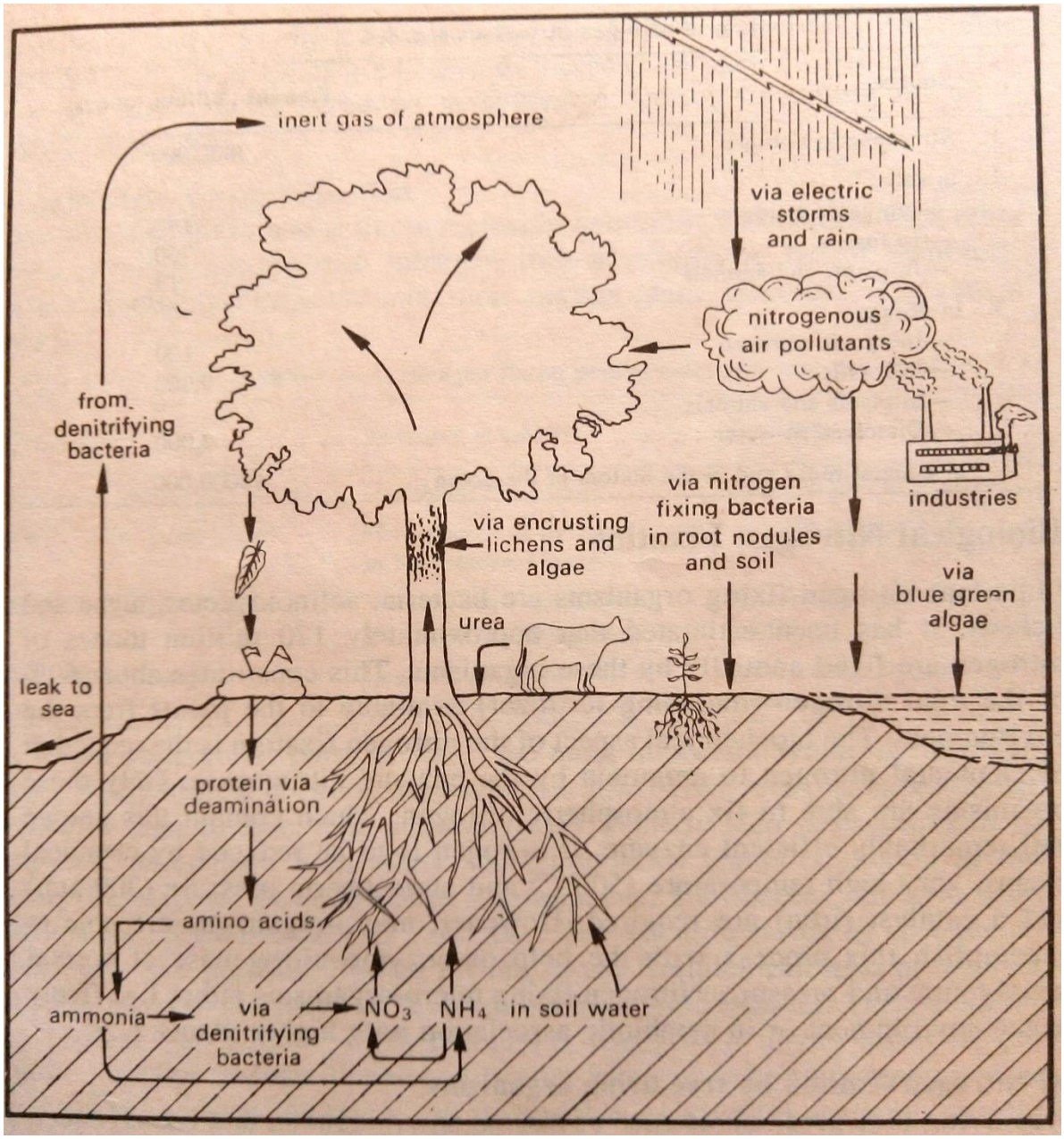
Subject: Genetics and Plant Breeding CBCS Pattern Practical Examination

M.Sc (Ag.) II Semester Course: Open Elective (Plant Physiology)

Nitrogen metabolism: Inorganic nitrogen species (N_2, NO_3 and NH_3) and their reduction to amino acids, protein synthesis and nucleic acids, nitrogen cycle.

