MINERAL NUTRITION

Introduction

The basic needs of all living organisms are essentially the same. They require macro-molecules, such as carbohydrates, proteins and fats and water and minerals for their growth and development.

Methods to study the mineral requirements of plants

History: In 1860 Julius Von Sachs a prominent German Botanist demonstrated, for the first time. That plants could be grown to maturity in a defined nutrient solution in complete absence of soil. "This technique of growing plants in a nutrient solution is known as hydroponics."

Aim of hydroponics : To determine the mineral nutrients essential for plants.

Requirements of hydroponics :

(i) Purified water

(ii) Mineral nutrient salts

(iii) The nutrient solution must be adequateiy aerated to obtain the optimum growth.

Q. Can you explain why purified water and mineral nutrient salts are essential into hydroponics ?

Ans. Because any contamination or impurity can cause infection in plant which result in growth retardation.

Methodology :

In this technique roots of the plants were immersed in nutrient solutions and wherein an element was added/removed or given in varied concentration. a mineral solution suitable for the plant growth was obtained.

Significance and application :

(i) Essential elements were identified ..

(ii) Deficiency symptoms of essential elements were discovered.

(iii) Hydroponics has been successfully employed as a technique for the commercial production of vegetables such as tomato, seedless cucumber and lettuce.

Q. What would happen if solutions are poorly aerated ?

Ans. Plant will not show normal/proper growth.

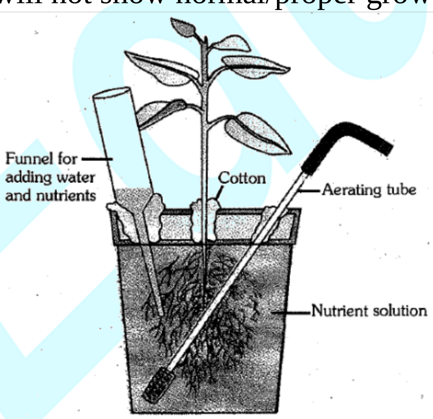
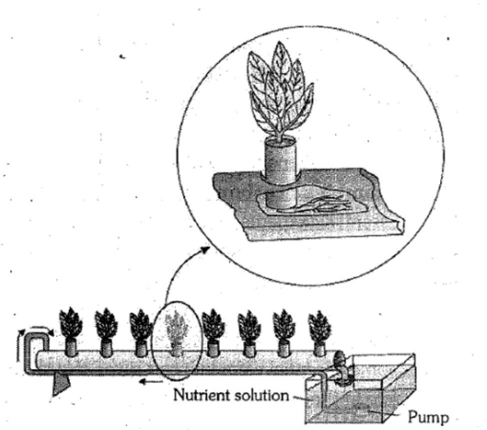


Diagram of a typical set-up for nutrient solution culture



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

Essential Mineral Elements

- Most of the minerals present in soil can enter plants through roots.
- In fact, more than sixty elements of the total discovered so far are found in different plants.
- Some plant species accumulate selenium (Astragalus), some others gold, while some plants growing near nuclear test sites take up radioactive strontium.
- There are techniques that are able to detect the minerals even at a very low concentration (10^{-8} g/mL).

Criteria for essentiality :The criteria for essentiality of an element are given below :

(a) The element must be absolutely necessary for supporting normal growth and reproduction. In the absence of the element the plants do not complete their life cycle or set the seeds.

(b) The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element can not be met by supplying some other element.

(c) The element must be directly involved in the metabolism of the plant.

Based upon the above criteria only 17 elements have been found to be absolutely essential for plant growth and metabolism.

These elements are further divided into two broad categories based on their guantitative reguirements.

(i) Macronutrients

(ii) Micronutrients

(i) Macronutrients :

- They are generally required in plants tissues in large amounts i.e. excess of 10 m mole kg^{-1} of dry matter.
- They are nine 1n number eg., carbon, hydrogen, oxygen, nitrogen, phosphorus, sulphur, potassium, calcium and magnesium.
- Out of these carbon, hydrogen and oxygen are mainly obtained from CO_2 and H_2O, while the others are absorbed from the soil as mineral nutrition.

(ii) Micronutrients or trace elements :

- They are needed in very small amounts i.e. less than 10 m mole Kg^{-1} of dry matter.
- They are eight in number. These include iron, manganese, copper, molybdenum, zinc, boron, chlorine and nickel.
- Fe is required in large amounts in comparision to other micronutrients, while Mo is required in minimum quantity.
- In addition to the 17 essential elements, there are some beneficial elements such as sodium (Atriplex), silicon (masses), cobalt (legumes) and selenium (Astragalus). They are required by higher plants.

