Mineral Nutrition 12

It is the study of source, mode of absorption, distribution and metabolism of various inorganic substances of minerals by plants for their growth, development, structure, physiology and reproduction.

* **Liebig (1840)** reported the essentiality of mineral nutrition & stated that productivity of soil depends upon the proportionate amount of that essential elements which is deficient in that soil.
* **Sachs and knopp (1960)** published a list of major elements of plants ash.

Ash analysis :

A desired sample of plant is dried at 100^0C in an oven. Now dry matter of plant is put in a muffle furnace between 600^0C to 700^0C.

Soil less culture or Hydroponics :

The nutrient solution composition proposed by **sachs, knops** but did not notice the importance of micronutrients. **Hogland** & **Arnon** proposed the formula of nutrient solution containing all the micronutrients.

The cultivation of plants by placing the root in nutrient solution is called **hydroponics (Gericke-1937).**

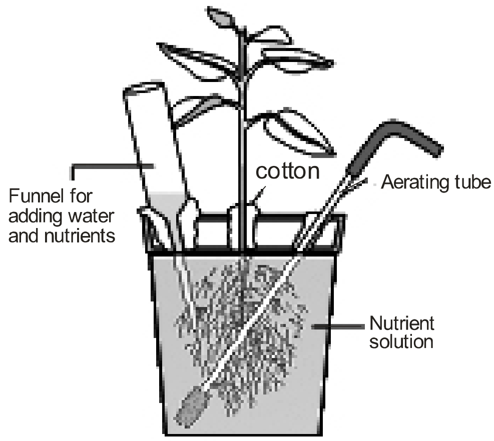


Fig : Diagram of a typical set-up for nutrient solution culture

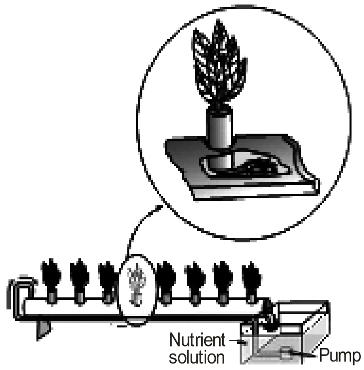


Fig : Hydroponic plant production. plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots are continuously bathed in aerated nutrient solution. The arrows indicates the direction of the flow.

Sand culture :

The plants are grown in sand supplemented with nutrients. It is better than solution culture because it provides solid medium and natural aeration.

Aeroponics :

It is a system of growing plants with their roots bathed in nutrient mist. The plants of citrus and olive have been successfully grown aeroponically (Zobel).

Essentiality of mineral elements :

Arnon and stout (1939) stated that.

(1) The element must be essential for normal growth and development.

(2) The elements can not be replaced by another elements.

(3) Element must be directly involve in the nutrition of a plant.

(4) The deficiency of the element forms disorders.

* Total 105 elements are found in plants out of them **17 (C,H, O, N, P , K, S, Mg, Ca, Fe, B, Mn, Cu, Zn, Mo, Cl & Ni) are essential elements**. Two categories of essential elements are as follow

(i) **Macro or Major elements :** These are required by plants in abund ant quantity **(more than 1 miligram / gram)** dry matter. They are **C, H, O, N, P , S, K, Mg, Ca.**

(ii) **Micro elements :** These are required by plants **in less amount (less than 1 miligram / gram** dry matter). They are **Fe, Zn, Cu, Mn, B, Mo, Cl, Ni.**