Nitrogen Cycle

Nitrogen present in plants and animals returns to the soil, water and atmosphere for the use of other organisms by various interrelated processes.
The process called 'nitrogen cycle' is outlined in figure 8.1. The element nitrogen in the gaseous form constitutes about 78% of the atmosphere.



Although higher plants can absorb this nitrogen through open stomata, they are unable to assimilate it and subsequently the gas re-enters the atmosphere by diffusion. But certain micro-organisms living either freely in the soil and water or in symbiotic association with higher plants are able to reduce this nitrogen to ammonia, a process called biological nitrogen fixation. The 'fixed' nitrogen can be taken in and assimilated by the plants. Alternatively, the nitrifying

bacteria present in the soil may convert ammonia into nitrate; a procèss called nitrification. Plants are able to absorb and assimilate nitrate form of nitrogen, Still there are other types of bacteria, called denitrifying bacteria, which convert nitrate into nitrite and elemental nitrogen which scapes out to the atmosphere. This process is called denitrification. In most soils, about 60% of the nitrogen applied as ammonia or nitrate fertilizer escapes to the atmosphere in 15 to 20 days. The process is accelerated in water logged and non-porous soils.

Nitrogen present in plants is mostly in the form of complex molecules in the animal world is also temporary and most of it is lost again to the environment in the form of urea and ammonia as excretory products of the animals. Further, after the death of living organisms, the organic molecules disintegrate and most of nitrogenis converted to ammonia by the process called ammonification. This process is also mediated by micro-such as amino acids, proteins, nucleic acids, chlorophylls, alkaloids etc. Animals receive nitrogen in these forms when they consume plants as food. Carnivorous animals receive nitrogen by eating smaller animals.

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Table 8.1. **Distribution of nitrogen in various sources.**

Source	Amount (Billion tonnes)
1. Atmospheric nitrogen	3800,000
2. In soil	
—Inorganic nitrogen	140
—In fossils	760
—In plants and animals	13
3. In oceans	
—Inorganic nitrogen	100
—In fossils	9,000
—In plants and animals	1
—Dissolved in water	2,000
4. In mineral rocks and in the bottom of the ocean	18000,000

Ammonia thus produced, re-enters the nitrogen cycle either through the soil or through the air. Approximate amount of nitrogen present in various sources is shown in table 8.1.

A small percentage of nitrogen in a form of nitrogen oxides (NO and NO_2,) and ammonia is released and is present in the atmosphere as a result of various industrial activities. These gases act as pollutants and their quantity is bound to inçrease in the atmosphere, as a result of increasing human activities and industrialization. Plants, soil and to some extent water reservoirs act as sinks for these gases.

Biological Nitrogen Fixation

Important nitrogen fixing organisms are bacteria, actinomycetes, algae and lichens. It has been estimated that approximately 170 million tonnes of nitrogen are fixed annually by these organisms. This constitutes about 69% of the total nitrogen (including fertilizer) available to the plants from the environment. The biochemical aspect of the nitrogen fixation is the reduction of elemental nitrogen to ammonia by the enzyme nitrogenase. Only those organisms are able to fix atmospheric nitrogen, which contain this unique and remarkably efficient enzyme. If nitrogen is to be reduced by chemical means, V very high. temperature (500C) and atmospheric pressure (300 atm) and a catalyst (iron) are required. However, micro-organisms are able to accomplish this process with the help of enzyme nitrogenase at normal temperature and pressure. Nitrogen fixing micro-organisms either live freely in the environment or in symbiotic association with higher plants.

[I] Nitrogen fixation by free living organisms

Free living blue green algae such as Nostoc and Anabaena and bacteria such as Chromatium, Chlorobium, Rhodospirillum etc. convert nitrogen to ammonia and eventually to organic nitrogenous compounds, with the help of nitrogenase enzyme. While most of these organisms derive energy for nitrogen reduction from photosynthetic reactions in light, some bacteria such as Azotobacter and Clostridium are able to fix nitrogen in darkness. These organisms derive energy for nitrogen fixation from the decomposition of several inorganic and organic compounds present in the soil.

The free living bacteria normally do not fix nitrogen, if nitrate, ammonia or other forms of combined nitrogen are present in soil. Moreover the fixed nitrogen (i.e. ammonia) is assimilated as fast as it is formed by these bacteria and it is available to the higher plants only after the death and decay of bacteria. Thus, the contribution of free living bacteria to the soil is rather negligible. However, the nitrogen fixing activity of some free living algae, seems to be useful for higher plants growing in the vicinity. Culture, of blue sgrecn algae in paddy fields are known to increase nitrogen availability to the crop, and it has been practiced in several countries (including India) to increase crop yield.