  Essential elements can also be grouped into four broad categories on the basis of their diverse functions.

These categories are :

(i) Essential elements that are components of biomolecules and hence structural elements of cells.

Examples : carbon, hydrogen, oxygen and nitrogen.

(ii) Essential elements that are components of energy-related chemical compounds in plants

Examples : Magnesium in chlorophyll and phosphorus in ATP.

(iii) Essential elements that activate enzymes. for example Mg^{2+} is an activator for both RuBisCO and PEP case, both of which are critical enzymes in photosynthetic carbon fixation; Zn^{2+} is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism.

(iv) Some essential elements can alter the osmotic potential of a cell. Potassium plays an important role in the opening and closing of stomata.

Deficiency symptoms of essential elements :

- The concentration of the essential elements, below which plant growth is retarded, is termed as critical concentration.
- The essential element is said to be deficient when present below the critical concentration.

DEFICIENCY SYMPTOMS

(In the absence or deficiency of any essential element. plants show certain morphological changes. These are indications of certain element deficiency and are called deficiency symptoms. They vary from element to element)

On the basis of mobility of the element in the plant

Appear first in the older tissues (Mobile elements)

- If elements are actively mobilised within the plants and exported to young developing tissues.

  Examples : M, P, K, Mg and S

Appear first in the younger tissues (Immobile elements)

- If elements are relatively immobile and are not transported out of the mature organs because they are part of the structural component of the cell and hence are not easily released.

  Example : Calcium

Deficiency symptoms disappear when the deficient mineral nutrient is provided to the plant. However, if deprivation continues, it may eventually lead to the death of the plant.

Prevalent deficiency symptoms in plants :

(i) **Chlorosis** : It is the loss of chlorophyll leading to yellowing in leaves. This symptom is caused by the deficiency of elements N, K, Mg, S, Fe, Mn, Zn and Mo.

(ii) **Necrosis** : It is the death of tissue, particularly leaf tissue, is due to the deficiency of Ca, Mg, Cu and K.

(iii) **Inhibition of cell division** : Deficiency of N, K, S and Mo causes an inhibition of cell division.

(iv) **Delay in flowering** : Deficiency of N, Sand Mo causes delay in flowering.

Therefor, the deficiency of any element can cause multiple deficiency symptoms and that the same deficiency symptom may be caused by the deficiency of one of several different elements.

Special points

- C, H, 0, N, P and S are called protoplasmic elements as they are main constituent of organic part of protoplasm.
- C, H and 0 are called frame work elements as they are main constituent of proteins, carbohydrates and fats.
- N, P and K are called critical elements as they are required by plants in high doses. To fulfil this great demand soil mostly faces a shortage of such elements. Therefore, farmers frequently supply them in the form of fertilisers· to obtain optimum crop yield.
- A very low concentration of gold reported in Equisetum and mustard plant.

- About 98 percent of the mass of every living organism is composed of six elements including carbon, hydrogen, oxygen, nitrogen, calcium and phosphorus.

Role of macro and micro nutrients

Essential elements perform several functions. They participate in various metabolic processes in the plant cells such as permeability of cell membrane, maintenance of osmotic concentration of cell sap, electron transport systems, buffering action, enzymatic activity and act as major constituents of macromolecules and co-enzymes.

- Some physiological deficiency diseases
  (i) Whiptail of cauliflower : caused by deficiency of Mo
  (ii) Marsh spot of pea : caused by deficiency of Mn
  (iii) Grey or speck spot of oat : caused by deficiency of Mn
  (iv) Die back of Citrus : caused by deficiency of Cu
  (v) Reclamation disease of cereals and legume crops : caused by deficiency of Cu
  (vi) Khaira disease of paddy : caused by deficiency of Zn
  (vii) Brown heart rot of beats : caused by deficiency of B
  (viii) Stout axis : caused by deficiency of B

Various forms, physiological and deficiency symptoms of mineral elements are given in table :-

NITROGEN

ABSORPTION	PHYSIOLOGICAL ROLE
It is absorbed mainly as NO_3^- though some are also taken up as NO_2^- or NH_4^+	• Nitrogen is required by all parts of the plant particularly the meristematic tissues and metabolically active cells. • Nitrogen is one of the constituent of proteins, nucleic acids, vitamins, chlorophylls, enzymes amides and hormones.

PHOSPHORUS

ABSORPTION	PHYSIOLOGICAL ROLE
Either as $H_2PO_4^-$ or HPO_4^{-2}	• Phosphorus is a constituent of cell membranes, certain proteins, all nucleic acids and nucleotides • Required for all phosphorylation reactions. • It is a component of energy related chemical like ATP.