S. No.	Macro Element	Absorbed as	Major functions	Deficiency Symptoms
1.	Nitrogen	NO_3^-, NH_4^- rarely NH_2^-	Part of proteins, enzymes, cytochromes,chlorophyll. ATP,some coenzymes-NAD, NADP, FMN, FAD, nucleotides of DNA & RNA, vitamins, hormones like IAA	(i) Chlorosis appears firstly in older leaves. (ii) Stunted growth. (iii) Premature leaf fall. (iv) Purple colouration of stem. (v) Reduced yield. (vi) Delaying flowering.
2.	Phosphorus	PO_4^{3-} $H_2PO_4^{2-}$	Phospholipids, Nucleic acids-DNA & RNA, NAD, NADP, TPP, ATP, AMP,ADP, nucleoproteins Energy transfer, cell division, phosphorylation reactions	(i) Chlorosis with necrosis. (ii) Premature leaf fall. (iii) Delayed flowering & seed germination. (iv) Poor vascular tissues.
3.	Potassium	K^+	Stomatal movements, Osmotic regulation and hydration, Membrane Permeability, Enzymes related with Photosynthesis, respiration, protein synthesis, nitrate reduction, translocation of sugars	(i) Mottled interveinal chlorosis. (ii) Loss of apical dominance. (iii) Die back. (iv) Loss of cambial activity. (v) Plastid disintegration, (vi) Bushy habit. (vii) Marginal or apical yellowing or scorch & curling. (viii) Shorter internodes. (ix) Lodging in cereals
4.	Calcium	Ca^{2+}	Calcium pectate of middle lamella, activator of enzymes related with chromosome formation,Spindle formation, Detoxification,activators of amylases, ATPase, adenyl kinase, Selective permeability of cell Membrane, meristematic activity	(i) Degeneration of meristems especially in root apex. (ii) Stunted growth. (iii) Chlorosis & necrosis. (iv) Curling appearing first in young leaves. (v) Blossom end rot of Tomato. (vi) Irregularity in chromosome. (vii) Premature flower abscission.
5.	Magnesium	Mg^{2+}	Association of ribosome subunits, formation of Chlorophyll,carotenoids activator of enzymes related with photosynthesis, phosphate transfer in respiration, Nitrogen metabolism, DNA and RNA synthesis, carbohydrate & fat metabolism	(i) Interveinal chlorosis with purple anthocyanin pigmentation. (ii) Marginal curling. (iii) Premature leaf abscission. (iv) Reduced growth. (v) Chlorotic areas may turn necrotic
6.	Sulphur	SO_4^{2-} & as SO_2 from air	Component of Amino acids-cysteine & methionine, ferredoxin, lipoic acid, CoA, vitamins like thiamine and biotin, nodule formation in legumes ,Chlorophyll formation,	(i) Chlorosis appearing first in young leave. (ii) Stunted growth. (iii) Accumulation of anthocyanins. (iv) Tea yellow disease. (v) Reduced nodulation in legumes.

S. No.	Micro Element	Absorbed as	Major functions	Deficiency Symptoms
1.	Iron	Fe^{3+} or Fe^{2+} in acidic soil	Component of ferredoxin, Cytochromes, nitrogenase, synthesis of chlorophyll, Electron transport in respiration photosynthesis,Activator of catalases, peroxidase & Aconitase	(i) Interveinal chlorosis in young leaves. (ii) Reduced growth.
2.	Copper	Cu^{2+}	Activator or Component of cytochrome Oxidase,ascorbic acid oxidase, tyrosinase, plastocyanin, RuBP carboxylase	(i) Die back of citrus. (ii) Exuding gummy substance. (iii) Exanthema. (iv) Reclammation disease. (v) Apical necrosis of young leaves. (vi) Blackening of potato tubers.
3.	Zinc	Zn^{2+}	Component or activator of some enzymes like carbonic anhydrase, dehydrogenases and carboxylases, synthesis of IAA, RNA and protein, formation of seed,	(i) Interveinal chlorosis. (ii) little leaf. (iii) Leaf rosettes. (iv) White bud. (v) Stunted growth. (vi) Khaira disease of rice. (vii) Whip tip of maize
4.	Boron	BO_3^{-3} or $B_4O_7^{2-}$	Translocation of sugars, seed, pollen and spore germination, synthesis of pectins, proteins and nucleic acids, uptake and utilization of calcium, nodule formation in legumes, flowering and fruiting	(i) Brown heart of Turnip. (ii) Internal cork of apple. (iii) Heart rot of sugar beet. (iv) Death of shoot & root tips. (v) Stunted growth. (vi) Small size of fruits. (vii) Decreased nodulation of legumes. (viii) Browning of cauliflower.
5.	Manganese	Mn^{2+}	Activator of enzymes of photosynthesis, respiration, and nitrogen metabolism, Photolysis of H_2O in photosynthesis, synthesis of chlorophyll	(i) Interveinal chlorosis. (ii) Marsh spot of pea. (iii) Grey speak of oat. (iv) Stunted growth. (v) Flowers sterile.
6.	Molybdenum	MoO_4^{2-}	Cofactor of nitrate reductase, Important in N_2 fixation, ascorbic acid synthesis.	(i) Mottled chlorosis with marginal necrosis. (ii) Whip tail of cauliflower. (iii) Loosening of inflorescence in Cauliflower.
7.	Chlorine	Cl^-	Anion-cation balance, transfer of electron from water to PS II (Photolysis)	(i) Bronze colour in leaves. (ii) Swollen root tips. (iii) Chlorosis and necrosis. (iv) Flower abscission reduced fruiting
8.	Nickel	Ni^{2+}	Activator of Urease and required for hydrogenase	(i) Necrosis of leaf tip

General function of Mineral elements :

(i) Frame work elements : They form cell walls and storage products of plants, **Ex: C, H, O.**

(ii) Protoplasmic elements : they form protoplasm **Ex: C,H, O, N, S, P, Mg, Fe.**

(iii) Balancing Elements : They minimise the toxic effect of heavy elements. **Ex: Ca^{++}, Mg^{++}, K^+.**

(iv) Critical elements : some macronutrients become commonly deficient in the soils that are called critical elements. The latter are three–**N, P & K**. Deficiency caused by a critical element is known as **primary deficiency.** Most fertilisers contain critical elements. They are called complete fertilisers. They are labelled 15 : 15 : 15, 18 : 17 : 19, etc, depending upon the proportions of N, P and K.

