Plant Physiology (Mineral Nutrition) - Part 2

Objectives

After going through this lesson, the learners will be able to understand the following:

- Describe the mechanism of absorption of elements in plants.
- Delineate the process of translocation of solutes in plants.
- Discuss the role of soil as a reservoir of essential elements in plants.
- Explain the process of Nitrogen metabolism in plants.

Content Outline

- Mechanism of Absorption of Elements
- Translocation of Solutes
- Soil as Reservoir of Essential Elements
- Metabolism of Nitrogen
- Summary

Mechanism of Absorption of Elements

Much of the studies on mechanism of absorption of elements by plants has been carried out in isolated cells, tissues or organs. These studies revealed that the process of absorption can be demarcated into two main phases. In the first phase, an initial rapid uptake of ions into the 'free space' or 'outer space' of cells – the apoplast, is passive. In the second phase of uptake, the ions are taken in slowly into the 'inner space' – the symplast of the cells. The passive movement of ions into the apoplast usually occurs through ion-channels, the transmembrane proteins that function as selective pores. On the other hand, the entry or exit of ions to and from the symplast requires the expenditure of metabolic energy, which is an active process. The movement of ions is usually called flux; the inward movement into the cells is influx and the outward movement, efflux. We have read the aspects of mineral nutrient uptake and translocation in plants in the earlier topics.

Point to Ponder

What is apoplast?

Inside a plant, the apoplast is the space outside the plasma membrane within which material can diffuse freely. It is interrupted by the Casparian strip in roots, by air spaces between plant cells and by the plant cuticle. Structurally, the apoplast is formed by the continuum of cell walls of adjacent cells as well as the extracellular spaces, forming a tissue level compartment comparable to the symplast. The apoplastic route facilitates the transport of water and solutes across a tissue or organ. This process is known as *apoplastic transport*. The apoplast is important for all the plant's interaction with its environment.

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.

Point to Ponder

What is a transpiration pull?

A transpiration pull is defined as a biological process in which the force of pulling is produced inside the xylem tissue. This force helps in the upward movement of water into the xylem vessels. In this process, loss of water in the form of vapours through leaves are observed. This biological process is carried out in all higher plants and trees as their stems are surrounded by bundles of fine tubes, which are made from a woody material known as xylem. Transpiration pull is also referred as suction force and this force is used to draw the water in an upward direction from the roots to the leaves. The amount of water received by the leaves are used for the photosynthesis and the excess amount of water is released into the atmosphere in the form of vapours through the openings in the leaves known as stomata.

Use of radioisotopes of mineral elements also substantiate the view that they are transported through the xylem. We have already discussed the movement of water in xylem in the earlier topics.

Point to Ponder

What are radioisotopes?

Radioisotopes are radioactive isotopes of an element. They can also be defined as atoms that contain an unstable combination of neutrons and protons, or excess energy in their nucleus. The unstable nucleus of a radioisotope can occur naturally, or as a result of artificially altering the atom.

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.

Point to Ponder

What are fertilizers?

Fertilisers are any materials of natural or synthetic origin other than liming materials that are applied to soils or to plant tissues to supply one or more plant nutrients essential to the growth of plants. Many sources of fertilisers exist, both natural and industrially produced. Fertilizers enhance the growth of plants. The nutrients required for healthy plant life are classified according to the elements, but the elements are not used as fertilizers. Instead compounds containing these elements are the basis of fertilizers. Fertilizers are commonly used for growing all crops, with application rates depending on the soil fertility, usually as measured by a soil test and according to the particular crop. Legumes, for example, fix nitrogen from the atmosphere and generally do not require nitrogen fertilizer. Fertilizers are applied to crops both as solids and as liquid. About 90% of fertilizers are applied as solids.

Metabolism of Nitrogen

I. Nitrogen Cycle

Apart from carbon, hydrogen and oxygen, nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls and many of the vitamins. Plants compete with microbes for the limited nitrogen that is available in soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural ecosystems. Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond. The process of conversion of nitrogen (N_2) to ammonia is termed as nitrogen fixation. In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO, NO_2, N_2O). Industrial combustions, forest fires, automobile exhausts and power-generating stations are also sources of atmospheric nitrogen oxides. Decomposition of organic nitrogen of dead plants and animals into ammonia is called ammonification.

Point to Ponder

What is ammonification, and when does it happen?