POTASSIUM

ABSORPTION	PHYSIOLOGICAL ROLE
K^+	• Potassium is required in more abundant quantities in the meristematic tissues, buds, leaves and root tips.

	• Potassium play key role in stomatal movement • It helps to maintain an anion-cation balance in cells and is involved in proteome synthesis. • It help in activation of enzymes and in the maintenance of turgidity of cells.

CALCIUM

ABSORPTION	PHYSIOLOGICAL ROLE
Ca^{++}	• Calcium is required by meristematic and differentiating tissues. • During cell division it is used in the synthesis of cell wall, particularly as Calcium pectate in the middle lamella. • It is also needed during the formation of mitotic spindle and chromosome synthesis. • It is involved in the normal functioning of the cell membrane (maintain permeability) and activates certain enzymes. • It plays an important role in regulating metabolic activities. • It accumulate in older leaves.

MAGNESIUM

ABSORPTION	PHYSIOLOGICAL ROLE
Mg^{++}	• It activates the enzymes of respiration (Hexokinase, phosphofuctokinase/PFKm pyruvate kinase etc.) and photosynthesis (RuBisCO, PEP case). • It is involved in the synthesis of DNA and RNA. • It is a constituent of the ring structure of chlorophyll. • It helps to maintain the ribosome structure.

SULPHUR

ABSORPTION	PHYSIOLOGICAL ROLE
SO_4^{-2}	• Sulphur is present in cysteine, methionine and cystine. • It is the main constituent of several co-

	enzymes, vitamins (Thiamine, biotin, Co-A) and ferredoxin.

IRON

ABSORPTION	PHYSIOLOGICAL ROLE
Fe^{+++} (Ferric ions)	• It is required in larger amounts in comparison to other micronutrients. • It is an important constituent of proteins involved in the transfer of electrons like Ferredoxin and Cytochromes. • It is reversibly oxidised from Fe^{++} to Fe^{+++} during electron transfer. • It activates catalase, perosidase and nitrogenise enzyme. • It is essential for synthesis of chlorophyll.

MANGANESE

ABSORPTION	PHYSIOLOGICAL ROLE
Manganous ions (Mn^{++})	• It activates many enzymes involved in photosynthesis, respiration and nitrogen metabolism. • The best defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis. (Transfer of e^{-} to PS-II from H_2O)

BORON

ABSORPTION	PHYSIOLOGICAL ROLE
BO_3^{-3} or $B_4O_7^{-2}$	• It is required for uptake and utilisation of calcium. • Play important role in carbohydrate translocation (Phloem transport). • It is also required for membrane functioning, pollen germination, cell elongation and cell differentiation. • It is the only micronutrient which is not associated with enzymes activation.

ZINC

ABSORPTION	PHYSIOLOGICAL ROLE
Zn^{++}	• It activates various enzymes (Carboxylases, carboxypeptidases,

	alcohol dehydrogenises) • It is also needed in the synthesis of auxin.

COPPER

ABSORPTION	PHYSIOLOGICAL ROLE
Cupric ions (Cu^{++})	• It is essential for the overall metabolism in plants. • It is associated with certain enzymes involved in redox reactions and is reversibly oxidised from Cu^{+} to Cu^{2+}. • It is essential for vitamin C (ascorbic acid) synthesis. • It is a constituent of plastocyanin and cytochrome a-a_3.

MOLYBDENUM

ABSORPTION	PHYSIOLOGICAL ROLE
Molybdate ions (MoO_2^{2+})	• It is a component of several enzymes, including nitrogenises and nitrate reductase.

CHLORINE

ABSORPTION	PHYSIOLOGICAL ROLE
Chloride ion (Cl^{-})	• Along with Na^{+} and K^{+}, it helps in determining the solute concentration and anion cation balance in cells. • It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.

NICKEL

ABSORPTION	PHYSIOLOGICAL ROLE
Ni^{2+}	• IT is the activator of enzyme urease (this enzyme is required to split urea in plants body to obtain nitrogen)

Toxicity of micronutrients

- The requirement of micronutrients is always in low amounts, there is a narrow range of concentration at which the micronutrients are optimum.
- Moderate decrease of micronutrient causes the deficiency symptoms and moderate increase causes toxicity.

- Any mineral ion concentration in tissues that reduces the dry weight of tissues by 10 percent is considered toxic.
- Toxic concentration is variable for the same micronutrient in different plants and also variable for different micronutrients in same plant.

