is the main amino acid from which other 17 amino acids are formed through transamination. With the help of enzyme transaminase, amino group of an amino acid (-CHNH2) is exchanged with keto group (-CO) of keto acid.Pyridoxal phosphate is required as coenzyme which is obtained from a vitamin.**



A very common α-ketoacid is α-ketoglutarate,an intermediate in the citric acid cycle .

In most of transamination glutamic acid is present as one of the reactants. The other reactant may be any one of a number of alpha-keto acids,. Which alpha keto acid receives the amino group from glutamic acid is determined by the specificity of the enzyme.

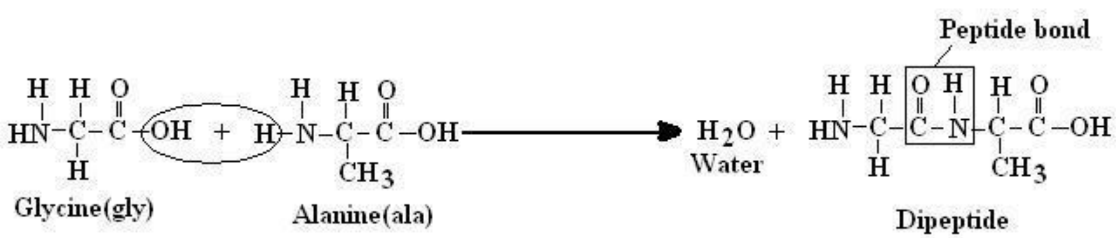
Amides :- Amides are nitrogen-containing organic compounds and contain more nitrogen than amino acids. Amides are formed when amino acids bond to each other to form proteins. Amino acids in which hydroxyl group (-OH) of carboxylic group is replaced by amino group (-NH2) from ammonia, or another amino acid.ATP is required. The two important amides found in plants are asparagine and glutamine.

$$NH4^{+} + \text{Aspartic acid} + ATP \xrightarrow[\text{synthetase}]{\text{Enzyme: Asparagine}} \text{Asparagine} + ADP + Pi$$

$$\text{Glutamine} + \text{Aspartic acid} + ATP \xrightarrow[\text{transamidase}]{\text{Enzyme: Glutamine-aspartate}} \text{Glutamic acid} + \text{Asparagine} + ADP + Pi$$

They are formed from two amino acids namely glutamic acid and aspartic acid. This reaction takes place in the presence of the enzymes glutamine synthetase or asparagine synthetase.

Proteins synthesis:- Proteins are made up of long chains of amino acids. Proteins are in the form of one or more chain called polypeptide chains. Amino acids bond to each other by peptide or amide bonds. The carboxyl group (-COOH) of one amino acid reacts with the amino group (-NH2)of the next amino acid ,releasing a molecule of water and as a result peptide bond (-CONH_) is formed.This may be illustrated with the two simplest amino acids, glycine and alanine:-



Combination of two amino acids are known as dipeptide and combination of more than two known as polypeptide

Chains of peptide- linked amino acids build up proteins

In a polypeptide, amino acids are arranged according to coded information contained in mRNA. Polypeptide synthesis occurs over ribosome where mRNA gets attached. Amino acids are brought there according to codons by means by tRNAs. The number of amino acids varies greatly among proteins and thus differs the molecular weight of proteins also.