.II] Symbiotic nitrogen fixation

Some bacteria, algae and actinomycetes, in symbiotic association with other plants, convert atmospheric nitrogen into ammonia.

A list of of symbiotic ofnitrogen fixing prokaryotcs and thcir higher plant symbiont is given inn table 8.2.

Table 8.2. **Symbioses between nitrogen fixing prokaryotes and vascular plants.**

Prokaryote	Vascular symbiont	Location
Anabaena (blue green algae)	Pteridophytes *Azolla*	Intercellular, in pockets
Nostoc (blue green algae)	Most genera of cycadales and in angiosperm *Gunnera* sp.	Inter or Intracellular
Frankia (Actinomycetes)	Various non-leguminous genera of angiosperms	Intracellular in root nodules
Rhizobium (bacteria)	Leguminous plants and in *Parasponia* of ulmaceae family	Intracellular in root nodules

From agricultural point of view, the most important symbiotic nitrogen fixing association is between the bacterium Rhizobium and plants belonging to family leguminosae. The actinomycetes Frankia also fixes nitrogen in symbiotic association with many non-leguminous plants belonging to the family Betulaceae, Casuarinaceae, Coriariaceae, Datiscaceae, Eleagnaceae, Myricaceae, Rhamnaceae and Rosaceae. The bacteria reside in specialized structures-the nodules, formed on the roots. Nodules can also be formed on aerial parts of the stems as in Aeschenomine and Sesbania. These nodules are profuse particularly when the plants are are grown under water-logged conditions. The nodules developed on the roots of leguminous plants as a result of Rhizobium infection, are of the size of mustard seeds or or slightly bigger and are brown, red or pink in colour. There may be hundreds of of nodules on the roots of a herbaceous plant (Fig. 8.2). Before the symbiotic association between the bacteria and the plant is able to fix nitrogen, the nodules have to develop and the bacteria are to develop into bacteroids.

Formation of root nodules.

Not all legumes can be infected by all rhizobia. Only a specific Rhizobium sp. infects a given leguminous species. Infection is initiated by the secretion of a special type of chemical from the roots of the plants. The chemical attracts the nitrogen fixing bacteria ir. the soil, towards the root surface. The binding site for the bacteria, in the host root, are believed to contain a special type of protein called 'lectin'. The plant lectin of a given species can interact with polysaccharides of only a given bacterium, and thus only a particular bacterium can infect a particular plant. In most cases, bacteria enter the host through root hairs. The root hair becomes deformed and curled around the rhizobia (Fig. 8.3).

At the point of contact, the host cell wall dissolves and the bacterium enters the root cortex 1n a thread like manner, multiplying as they move along the thread. The bacteria then enlarge upto forty folds and simultaneously undergo structural changes to become bacteroids. Meanwhile the

root cortex invaded by the bacteria and adjoinıng cells start dividing rapidly. Most of these cells become diploid or triploid, because of rapid division of their chromosomes. These cells later on modify and differentiate to form nodules, in which the bacteria reside ()

It is assumed that nodule initiation and development are controlled by growth substances provided by both partners of the symbiont. Auxin, gibberellins, cytokinins, abscişic acid and several unidentified factors seem to be involved, Although their exact physiology is not known. The bacteroids in the nodules are encloséd within a membranous envelope, which is probable site of occurrence of leghaemoglobin. Leghaemoglobin is a proteinaceous pigment, red in colour. The characteristic red, pink or brown colour of the nodule is due to this pigment. It constitutes about 25-30% of the total soluble nodule protein, and is actively involved in the fixation of nitrogen. It acts as an oxygen carrying pigment, as does bacteroidś to carry on oxiative ATP generation which is essential for oof nitrogen to ammonia is catalysed be by an enzyme-complex known asleghaemoglobin in blood. In doing so, the pigment protects the key enzymenitrogenase, against oxidative inactivation and at the same time allows thenitrogen fixation. The pigment is a symbiotic compound' made up of aapoprotein part and heme molecule. The apoprotein art is synthesized under the genetic direction of the plant and the heme part under rhizobial gene. Thus, the total leghaemoglobin molecule is synthesized only when Rhizobiucolonises the root and it is not present when Rhizobium is free living or in uninfected plant (host) root.