Example : Mn toxicity followed by appearance of brown spots surrounded by chlorotic veins.
Impacts of Mn toxicity :
(i) Manganese competes with iron and magnesium for uptake.
(ii) Manganese competes with magnesium for binding with enzymes.
(iii) Manganese inhibits calcium translocation in shoot apex.

Conclusion: Excess of manganese may, infact, induces deficiencies of iron, magnesium and calcium. Thus, what appears as symptoms of manganese toxicity may actually be the deficiency symptoms of iron, magnesium and calcium.

Uptake and transport of mineral nutrients

Plants obtain their carbon and most of their oxygen from CO_2 in the atmosphere. However, their remaining nutritional requirements are obtained from minerals and water for hydrogen in the soil.

Uptake of mineral ions :

All minerals can not be passively absorbed by the roots. Two factors account for this :

(i) Minerals are present in the soil as charged particles (ions) which can not move passively across cell membranes.

(ii) The concentration of minerals in the soil is usually lower than the concentration of minerals in the root.

- Therefore, most minerals must enter the root by active absorption into the cytoplasm of epidermal cells. Although some ions also move into the epidermal cells passively.
- The active uptake of ions is partly responsible for the water potential gradient in roots and therefore for the uptake of water by osmosis.

Studies on mechanism of absorption of elements revealed that the process of absorption can be demarcated into two main phases :
(i) Initial phase (ii) Metabolic phase

Initial	Metabolic
• This phase of uptake involve an initial rapid uptake of ions into the outer free space (this space comprises cell wall and inter cellular spaces i.e. apoplast). • It is a passive process. • It is non selective and expenditure of ATP is not required.	• In this phase of uptake, the ions are taken in slowly into the inner space (space enclosed by plasma membrane i.e. simplest). • It is both passive and active. • Passive movement occurs through transmembrane proteins (ion channels) • Active movement occurs through protein pump and expenditure of ATP is required. • It is selective process.

The movement of ions is called flux: the inward movement into the cells is called influx and the outward movement is called efflux.

Methods of mineral absorption :

(I) Passive absorption (II) Active absorption

(I) Passive absorption :

It occurs without expenditure of ATP.

(a) By diffusion : According to this method minerals may diffuse into root cell from the soil solution.

(b) By mass flow : According to this method minerals move along with bulk flow of water under the high rate of transpiration.

(II) Active absorption :

In this method ions move from less concentrated soil solution to more concentrated cell sap. Such movement is against concentration gradient hence ATP as a driving force required.

- Specific proteins in the membranes of root hair cells actively pump ions from the soil into the cytoplasms of the epidermal cells.
- Like all cells, the endodermal cells have many transport proteins embedded in their plasma membrane; they let some solutes cross the membrane, but not others.
- Transport proteins of endodermal cells are control points, where a plant adjusts the quantity and types of solutes that reach the xylem. The root endodermis because of the layer of suberin has the ability to actively transport ions in one direction only.
- Active uptake of minerals depends upon dephosphorylation (ATP $\rightarrow$ ADP + iP)

Translocation of mineral ions :

- First of all ions have reached xylem through active or nassive untake. or a combination of the two.
- Further transport for the stem to all parts of the plant is through the transpiration stream.
- The chief sinks for the mineral elements are the growing regions of the plant such as the apical and lateral meristems, young leaves, developing flowers, fruits, seeds and the storage organs.
- Unlading of mineral ions occurs at the fine vein endings through diffusion and actively uptake by cells of sink.
- Mineral ions are frequently remobilised, particularly from older, senescing parts. Older dying leaves export much of their mineral content to younger leaves. Similarly, before leaf fall in deciduous plants, minerals are removed to other parts.
- Elements most readily mobilised are phosphorus, sulphur, nitrogen and potassium. Some elements that are structural components like calcium are not remobilised.

An analysis of the xylem exudates shows that though some of the nitrogen travels as inorganic ions, much of it is carried in the organic form as amino acids and related compounds. Similarly, small amounts of P and S are carried as organic compounds. In addition, small amount of exchange of materials does take place between xylem and phloem.

Conclusion : Hence it is not that we can clearly make a distinction and say categorically that xylem transports only inorganic nutrients while phloem transports only organic materials, as was traditionally believed.

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. Thus, soil play several roles :

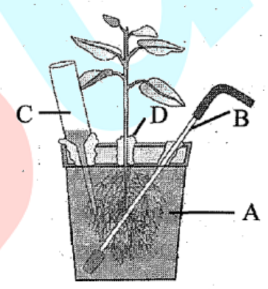
(i) Soil is reservoir of water for plants.
(ii) Soil supplies minerals and harbours nitrogen-fixing bacteria.
(iii) Soil supplies air to the roots.
(iv) Soil acts as a matrix that stabilises the plant.
(v) In terrestrial habitat soil is the site for decomposition (decomposition is a process to recycle minerals from detritus). 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.