(v) Storage elements : Ex: C, N, P, S.

(vi) Toxic elements : Ex: Pb, Hg, As, Al , Ag.

(vii) Permeability : Monovalents like **Na^+, K^+** increase membrane permeability whereas Calcium and other divalents decrease it.

(viii) Catalytic Effects : Activation of many enzymes require mineral elements as cofactors. **Ex : K, Mg, Fe, Mn, Zn, Cu, Mo.**

(ix) Osmotic potential : Inorganic salts cause Osmotic potential of cell sap. the latter is required for absorption of water and maintenance of cell turgidity.

(x) Constituents of Biomolecules : C, H, are components of all organic substances. Nitrogen is a constituent of all amino acids, proteins, nucleic acid, chlorophyll, auxin, cytokinins and vitamins. Phosphorus is present in nucleotides, higher nucleotides **(eg. ADP, ATP, CDP, CTP),** coenzymes like NAD^+ and NADP, RNA, DNA and phospholipids.

MINERAL ABSORPTION

* Soil is the main source of mineral salts. These mineral salts are mainly absorbed by the (Sub terminal) **meristemetic region of roots.**
* Mineral salts are present with soil particles in colloidal form and in water as soil solution. Conduction of mineral salts is done through the xylem.
* Mineral absorption is done in form of ions mainly form meristemetic zone.
* Absorption of mineral in plant is an active process.
* There are two methods of absorption of mineral salts:-

(A) Passive absorption of minerals :

(Without expenditure of ATP)

(1) By Simple diffusion : According to this method mineral ions may diffuse in root cells from the soil solution.

(2) By mass flow : According to this method mineral ions absorption occurs with flow of water under the influence of transpiration.

(3) By ion exchange : This is exchange of mineral ions with the ions of same charge.

(i) By contact exchange : When the mineral ion exchange occurs with the H^+ and OH^- ions.

(ii) carbonic acid exchange : When the mineral ion exchange takes place with the ions of carbonic acid.

(4) By Donnan equilibrium : This theory explains the passive accumulation of ions against the concentration gradient or electrochemical potential (ECP) without ATP. At the inner side of cell membrane, which separates from outside (external medium), there are some anions, which are fixed or non diffusible and membrane is impermeable to these anions. While cations are diffusible.

* In such condition, for maintenance of equilibrium additional cations are needed to balance negative charges of anions (at inner side of membrane).Thus some cations moves, inside the cell from soil solution.
* So according to this theory Donnan equilibrium is attained, if the anions and cations in the internal solution become equal to the anions and cations in external solution.

Objections for passive mineral absorption / evidences in favour of avtive mineral absorption:

(1) Absorption of K^+ ions in **Nitella** algae is observed against the concentration gradient.

(2) Rate of respiration of a plant is increases, when plant transferred to mineral solution.(Salt respiration)

(3) Factors like deficiency of oxygen, CO, CN, which inhibits rate of respiration, these factors also inhibit the absorption of mineral ions in plants.

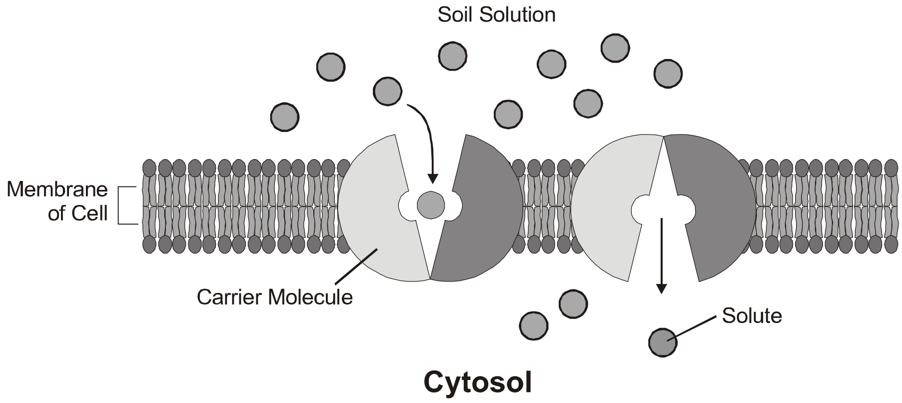
Thus ion absorption in plants is considered mainly as an active process.

(B) Active ion absorption : (By expenditure of ATPs)

(1) Cytochrome pump theory : By **Lundegardh and Burstrom** (1933) according to this theory, **only anions** are absorbed by active mechanism through cytochrome pumping and absorption of cation is passive process.