Biochemistry of nitrogen fixation.

As mentioned earlier, the reduction of nitrogen to ammonia is catalysed by an enzyme-complex known as nitrogenase.

$$N_2 + 6e^- + 12\ ATP + 8H^+ \xrightarrow{\text{Nitrogenase}} 2NH^+_4 + 12\ ADP + 12\ Pi$$

Nitrogenase is able to reduce many other molecules which resemble dinitrogen, such as acetylene, protons, azides and cyanides. The reduction of acetylene to ethylene is often used as a method for determining the enzyme activity, as ethylene can be detected rapidly and with great sensitivity, by using a gas chromatograph.

The enzyme nitrogenase has been isolated and characterised from different sources and all show similar properties. The enzyme has been fractionated into two components:
(1) A larger Mo-Fe protein consisting of four sub-units, referred to asnitrogenase and

(2) A smaller Fe-protein consisting of two sub-units, referred as nitrogenase reductase.

Both components are coloured due to the presence of Fe atom, and both are essential for the reduction of nitrogen to ammonia. Some properties of these two components are given in table 8.3.

Table 8.3. **Some properties of two components of nitrogenase.**

Parameter	Mo-Fe protein (nitrogenase)	Fe-protein (nitrogenase reductase)
Molecular weight	218,000	66,800
Number of sub-units	Four (two types) 2 × 59,000 (m.w.) 2 × 51,000 (m.w.)	Two identical sub-units 2 × 34,000 (m.w.)
Mo content g-atom/mol	2	None
Fe content g-atom/mol	24–34	4
S content g-atom/mol	24	4

During nitrogen fixation and also during the reduction of other substrates reaction. In vitro studies have shown that about 12-15 moles of ATP are utilised per mole of nitrogen reduced to ammonia. This ATP comes from the respiratory process of the organism. The major carbon substrates found in nodules are carbohydrates, polyols (carbohydrates with alcohol side groups) and organic acids. The reducing power (source of electrons) for the reaction is provided by reduced ferredoxins and flavodoxins. These proteins are strong reducing agents and are capable of reducing many other biological molecules provided that the appropriate enzyme is available. NADPH can also act indirectly as reducing power as it can reduce the ferredoxin in the presence of NADP-ferredoxin reductase. In Azotobacter vinelandii, following sequence of electron transfer has been identified, during reduction of nitrogen to ammonia by the enzyme nitrogenase NADPH Ferredoxin- Azotoflavin -Fe-protein- Mo-Fe -protein- N_2

The nitrogenase enzyme is irreversibly destroyed by O_2. As indicated earlier, leghaemoglobin combines with the O_2 in the nodules and removes it away from nitrogenase.

Assimilation of Nitrogen and Sulfurin the fixation of nitrogen. It acts as an oxygen carrying pigment, as does leghaemoglobin in blood. In doing so, the pigment protects the key enzyme nitrogenase, against oxidative inactivation and at the same time allows the bacteroidś to carry on oxidative ATP generation which is essential for nitrogen fixation. The pigment is a 'symbiotic compound' made up of a apoprotein part and heme molecule. The apoprotein part is synthesized under the genetic direction of the plant and the heme part under rhizobial gene. Thus, the total leghaemoglobin molecule is synthesized only when Rhizobium colonises the root and it is not present when Rhizobium is free living or in uninfected plant (host) root.

Biochemistry of nitrogen fixation.

As mentioned earlier, the reductionof nitrogen to ammonia is catalysed by an enzyme-complex known as nitrogenase. The overall reaction can be represented as follows Nitrogenase

Molecular mechanism of nitrogenase action.

Although the precise mechanism by which nitrogenase reduces dinitrogen to ammonia, is yet to be elucidated, some details of the reaction are known. The process can be divided into four groups of reactions

(i) In the first step, an electron donor (such as reduced ferredoxin) donates an electron and reduces the smaller component (Fe-protein) of the enzyme

[Fe-protcin] ox. + e^- [Fe-protein] red

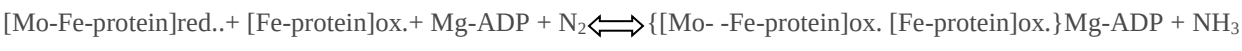
(ii) In the second step, the reduced Fe-protein forms a complex with the other component of the enzyme, the larger Mo-Fe protein and with Mg-ATP