BEGINNER'S BOX-1

1. Which of the following essential elements is required by plants less than 10m mole Kg^{-1} of dry matter?
(1) Manganese (2) Magnesium (3) Phosphorus (4) Potassium

2. Given below is the diagrammatic sketch of nutrient solution culture. Identify the parts labelled A, B, C and D and select the right option about them :
Options :-

	A	B	C	D
(1)	Funnel	Aerating tube	Nutrient solution	Cotton
(2)	Nutrient solution	Cotton	Aerating tube	Funnel
(3)	Nutrient solution	Aerating tube	Funnel	Cotton
(4)	Cotton	Funnel	Aerating tube	Nutrient solution



3. Except which of the following, all the other essential nutrients are absorbed in the form of minerals?
(1) C, H, O (2) N, P, K (3) S, Ca, Mg (4) Mn, Cl, Zn

4. Which of the following elements is involved in opening and closing of stomata and in maintenance of the turgidity of cells?
(1) Magnesium (2) Potassium (3) Sulphur (4) Iron

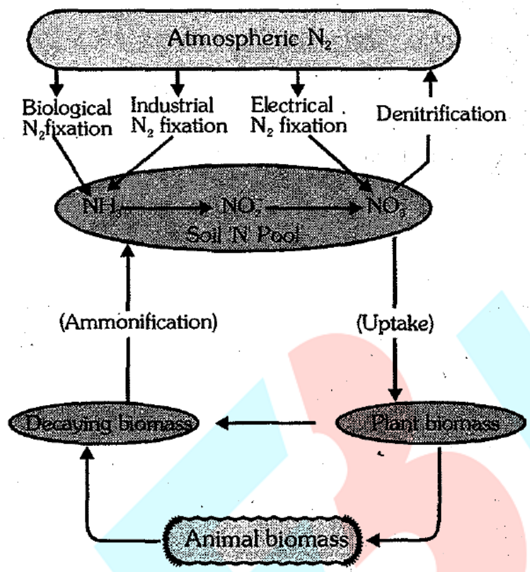
5. Which of the following micronutrients is an important constituent of proteins involved in the transfer of electrons and is required in larger amount in comparison to other micronutrients ?
(1) Iron (2) Molybdenum (3) Manganese (4) Zinc

METABOLISM OF NITROGEN

- Apart from carbon, hydrogen and oxygen, nitrogen is the most prevalent element in living organisms.
- Nitrogen is a constituent of amino acids, amides, nucleic acids, enzymes, proteins, hormones, chlorophylls and many of the vitamins.
- Nitrogen is a limiting nutrient for both natural and agricultural eco-systems because plants compete with microbes for the limited nitrogen that is available in soil.
- Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond ($N \equiv N$)

Constituent of nitrogen cycle :

(1)	N_2 fixation	$N_2 \rightarrow NH_3$	Reduction	Biological as well as a biological
(2)	Nitrification	$NH_3 \rightarrow NO_3^-$	Oxidation	Biological only
(3)	Ammonification	Protein $\rightarrow NH_3$	Hydrolysis	Biological only



The nitrogen cycle showing relationship between the **three main nitrogen pools - atmosphere, soil and biomass**

(1) **N_2 fixation :** It is of two types :

(I) Abiological (II) Biological

(I) **Abiological** : It is again of two types :

(i) Natural/Electrical (ii) Industrial Artificial.

(i) **Natural/Electrical** : In nature, lightning and ultraviolet radiations provide enough energy to convert nitrogen to nitrogen oxides.

Atmospheric N_2 + Atmospheric O_2 $\xrightarrow[\text{UV radiations and lightning}]{\text{Energy from}}$ Nitrogen oxides (NO, NO_2, N_2O)

- Industrial combustions, forest fires, automobile exhausts and power -generating stations are also sources of atmospheric nitrogen oxides.

Nitrogen oxides (NO, NO_2, N_2O) + Water vapours $\longrightarrow$ HNO_3, HNO_2, HNO

These acids of nitrogen enter into the soU along with rain and dissociate.

$HNO_3 \longrightarrow NO_3^- + H^+$; $HNO \longrightarrow NO^- + H^+$; $HNO_2 \rightarrow NO^- + H^+$

These NO^-_3, NO_2^- and NO^- are uptake by plants and utilised.

(ii) **Industrial/ Artificial :**

Haber method of NH_3 preparation :

$N_2 + 3H_2 \longrightarrow 2NH_3$

This ammonia is further incorporated into urea ($NH_2.CO.NH_2$). Urea used as a fertiliser by farmers.