When a plant or animal dies or an animal expels waste, the initial form of nitrogen is organic. Bacteria or fungi convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization.

Some of this ammonia volatilises and re-enters the atmosphere, but most of it is converted into nitrate by soil bacteria in the following steps:

$$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H^+ + 2H_2O \quad \text{.... (I)}$$

$$2NO_2^- + O_2 \longrightarrow 2NO_3^- \quad \text{...... (II)}$$

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 (Figure 1). These nitrifying bacteria are chemoautotrophs.

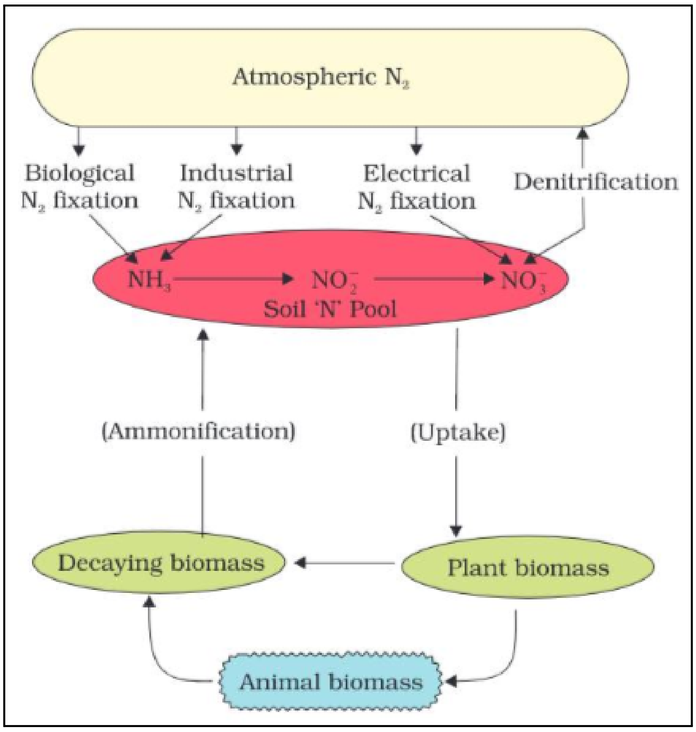


Figure 1: The nitrogen cycle showing relationship between the three main nitrogen pools – atmospheric soil, and biomass

Point to Ponder

What are Chemoautotrophs?

Chemoautotrophs derive energy from chemical reactions, synthesizing all necessary organic compounds from carbon dioxide. Chemoautotrophs use inorganic energy sources such as hydrogen sulphide, elemental sulphur, ferrous iron, molecular hydrogen, and ammonia. Most chemoautotrophs are extremophiles, bacteria or archaea that live in hostile environments (such as deep sea vents) and are the primary producers in such ecosystems. Chemoautotrophs generally fall into several groups: methanogens, halophiles, sulfur oxidizers and reducers, nitrifiers, anammox bacteria, and thermoacidophiles. An example of one of these prokaryotes is *Sulfolobus*.

The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of denitrification. The denitrification is carried by bacteria *Pseudomonas* and *Thiobacillus*.

II. Biological Nitrogen Fixation

Very few living organisms can utilise the nitrogen in the form N_2, available abundantly in the air. Only certain prokaryotic 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.

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

The nitrogen-fixing microbes could be free-living or symbiotic. Examples of free-living nitrogen-fixing aerobic microbes are *Azotobacter* and *Beijerinckia* while *Rhodospirillum* is anaerobic and Bacillus free-living. In addition, a number of cyanobacteria such as *Anabaena* and *Nostoc* are also free-living nitrogen-fixers.

Point to Ponder

What is symbiosis?

Symbiosis is a biological relationship in which two species live in close proximity to each other and interact regularly in such a way as to benefit one or both of the organisms. When both partners benefit, this variety of symbiosis is known as *mutualism.* The name for a situation in which only one of the partner's benefits is far more well known. Such an arrangement is known as *parasitism,* and a parasite is an organism that obtains nourishment or other life support from a host, usually without killing it. By their very nature, parasites are never beneficial, and sometimes they can be downright deadly. In addition to the extremes of mutualism and parasitism, there is a third variety of symbiosis, called *commensalism.* As with parasitism, in a relationship characterized by commensalism only one of the two organisms or species derives benefit, but in this case it manages to do so without causing harm to the host.

a) Symbiotic biological nitrogen fixation

Several types of symbiotic biological nitrogen fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* has such a relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, *Frankia*, also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., *Alnus*). Both *Rhizobium* and *Frankia* are free living in soil, but as symbionts, can fix atmospheric nitrogen. Uproot any one plant of a common pulse, just before flowering. You will see near-spherical outgrowths on the roots. These are nodules. If you cut through them you will notice that the central portion is red or pink. What makes the nodules pink? This is due to the presence of leguminous haemoglobin or leghaemoglobin.