According to cytochrome pump theory **salt respiration** is called as **anion respiration**.

(2) Carrier concept : By **Vanden Honert**. According to this theory some specific carrier molecules made up of **proteins** are present in cell membrane of root cell, which absorb both the ions and forms **ion-carrier complex.** This complex is broken inside the cell membrane with the use energy.



CARRIER CONCEPT MECHANISM OF MINERAL ABSORPTION

(3) **Protein – lecithin theory** : By **Bennet** and **Clark**

According to this theory a **phospholipid lecithin** in root cell membrane works as carrier for both type of ions.

Lecithin has two type of groups.

Lecithin → **Phosphate** group (⊕ **ions** absorption)
Lecithin → **Choline** group (Θ **ions** absorption)

* **Goldacre –** A contractile protein is associated, with absorption of minerals.

Mineral absorbed by the roots of plants are carried by xylem by two pathways, apoplastic and symplastic pathway.

* **P.R. Stout** and **Hoagland** (1939) proved that **mineral salts are translocated through xylem** along with transpiration pull (exp. with help of radioisotopes).

TRANSLOCATION OF SOLUTES

* Mineral salts are translocated through xylem along with the ascending stream of water, which is pulled up through the plant by transpirational pull.
* Analysis of xylem sap shows the presence of mineral salts in it. Use of radioisotopes of mineral elements also substantiate the view that they are transported through the xylem. You have already discussed the movement of water in xylem.

SOIL AS RESERVOIR OF ESSENTIAL ELEMENTS

* Majority of the nutrients that are essential for the growth and development of plants become available to the roots due to weathering and breakdown of rocks. These processes enrich the soil with dissolved ions and inorganic salts. Since they are derived from the rock minerals, their role in plant nutrition is referred to as mineral nutrition.
* Soil consists of a wide variety of substances.
  Soil not only supplies minerals but also harbours nitrogen-fixing bacteria, other microbes, holds water, supplies air to the roots and acts as a matrix that stabilises the plant.
* Since deficiency of essential minerals affect the crop-yield, there is often a need for supplying them through fertilisers. Both macro-nutrients (N, P, K, S, etc.) and micro-nutrients (Cu, Zn, Fe, Mn, etc.) form components of fertilisers and are applied as per need.

Nitrogen Metabolism

Role of Nitrogen in Plants :– Constituent of proteins, nucleic acids ATP, GTP, Chitin, Vitamins, chlorophyll, alkaloids, cytochromes, hormones. Nitrogen is necessary to plants for heridity, reproduction, growth metabolism and development.

Sources of Nitrogen to plants :

(1) **Atmospheric nitrogen :**

$N \equiv N$ (Molecular, inert or elemental form) used by Rhizobium(Legumes), BGA, Lichens, Yeast, Pullularia.

* These **converts atm. N_2 into metabolically usefull ammonaium ions (NH_4^+)**. This process is called as biological nitrogen fixation.

(2) NO_3^-, NO_2^-, NH_4^+ in soil :

These are major source of nitrogen to plants.

* **Nitrate ions (NO_3^-)** are **cheif form of nitrogen used by majority of plants.**

Plants grow in acidic soil & found in forest use ammonium ions (NH_4^+) as major N_2 source.

Nitrate ions are cheif source of N_2 for plants but they can not be used directly in metabolic pathway in plant cells, as it is highly oxidised form. so **NO_3^- (Nitrate) first converted into NH_4^+ (ammonium ions)** called **nitrate reduction**. So NH_4^+ ions enters in plant metabolism.

(3) Organic nitrogen in soil : as amino acids, protein body.

Due to death & decay of organisms. This is not a major source of N_2.

(4) Insect bodies : for some plants (insectivorous plants)

(5) Urea as chemical/artificial fertilizers

Nitrogen (N_2) Cycle :

(1) Biological Nitrogen Fixation / Diazotrophy ($N_2 \rightarrow NH_3$) **:–** Conversion of molecular or elemental nitrogen ($N \equiv N$) into inorganic nitrogenous compounds (NH_4^+) through agency of living organisms is called as **biological nitrogen fixation** or **Diazotrophy**

Nitrogen Fixing organisms (Diazotrophs) :–

(A) Free living organisms or non–symbiotic organisms/bacteria :– Azotobacter, Aerobacter, Beijerinckia, Heliobacterium (**Clostridium, Rhodospirillum, Chromatium, Chlorobium, Rhodopseudomonas**.

* **Cyanobacteria** (Blue = green algae) **– Nostoc, Anabaena, Caulothrix, Cylindriospermum, Tolypothrix, Aulosira** etc.