(II) **Biological N_2 fixation/diazotrophy:**

- Only certain prokatyotic species are capable of fixing nitrogen. Reduction of nitrogen to ammonia by living organisms is called biological nitrogen fixation.

- The enzyme, nitrogenase which is capable of nitrogen reduction is present exclusively in prokaryotes. Such microbes are called N_2 -fixers or diazotrophs.

$$N \equiv N \xrightarrow{\text{Nitrogenase}} NH_3$$

(i) BY BACTERIA : They fix nitrogen in three different ways :

(a) Free living nitrogen fixing bacteria : They fix nitrogen in free living way.

Examples : Azotobacter, Beiiernickia, Rhodospirillum, Bacillus, Clostridium, Rhodopseudomenas etc.

(b) Symbiotic nitrogen fixing bacteria :

Several types of symbiotic biological nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. The most common association on root is as nodules. These nodules are small outgrowths on root.

Bacteria	Type of host
Rhizobium	Roots of several legumes such as alfalfa, sweet clover. Sweet pea, Lentils, garden pea, broad bean. clover beans etc.
Frankia	Roots of Alnus, Casuarina (non: legumes)
Azorhizobium	Stem nodules of Sesbania
Bradyrhizobium and Sinorhizobium	Root nodules of soya bean

Both Rhizobium (rod shaped) and Frankia (filamentous) are heterotrophic, free living forms in soil, but as symbionts, can fix atmospheric nitrogen.

(c) By loose symbiotic bacteria :

This is symbiosis without nodule formation e.g., Azospirillum with maize root.

(ii) BY CYANOBACTERIA OR BLUE GREEN ALGAE : They fix nitrogen inside specialised cells called, heterocyst. BGA fix nitrogen by two ways :

(a) Free living N2 fixing BGA : They fix nitrogen without help of host. e.g., Nostoc. Anabaena, Oscillatoria, Aulosira, Cylindrospermum etc: Oscillatoria and Aulosira are active nitrogen fixer in paddy field and Cylindrospermum is an active nitrogen fixer in sugarcane and maize field.

(b) Symbiotic N_2 fixing BGA : They fix nitrogen with symbiotic association. BGA make symbiotic association almost with every group of plants in plantae kingdom.

BGA	Type of host
Anabaena anthocerotae	Associate with thallus of Anthoceros (bryophyte)
Anabaena azollae	Associate with frond (compound leaf) of Azolla pinnata (pteridophyte, a fern)
Anabaena cycacfae	Associate with coralloid roots of Cycas
Nostoc species	Associate with Gunner (angiosperm) stem and Trifolium (angiosperm) root.

BIOLOGICAL NITROGEN FIXATION

Nodule formation :

- This is a necessity during nitrogen fixation by bacterial symbiosis only.
- Nodule formation involves a sequence of multiple interactions between Rhizobium and roots of the host plant.

  Principle stages in the nodule formation are summarised as follows :

Stage-1 : Rhizobium contact a susceptible root hair. Rhizobia multiply and colonise the surroundings of roots, and get attached to epidermal and root hair cells.

- This attraction is chemotclctic (rhicadhesin protein of bacterial cell identify host root). To attract rhizobia root of legume releases amino acid, sugars, organic acids and flavonoids.

Stage-II : Bacteria invade the root hair (infection). Successful ~nfection of root hair causes it to curl.

- Curling of root hair is induced by specific complex polysaccharides found on the surface of Rhizobia, recognised by lectins (small proteins of host plant root.)

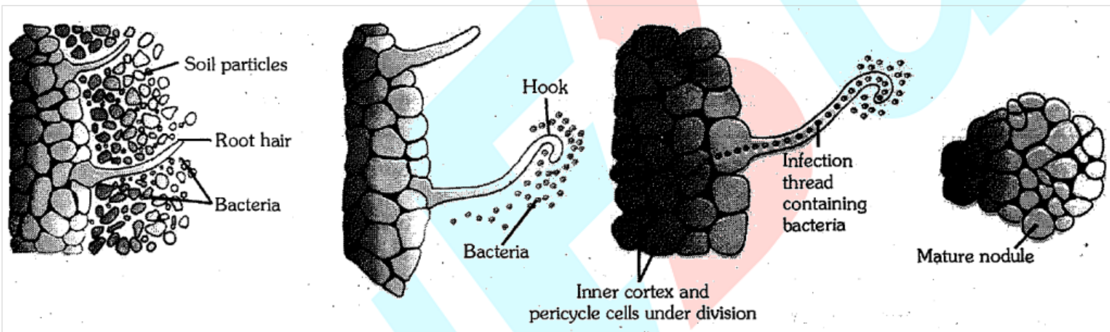
Stage-III : An infection thread is produced carrying the bacteria into the inner cortex of the root.