Point to Ponder

What is leghaemoglobin?

Leghaemoglobin (also leghemoglobin or legoglobin) is an oxygen carrier and hemoprotein found in the nitrogen-fixing root nodules of leguminous plants. It is produced by legumes in response to the roots being colonized by nitrogen-fixing bacteria, termed rhizobia, as part of the symbiotic interaction between plant and bacterium: roots not colonized by *Rhizobium* do not synthesise leghemoglobin. The Leghemoglobin has close chemical and structural similarities to hemoglobin, and, like hemoglobin, is red in colour. The holoprotein (protein + heme cofactor) is widely believed to be a product of both plant and the bacterium in which the apoprotein is produced by the plant and the heme (an iron atom bound in a porphyrin ring) is produced by the bacterium.

b) Nodule Formation

Nodule formation involves a sequence of multiple interactions between Rhizobium and roots of the host plant. Principal stages in the nodule formation are summarised as follows:

Rhizobia multiply and colonise the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair. An infection thread is produced carrying the bacteria into the cortex of the root, where they initiate the nodule formation in the cortex of the root. Then the bacteria are released from the thread into the cells which leads to the differentiation of specialised nitrogen fixing cells. The nodule thus formed, establishes a direct vascular connection with the host for exchange of nutrients. The events of development of root nodules are depicted in Figure 2.

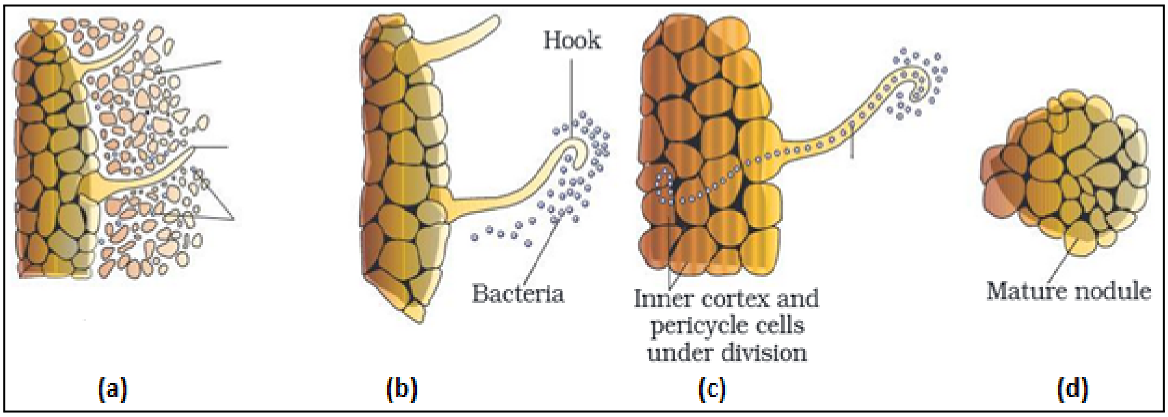


Figure 2: Development of root nodules in soybean:

(a) Rhizobium bacteria contact a susceptible root hair, divide near it,

(b) Successful infection of the root hair causes it to curl,

(c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroides and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation,

(d) A mature nodule is complete with vascular tissues continuous with those of the root

The nodule contains all the necessary biochemical components, such as the enzyme nitrogenase and leghaemoglobin. The enzyme nitrogenase is a Mo-Fe protein and catalyses the conversion of atmospheric nitrogen to ammonia, (Figure 3) the first stable product of nitrogen fixation.