(B) Symbiotic Nitrogen fixers :–

* **Rhizobium leguminosarum** (= **Bacillus radicicola**) is association with the root nodules of leguminous plants. None of these two partners alone can fix atm. nitrogen.

(i) Root nodules of non–legume (15–genera) Angiosperms :– Casuorina, Myrica, Alnus, Ceanothus, Elaiagnus, Hippophae, Coriaria.

Filamentous, Actinomycetous bacteria **Frankia** live in root nodules of these non-legume plants.

Both **Rhizobium** & **Frankia live freely in soil** but **fix nitrogen only when in symbiotic association** with host plant.

(ii) Root nodules of gymnosperms :– Cycadaceae, Podocarpus.

(iii) Leaf Nodules (Phyllosphere) **:– Psychotria, Dioscoria** (**Klebsiella**), Rubiaceae (**Mycobacteria**).

(iv) Lichens :– Cyanobacteria (BGA) partner

(v) Non nodule forming associations : Between **Azolla** (aquatic fern) & Cyanobacteria **Anabaena.**

(vi) Paspalum-notatum grass have loose symbiosis (associative symbiosis) with Azotobacter pospali in roots and Azospirillum with wheat, maize, sorghum etc.

(2) Ammonification : Conversion of dead organic nitrogenous compounds into ammonia. **Bacillus mycoides, B. ramosus.**

(3) Nitrification : Oxidation of ammonia, produced by ammonification **into nitrates** by **nitrifying bacteria** is called as **nitrification.**

(i) $$\underset{\text{Ammonia}}{2NH_3} + 2O_2 \xrightarrow{\text{Nitrosomonas}} \underset{\text{Nitrite ion}}{2NO_2^-} + 2H_2O + 2H^+$$

(ii) $$2NO_2^- + O_2 \xrightarrow{\text{Nitrobacter}} \underset{\text{Nitrate ions}}{2NO_3^-}$$

Some fungi like Aspergillus, Penicillium can also carry out this process.

(4) Denitrification : Nitrates or nitrites converts back into molecular or atm. nitrogen by **denitrifying bacteria** is denitrification. Ex. **Pseudomonas**

(5) Nitrate reduction :

* Plants take nitrogen from soil, chiefly in nitrate forms which is highly oxidised form.

  so **NO_3 converts in ammonia by following method**

  (a) $NO_3^- + NADH + H^+ \xrightarrow{\text{Nitrate reductase}} NO_2 + NAD + H_2O$

  (b) $NO_2^- \xrightarrow{2e^-,\, 2H^+} NOH \xrightarrow{2e^-,\, 2H^+} NH_2OH \xrightarrow{2e^-,\, 2H^+} NH_4^+$

  or $2NO_2^- + 6H_2O + 4H^+ \xrightarrow{\text{Nitrate reductase}} 2NH_4^+ + 3O_2 + 4H_2O$

* Nitrate reductase is Molybdo flavoprotein isolated by Evans and Nason 1953 from Neurospora and Glycin max leaves.

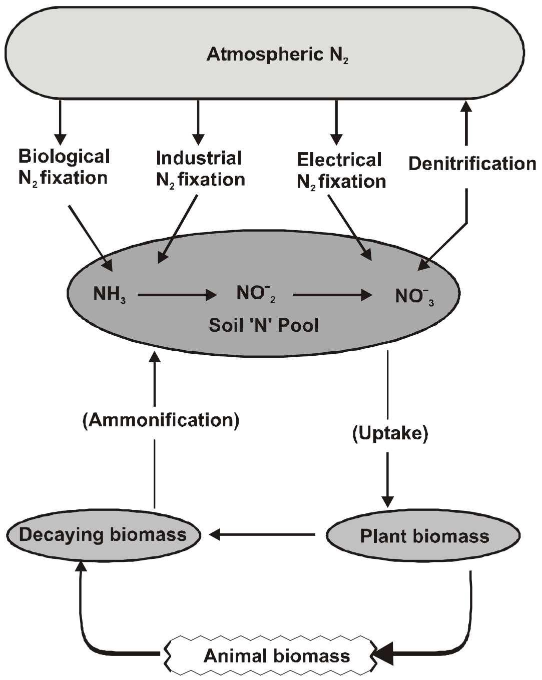


Fig : Nitrogen Cycle

BIOLOGICAL N_2 FIXATION

It is done by symbiotic bacteria & free living bacteria.

Symbiotic N_2 fixation (Diazotrophy) : In **leguminous plants (Fabaceae)** by symbiotic bacterium **Rhizobium**, which form nodules in their roots.

* **N_2** directly **convert into NH_4^+ ion**, which is used in plant metabolism.

* Root nodules act as site for N_2 fixation. It contains all necessary biochemical components like enzyme Nitrogenase, Leghaemoglobin, required in N_2 fixation.