[Mo-Fe-protein] ox. + [Fe-protein] red.+ Mg-ATP [Mo-Fe Fe-protein] ox. [Fe-protein] red. Mg-ATP)

(iii) In the third step, electrons are transferred from Fe-protein part to Mo- -Fe-protein part of the enzyme. ATP (of Mg-ATP) is hydrolysed in this reaction to provide energy:

{[Mo-Fe-protein] ox. + [Fe-protein] red+ Mg-ATP}{[Mo- -Fe-protein] red. [Fe-protein] ox. Mg-ADP]+HPO_4^{--}

(iv) In the fourth and final step, electrons are transferred from [Mo- -Fe-protein] red. to nitrogen, or other substrates of nitrogenase reaction.



[Mo-Fe-protein]red..+ [Fe-protein]ox.+ Mg-ADP + N_2 {[Mo- -Fe-protein]ox. [Fe-protein]ox.}Mg-ADP + NH_3

The reduction of nitrogen to ammnonia is also a multistep process. It involves three steps forming intermediates diamide and hydrazine.

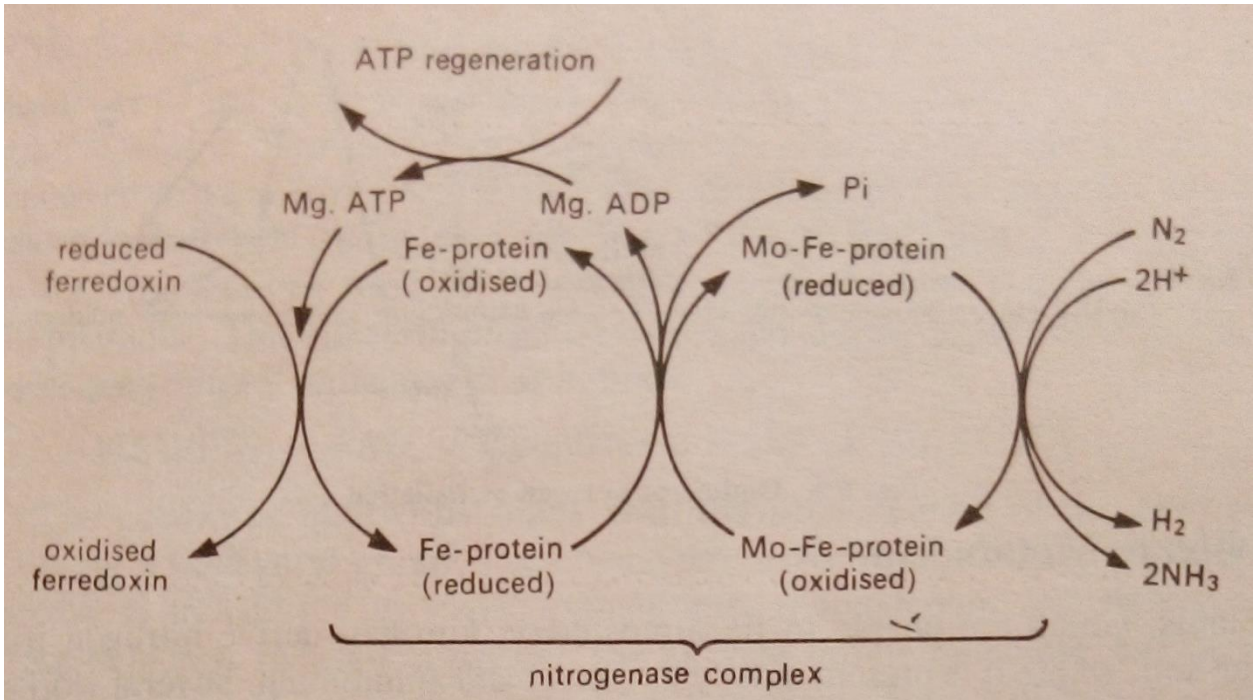


Fig. 8.4. Reactions catalysed by nitrogenase complex.