Then bacteria are released frorn the thread into the cells. The bacteria get modified into rod shaped bacteroids and cause inner cortical and pericycle cells to divide.

For dedifferentiation of cortical and pericycle cell PGRs (auxin and cytokinin) are required. Auxin is provided by host root while cytokinin by bacteria.

- Division and growth of cortical and pericycle cells lead to nodule formation.

Stage-IV : The mature nodule is formed and establishes a direct vascular connection with the host for exchange of nutrients.



Different stages of nodule formation

Mechanism of biological nitrogen fixation

Requirements for biological nitrogen fixation :

(i) Substrate - nitrogen

(ii) An enzyme- nitrogenise

The enzyme nitrogenise is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia.

This enzyme is activated by both molybdenum and iron (mainly by molybdenum).

(iii) Source of energy (ATP)

  (a) During free living nitrogen fixation, prokaryote itself is the source of ATP.

  (b) During symbiosis ATP is obtained from the respiration of the host cells.

(iv) Source of reduction power (NADH)

  (a) During free living N_2 fixation prokaryote itself is the source of NADH.

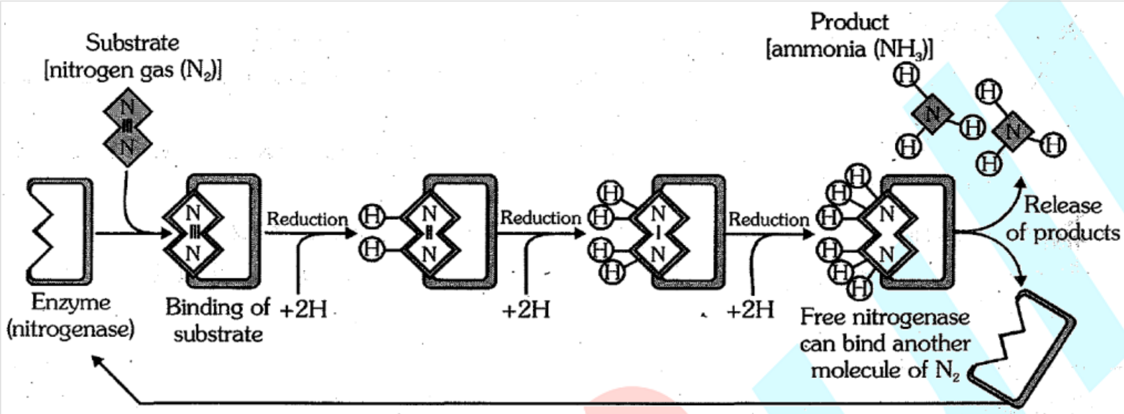
  (b) During symbiosis NADH provided by the respiration of host cells.

(v) Oxygen free environment.

- The enzyme nitrogenise is highly sensitive to the molecular oxygen thus its operation/activation requires anaerobic conditions. To protect this Erozyme, the nodule contains an oxygen scavenger called leg-haemoglobin.

- Leg-haemoglobin (holoprotein) is a joint product of both plant and the bacterium in which the globin (apoprotein) is produced by the plant (legume) and the heme (an iron atom bound in a porphyrin ring) is produced by the bacterium (Rhizobium).

(vi) Genes (nod, nif, fix) : Nod gene present in both plant and bacterium while nif (nitrogenise inducing factor) and fix present only in bacterium.



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

- The mechanism of nitrogen fixation is summarised by following reaction :
  $N_2 + 8e^- + 8H^+ + 16ATP \xrightarrow{\text{Nitrogenise}} 2NH_3 + H_2 + 16ADP + 16\ Pi$
- In above reaction the ammonia synthesis by nitrogenise requires a very high input of energy (8 ATP for each NH_3 produced).

Fate of ammonia :

At physiological pH, the ammonia is protoriated to form NH_4^+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions. Ammonium ions are quite toxic to plants and hence cannot accumulate in them. Let us now see how the NH_4^+ is used to synthesise amino acids in plants.

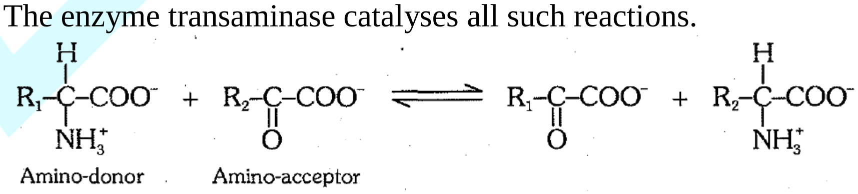
There are two main ways in which this can take place:

(i) **Reductive amination** : In this process, ammonium ion reacts with a.-ketoglutaric acid and forms glutamic acid.