* Enzyme **nitrogenase** is a **Mo–Fe protein** & catalyse the conversion of atm. N_2 to NH_3.

  It posses two units **unit-Ist is Mo-Fe protein** & **unit-IInd is Ferredoxin** (fe-s protein)**.**

* Nitrogenase is extremely sensitive to oxygen. So to protect it from oxygen, nodules contains an **O_2 scavanger** called **leghaemoglobin (Lhb)** and combined with O_2 to form oxyleghaemoglobin(Olhb)

* Leghaemoglobin is **pink** in colour & similar to haemoglobin of vertebrates. It is synthesised by plant gene (Globin part by plant and heam part given by bacteria).

(A) NODULE FORMATION :

It is due to interactions between bacteria and host root. It occurs in following steps :

(i) **Multiplication & colonization of Rhizobia** at Rhizosphere and attachment to epidermal root hair cells. Initial attraction of Rhizobia to host root is **chemotactic** (Rhicadhesin protein of bacterial cell identify host root) as **root exude amino acids, sugars, organic acids** and **flavonoids.**

(ii) **Characterstic curling of root hairs and invasion of the bacteria** to form an **infection thread,** by the invagination of plasma membrane of root hair cells and it reaches up to the cortex of roots.

Curling of root hairs is stimulated by **specific complex polysaccharides found on the surface of rhizobia**, recognised by **Lectins (small proteins of host plant root).**

(iii) **Nodule initiation** & **development in root cortex. Mitogenic agents secreted (Kinetin) by bacteria & auxin produced by plant cell promotes cell division & extension** leading to nodule formation. Nodule establishes direct vascular connection with host for exchange of nutrients. Root nudule cells have chromosome in double to other somatic cells. Thus nodule cells are polyploid specially **Tetraploid**.

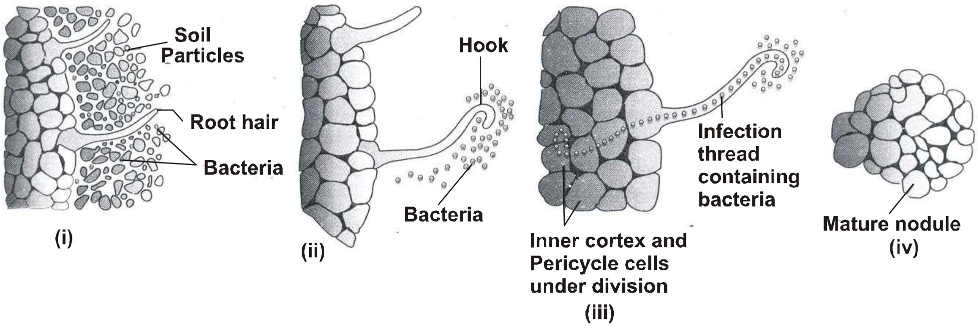


Fig:- Deveploment of root nodules in soyabean

(iv) **Release of bacteria from infection thread** and they differentiate as specilized nitrogen fixing cell.

* Bacteria continue to multiply during it's path in root hair cells & bacteria distribute in most of cells.

* The membrane of infection thread bud off to form small vesicles which containing one or more bacteria. Then bacteria stop dividing & enlarge & differentiate in group of **nitrogen fixing cells and called as bacteroid** & it's membrane is called as **peribacteroid membrane.**

(B) MECHANISM OF BIOLOGICAL N_2 FIXATION :

By Burris. The atm. N_2 is reduce by the addition of hydrogen atoms.

* The three bonds between two nitrogen atoms $N \equiv N$ or dinitrogen are broken & ammonia **(NH_3) is formed by reduction of $N \equiv N$** and then **reduction of ammonia (NH_3) to form ammonium ions (NH_4^+).**

N_2 fixation requires 3 components :

(i) **A strong reducing agent - $NADPH_2$/$FADH_2$/ $NADH_2$** – from photosynthesis & respiration.

(ii) **ATP** to transfer hydrogen atom to dinitrogen – from respiration & photosynthesis.

(iii) **Nitrogenase enzyme.**

* **Steps of N_2 fixation :**

(a) Reduction of N_2 to NH_3 :

$N \equiv N + 8e^- + 8H^+ + 16\ ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16ip$

(b) Reduction of NH_3 to NH_4^+ :

$2NH_3 + H_2 \longrightarrow \mathbf{2NH_4^+} + 2e^-$

Over all reaction :

$\mathbf{N_2 + 6e^- + 8H^+ + 16\ ATP \longrightarrow 2NH_4^+ + 16\ ADP + 16ip}$

* Biological N_2 fixation is controlled mainly by four genes :

(i) **NOD gene of host plant :** These encodes no. of nodule specific protein called nodulins, synthesis, requires for active nodule devlopment.

(ii) ***nod, nif*** and ***fix*** gene of bacteria.