(i) $N_2 + 2H^+ + 2e^- \rightarrow HN{=}NH$
Diimide

(ii) $HN{=}NH + 2H^+ + 2e^- \rightarrow H_2N{-}NH_2$
Hydrazine

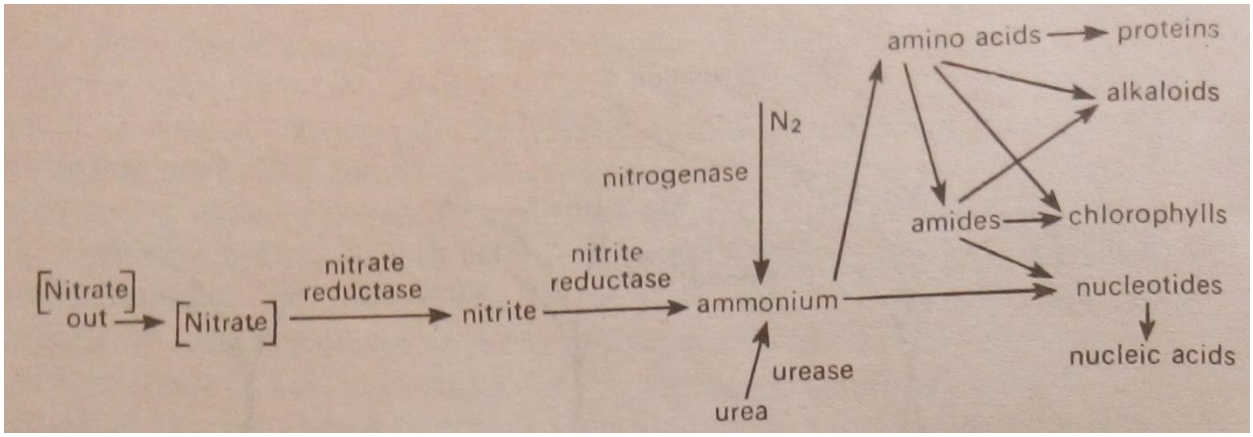
(iii) $H_2N{-}NH_2 + 2H^+ + 2e^- \rightarrow 2NH_3$
Ammonia

Recently, it has been shown that N_2, attaches itself to Mo atom of the Mo-Fe-protein component of the enzyme complex (in step iv mentioned earlier). First one of the two nitrogen atoms of N_2, molecule attaches to Mo. This makes the other nitrogen atom of N_2 molecule attractive to protons (H^+). Protons come in one by one, until the nitrogen has been reduced to NH_3 in three successive steps. Then the NH_3 is released from the enzyme complex. Attack on the second nitrogen atom starts then, until it is also reduced and released as NH_3

Regulation of nitrogenase.

Nitrogenase is rather an expensive enzyme as it requires a continuous and plentiful supply of the energy currency, the ATP. Consequently, the nitrogen fixing organisms have developed a means of regulating its synthesis and activity. In most organisms, when NO_3^{--} or NH_4^+ is available as a source of external nitrogen, the enzyme is not synthesized. The enzyme glutamine-synthetase which converts ammonia into glutamine, also controls nitrogenase activity The enzyme is known to exist in two forms: 'active' form and 'inactive' form. In active' form, it induces the synthesis of nitrogenase enzyme, while in inactive fom it inhibits the same. It is understandable, as the

product of nitrogenase action (i.e. ammonia) is the substrate for glutamine synthetase. The ammonia is required only when active glutamine synthetase is present to take care of it. This is an unique example of one enzyme regulating the formation of another enzyme. Other molecules such as ATP, ADP and O, also regulate the level and activity of nitrogenase enzyme.



156 **Nitrate Metabolism**

Plants, which are unable to fix atmospheric nitrogen derive nitrogen from the soil, where it is present mostly as nitrate and ammonium. Several workers have reported that plants, specially the cereals utilize nitrate better than ammonium, although some plants such as potato, pine apple and rice prefer ammonium over nitrate. However, the uptake and utilization of different nitrogenous salts depend upon the pH and perhaps other conditions of the soil. Generally, the alkaline or neutral pH prefers ammonium over nitrate, while acidic pH favours nitrate utilization, although in many cases both ions can be utilised over a wide range of pH.