α - Ketoglutaric acid + NH_4^+ + NADPH $\xrightleftharpoons{\text{Glutamate dehydrogenise}}$ Glutamate + H_2O + NADP

(ii) **Transamination** : It involves the transfer of amino group from one amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH_2 (amino group) takes place and other amino acids are formed through transamination.

The enzyme transaminase catalyses all such reactions.



Glutamic acid + Oxalo acetic acid $\longrightarrow$ α-Ketoglutaric acid + Aspartic acid

Glutamic acid + Pyruvic acid $\longrightarrow$ α-Ketoglutaric acid + Alanine

Amides : Amides are double aminated keto acids. The two most important amides -asparagine and glutamine found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid, respectively, by addition of another amino group to each.

The hydroxyl part of the acid is replaced by another NH_2^- radicle. Since amides contain more nitrogen than the amino acids. they are transported to other parts of the plant via xylem vessels. In addition, along with the transpiration stream the nodules of some plants (e.g., soyabean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

(2) Ammonification : Decomposition of organic nitrogen of dead plants and animals into ammonia is called ammonification. It is carried by biological agencies like Bacillus ramous, Bacillus mycoides etc.

Protein (detritus) = $\xrightarrow{\text{Proteases}}$ Amino acids $\longrightarrow NH_3$ + organic acids

$NH_3 \longrightarrow NH_4^+$

- This breakdown is both anaerobic as well as aerobic. Anaerobic breakdown of protein is called putrefaction while aerobic breakdown is called decay.
- Some of this ammonia volatilises and re-enters the atmosphere but most of it is converted into nitrates by soil bacteria. (Nitrification)

(3) Nitrification :

$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H^+ + 2H_2O$ + Energy

$2NO_2^- + O_2 \longrightarrow 2NO_3^-$ + Energy

Ammonia is first oxidised to nitrite by the bacteria Nitrosomonas and/or Nitrococcus. The nitrite is further oxidised to nitrate with the help of the bacterium Nitrobacter. These steps are called nitrification and these nitrifying bacteria are chemoautotroph's.

Nitrogen is absorbed mainly as NO_3^-. Though some is also taken up as NO_2^- or NH_4^+. After uptake plants assimilate these nitrates.

- This is a reductive process.

$NO_3^- \xrightarrow{\text{Nitrate reductase (cytoplasm)}} NO_2^-$

$NO_2^- \xrightarrow{\text{Nitrate reductase (plastid)}} NH_4^+$

(4) Denitrification :

Nitrate present in the soil is also reduced to nitrogen. This process is called denitrification. Denitrification is carried by bacteria (Pseudomonas. Thiobacillus) and high energy radiations.

BEGINNER'S BOX-2

1. In root nodules of legumes, leg haemoglobin is important because it :-
(1) acts as a catalyst in transamination
(2) transports oxygen to the root nodule
(3) provide energy to the nitrogen fixing bacteria
(4) acts as an oxygen scavenger

2. Which of the following processes are responsible for the release of nitrate (NO_3^-) in soil during nitrogen cycle?
(1) Biological nitrogen fixation and Denitrification
(2) Nitrification and Industrial nitrogen fixation
(3) Electrical nitrogen fixation and nitrification
(4) Ammonification and Denitrification

3. In biological nitrogen fixation, the ammonia synthesis by nitrogenase enzyme requires a very high input of energy and that is :-
(1) 16 ATP for each NH_3 produced (2) 32 ATP for each NH_3 produced
(3) 8 ATP for each NH_3 produced (4) 4 ATP for each NH_3 produced

4. $2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O$
During nitrogen cycle, above process is performed by which of the following microbes?
(1) Nitrosomonas or Nitrobacter (2) Pseudomonas and Thiobaciilus
(3) Nitrococcus or Nitrosomonas (4) Rhizobium and Frankia

5. Which of the following is a free living aerobic nitrogen fixing microbe ?
(1) Pseudomonas (2) Azotobacter (3) Rhodospirillum (4) Thiobacillus

ANSWER KEY

BEGINNER'S BOX-1

1.	(1)	**2.**	(3)	**3.**	(1)	**4.**	(2)	**5.**	(1)

BEGINNER'S BOX-2

1.	(4)	**2.**	(3)	**3.**	(3)	**4.**	(3)	**5.**	(2)