SYNTHESIS OF AMINO ACIDS & NITROGEN ASSIMILATION

Nitrogen assimilation :– Inorganic NH_3 (Produced by nitrate reduction or biological fixation or obtained from soil as NH_4^+) reacts with a TCA cycle intermediate – α–ketoglutaric acid to form an amino acid **glutamic acid**. This process known as **Reductive amination or Amino acid Biosynthesis.**

α – Ketoglutaric acid + NH_4^+ + $NADPH_2$ $\xrightleftharpoons[\text{dehydrogenase}]{\text{Glutamate}}$ Glutamic acid + H_2O + NADP

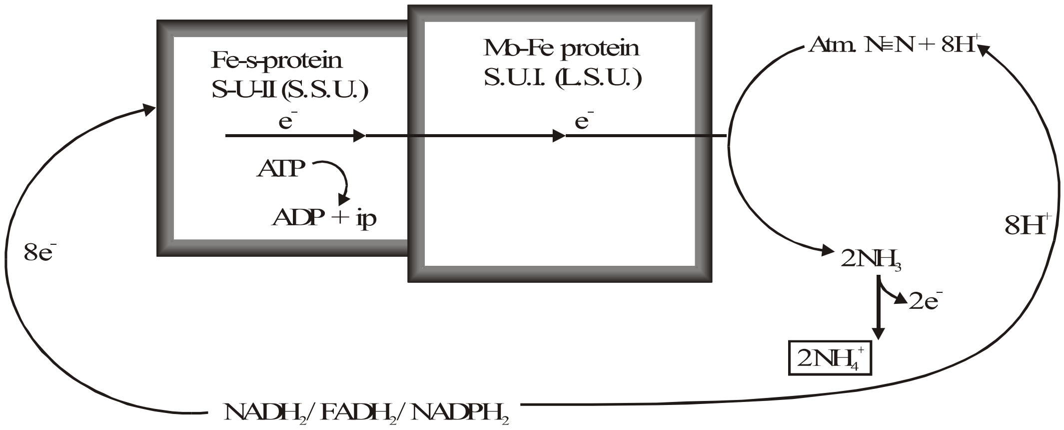
Transamination :– Transfer of Amino group from glutamic acid to other keto acid is known as transamination. This is a process of formation of other amino acids in plants. **(transaminase enzyme)**

Ex. Glutamic acid + Pyruvic acid $\rightleftharpoons$ **Alanine** + α-ketoglutarate

Glutamic acid + OAA $\rightleftharpoons$ **Aspartic acid** + α–keto glutaric acid.

* **Glutamic acid is first formed amino acid in plants** & can synthesize different amino acids by transamination.

NITROGENASE



Biological N_2 fixation

Mechanism of nitrogen fixation

(a) The 2nd unit (ferredoxin) of nitrogenase, receive electrons from e^- donar ($FADH_2NADH_2\backslash NADPH_2$) and become reduced.

(b) This reduced 2nd unit is now activated by ATP and form a complex called **ferredoxin ATP complex.**

(c) On other side unit 1st (Fe-Mo protein) of nitrogenase, reacts with molecular nitrogen to form **nitrogenase-nitrogen complex.**

(d) Ferredoxin ATP complex then transfer electron to nitrogenase-nitrogen complex, so that the later gets reduced. This reaction is catalysed by hydrolysis of ATP.

(e) The reduced nitrogenase-nitrogen complex now receives proton (H^+) resulting in formation of ammonium (NH_4^+) ion.

Catalytic Amidation :

Transportation of fixed N_2/Assimilated N_2 in plants occurs mainly in form of amides especially in leguminous plants as amides are more stable than amino acids and posses high Nitrogen to Carbon ratio (2N to 4C - in Asparagine, 2N to 5C in glutamine (as glutamate posses 1N to 5C)

Formation of amides from amino acids catalysed by enzymes called as catalytic amidation.

In legumes of temperate origine like pea and clover- Asparagine is translocated in non nodulated plant parts.

In legumes of tropical origin like soyabean and cow-pea-ureides are translocated in non nodulated plant parts.