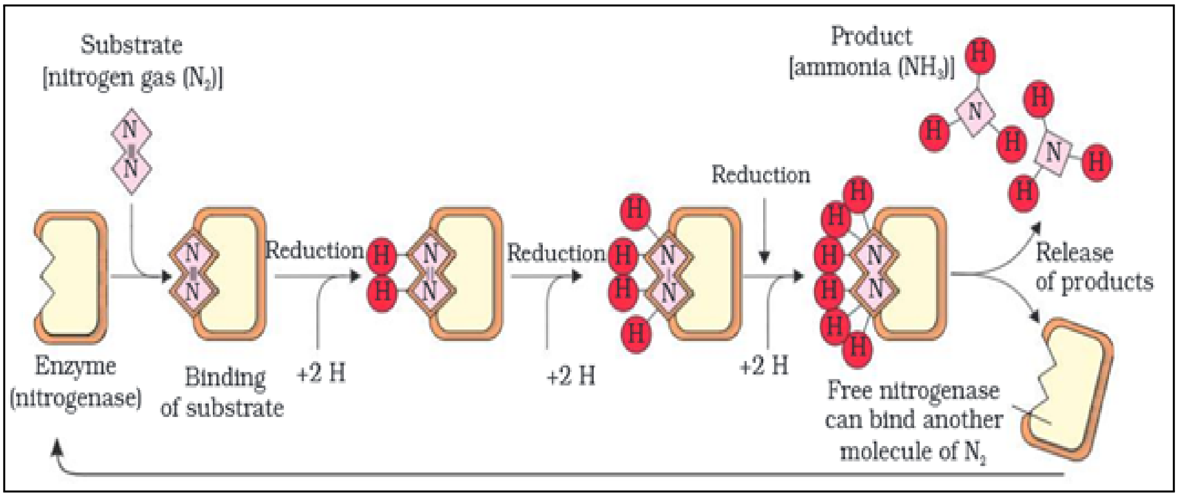


Figure 3: Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixation bacteria.

The reaction involved is as follows:

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

c) Types of Nodules

Two main types of root nodule have been described: determinate and indeterminate.

i. **Determinate nodules** are found on certain tribes of tropical legume such as those of the genera *Glycine* (soybean), *Phaseolus* (common bean), and *Vigna* and on some temperate legumes such as Lotus. These determinate nodules lose meristematic activity shortly after initiation, thus growth is due to cell expansion resulting in mature nodules which are spherical in shape. Another type of determinate nodule is found in a wide range of herbs, shrubs and trees, such as Arachis (peanut). These are always associated with the axils of lateral or adventitious roots and are formed following infection via cracks where these roots emerge and not using root hairs. Their internal structure is quite different from those of the soybean type of nodule.

ii. **Indeterminate nodules** are found in the majority of legumes from all three subfamilies, whether in temperate regions or in the tropics. They can be seen in papilionoid legumes such as *Pisum* (pea), *Medicago* (alfalfa), *Trifolium* (clover), and *Vicia* (vetch) and all mimosoid legumes such as acacias (mimosas), the few nodulated caesalpinioid legumes such as partridge pea. They earned the name "indeterminate" because they maintain an active apical meristem that produces new cells for growth over the life of the nodule. This results in the nodule having a generally cylindrical shape, which may be extensively branched. Because they are actively growing, indeterminate nodules manifest zones (Figure 4) which demarcate different stages of development/ symbiosis:

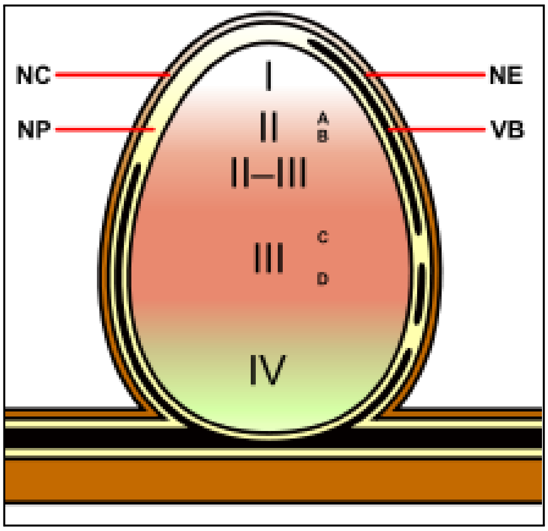


Figure 4: Different zones of an indeterminate root nodule

I - meristem

II - infection zone

A - invasion zone

B - pre-fixing zone

II–III - intermediate zone between the infection and nitrogen fixation zones

III - nitrogen fixation zone

C - efficient zone

D - inefficient zone

IV - senescent zone

The image also demonstrates some of the cellular architecture of the nodule:

NC - nodule cortex

NE - nodule endodermis

NP - nodule parenchyma

VB - vascular bundle

Source: https://en.wikipedia.org/wiki/Root_nodule#/media/File:Indeterminate_Nodule_Zones_Diagram.svg

Zone I - the **active meristem**. This is where new nodule tissue is formed which will later differentiate into the other zones of the nodule.