Glutamine synthesis :

$$\text{Glutamic acid/Glutamate} + NH_4^+ + \text{ATP} \xrightarrow{\text{Glutamine (G.S)Synthase}} \text{Glutamine} + \text{ADP} + \text{iP}$$

Asparagine syntnesis :

$$\text{Glutamine/Glutamate} + \text{Aspartate} + \text{ATP} \xrightarrow{\text{Asparagine(A.S)Synthase}} \text{Asparagine} + \text{ADP} + \text{iP} + \text{Glutamate}$$

/α-Ketoglutaric acid

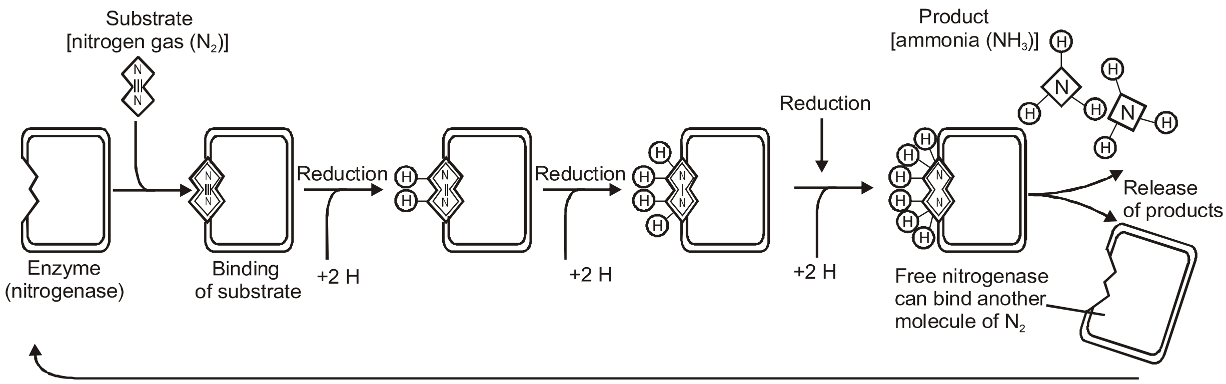


Fig : Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria

* Symbiotic nitrogen fixation requires **Nod genes** of legume, **nod, fix** and **nif gene** clusters of bacteria.

(B) Ammonification and Nitrification :

* Free living nitogen fixers do not immediately enrich the soil. It is only after their death the fixed nitrogen transfer to the cycling pool. It occurs in two steps, **amonification** and **nitrification**.

(1) Ammonification : Decomposers **(Bacillus ramosus, B. vulgaris)** degrade nitrogen excretions and proteins of dead organisms. They first form amino acids and then ammonia.

$$\text{Proteins} + H_2O \longrightarrow R-\underset{}{\overset{\displaystyle NH_2 \atop |}{CH}}-COOH \xrightarrow{+H_2O} \underset{\text{Organic acid}}{RCOOH} + \underset{\text{ammonia}}{NH_3}$$

(2) Nitrification : Conversion of ammonia into nitrate involves two steps

(i) $$2NH_3 + 3O_2 \xrightarrow[\text{Aspergillus flavus}]{\text{Nitrosococcus, Nitrosomonas}} 2NO_2^- + 2H^+ + 2H_2O + \text{energy}$$

(ii) $$2NO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2NO_3 + \text{Energy}$$

(C) Nitrate Assimilation :

(i) Reduction of Nitrate to Nitrite : After absorption, Nitrate is reduced by nitrate reductase with the help of NADPH & FAD/FMN.

$$NO_3 + NAD(P)H + H^+ \xrightarrow[\text{FAD/FMN}]{\text{Nitrate Reductase}} NO_2^- + H_2O + NADP^+$$

(ii) Reduction of Nitrite : In the presence of NAD(P)H and ferredoxin, Nitrite is further reduced by enzyme nitrite reductase.

$$2\,NO_2^- + 7NAD(P)H + 7H^+ \xrightarrow[\text{Ferredoxin}]{\text{Nitrite reductase}} 2NH_3 + 4H_2O + 7NAD(P)$$

Now Ammonia is fused with organic acids to form amino acids

(D) Synthesis of Amino Acids : They are synthesised by three methods.

(i) Reductive amination :

(a) α–ketoglutaric acid + NH_4^+ + NAD (P)H $\xrightarrow[\text{dehydrogtenase}]{\text{glutamate}}$ Glutamate + H_2O + NAD (P)

(b) Oxaloacetic acid + NH_4^+ + NAD (P)H $\xrightarrow[\text{dehydrogenase}]{\text{aspartate}}$ Aspartate + H_2O + NAD (P)

(ii) Transamination :

Glutamic acid + Oxaloacetic acid $\xrightarrow[\text{Aminotransferase}]{\text{Glutamate aspartate}}$ α–ketoglutaric acid + Aspartic acid

(iii) Catalytic Amidation :

Glutamate + NH_4^+ + ATP $\xrightarrow[\text{synthetase}]{\text{glutamine}}$ Glutamine + ADP + Pi

Glutamine + α-ketoglutaric acid + NAD (P)H $\xrightarrow[\text{synthetase}]{\text{glutamate}}$ 2 Glutamate + NAD (P)

(E) Denitrification : Dentirifying bacteria like, **Pseudomonas denitrificans, Thiobacillus denitrificans** convert nitrate into gaseous nitrogen in the soil.

$$2NO_3' \longrightarrow 2NO_2' \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2$$