Zone II - the **infection zone**. This zone is permeated with infection threads full of bacteria. The plant cells are larger than in the previous zone and cell division is halted.

Interzone II–III - Here the bacteria have entered the plant cells, which contain amyloplasts. They elongate and begin terminally differentiating into symbiotic, nitrogen-fixing bacteroids.

Zone III - the **nitrogen fixation zone**. Each cell in this zone contains a large, central vacuole and the cytoplasm is filled with fully differentiated bacteroids which are actively fixing nitrogen. The plant provides these cells with leghemoglobin, resulting in a distinct pink color.

Zone IV - the **senescent zone**. Here plant cells and their bacteroid contents are being degraded. The breakdown of the heme component of leghemoglobin results in a visible greening at the base of the nodule. This is the most widely studied type of nodule, but the details are quite different in nodules of peanut and relatives and some other important crops such as lupins where the nodule is formed following direct infection of rhizobia through the epidermis and where infection threads are never formed. Nodules grow around the root, forming a collar-like structure. In these nodules and in the peanut type the central infected tissue is uniform, lacking the uninfected cells seen in nodules of soybean and many indeterminate types such as peas and clovers.

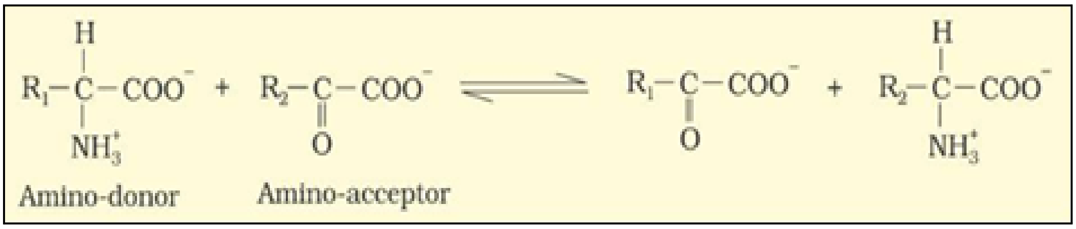
The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contains an oxygen scavenger called leghaemoglobin. It is interesting to note that these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they become anaerobic (thus protecting the nitrogenase enzyme). You must have noticed in the above reaction that the ammonia synthesis by nitrogenase requires a very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.

Fate of ammonia: At physiological pH, the ammonia is protonated to form NH_4^+ (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the latter is 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 synthesis of amino acids can take place:

I. **Reductive amination:** In these processes, ammonia reacts with a-ketoglutaric acid and forms glutamic acid as indicated in the equation given below:

$$\alpha - \text{ketoglutaric acid} + NH_4^+ + NADPH \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{glutamate} + H_2O + NADP$$

II. **Transamination:** It involves the transfer of an 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, the amino group takes place and other amino acids are formed through transamination. The enzyme transaminase catalyses all such reactions. For example,



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-} radical. 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., soybean) export the fixed nitrogen as ureides. These compounds also have a particularly high nitrogen to carbon ratio.

Summary

Plants absorb minerals through roots by either passive or active processes. They are carried to all parts of the organism through xylem along with water transport. Nitrogen is very essential for the sustenance of life. Plants cannot use atmospheric nitrogen directly. But some of the plants in association with N_2^- fixing bacteria, especially roots of legumes, can fix this atmospheric nitrogen into biologically usable forms. Nitrogen fixation requires a strong reducing agent and energy in the form of ATP. N_2^- fixation is accomplished with the help of nitrogen fixing microbes, mainly *Rhizobium*. The enzyme nitrogenase which plays an important role in biological N_2 fixation is very sensitive to oxygen. Most of the processes take place in anaerobic environment. The energy, ATP, required is provided by the respiration of the host cells. Ammonia produced following N_2 fixation is incorporated into amino acids as the amino group.