A general outline of the pathway of nitrate assimilation is shown in figure 8.5. The major components of the pathway are described in the following paragraphs :

1. **Nitrate reduction**. Assimilation of nitrate starts with its reduction to nitrite. This may take place either in the root itself or in the leaves, and the process can casily be demonstrated in vivo by incubating plant tissues in the presence of nitrate in the dark, under anaerobic conditions. The nitrite produced is accumulated in the root/leaf tissues and in the medium and can be.estimated colorimetrically.
   The reduction of nitrate to nitrite is catalysed by the enzyme nitrate reductase, which uses reduced pyridine nucleotides as electron donor (cocnzyme).

$$\mathbf{NO_3^-} + \mathbf{2e^-} \xleftarrow[\text{NAD(P)H+ H}^+]{\text{Nitrate reductase}} \mathbf{NO_2^-} + \mathbf{H_2O}$$

The enzyme nitrate reductase was first studied by A. Nason and H.J. Evans (1953) in the mold, Neurospora crassa, Since then, it has been demonstrated in almost all plants examined. It has been isolated and purified from higher plants, as well. The enzyme is a homodimer with monomer size100 to 120 kDalton. although it may very considerably among different species. Molybdenum is an essential constituent of the enzyme and flavin adenine dinucleotide (FAD) is present as a tightly bound prosthetic group of the enzymic protein. Heme in the form of cytochrome b-557 also seems to be an integral omponent of higher plant enzyme.

In addition to NAD(P)H, many non-physiological chemicals, such as reduced viologen dyes or dithionite can also serve as electron donors for nitrate reduction. Purified nitrate reductase can reduce cytochrome-c also. It is believed that the enzyme consists of two components: a component which transfers electrons (reductant) for NADP)H to FAD and a component transferring electrons from FAD to nitrate by the way of cytochrome and molybdenum. The direction of electron transfer during reduction of nitrate to nitrite by nitrate reductase is as follows

$$NAD(P)H \longrightarrow FAD \longrightarrow \text{Cytochrome b-557} \longrightarrow Mo \longrightarrow NO_3^-$$

The coenzyme NAD(P)H binds with the active center of the enzyme .involving a sulfhydryl group. The coenzyme is oxidised to NAD(P) and the electron is transferred to FAD, cytochrome, molybdenum and finally to nitrate, which is reduced to nitrite. The enzyme nitrate reductase, has been described as a rate limiting enzyme in nitrate assimilation pathway, that is, its activity will determine the overall assimilation of nitrate. The regulation of enzyme by various environmental and nutritional factors has been extensive ely studied in higher plants (Srivastava, 1980), and the enzyme has been used as a model for regulatory studies by many workers. One of the most important aspect of its regulation is the substrate inducibility; the enzyme increases in almost all plant parts when its substrate (nitrate) is fed to the plant or to a specific tissue.

2. Reduction of nitrite to ammonia.

The nitrite produced by the activity nitrate reductase is converted to ammonia by the enzyme nitrite reductase, which is present in plant tissues in amounts considerably higher than that Nitrate reductase

$$\mathbf{NO_2^- + 6e^- \xrightarrow[\text{Nitrite reductase}]{\text{6 Fd (red)}} NH_4^+ + 2H_2O}$$

NH 2H,0NO + 6e-, reduced ferredoxinorganis

In higher plants, and other photosynthetic organisms, reduced ferredoxin (Fd red) is used as an electron donor, while in non-photosynthetic organisms, the electron donor is NADPH. In many systems, flavodoxin can substitute ferredoxin as electron donor.

The enzyme appears to be made up of a single polypeptide chain of 600 amino acids with molecular weight ranging between 60,000 to 70,000. The prosthetic group of the enzyme has been identified as a iron containing porphyrin called Siroheme besides a tetranuclear cluster of 4 iron and 4 sulfur atoms, in the protein. The direction of flow of electrons during enzymic action seems to be as follows :

Fd (red) $\Longrightarrow$ 4Fe-4S $\Longrightarrow$ Siroheme $\Longrightarrow$ NO_2^-

In leaves and other green tissues, reduction of nitrite to ammonia is accelerated by light. Apparently light reactions of photosynthesis in green leaves generate reduced ferredoxin, which acts as a coenzyme for nitrite reduction. However, nitrite is readily metabolised by non-photosynthetic root tissues also which lack ferredoxin. Apparently, the roots are able to use NADPH (generated from carbohydrate metabolism) as electron donor for nitrite reduction. In these tissues, nitrite reduction is greatly inhibited by anaerobiosis.

Entry of ammonia into organic compounds.

Ammonia, either produced from the reduction of nitrate, or absorbed from soil is incorporated to glutamic acid by two alternative pathways

(i) By reductive amination of 2-oxo-glutarate and
(ii) Via glutamine synthesisis

A limited amount of ammonia is also absorbed by the leaves from the moderately polluted atmosphere. In leguminous and other plants which have a symbiotic association with the nitrogen fixing micro-organisms, ammonia is supplied to the plant by microbes. The two pathways of glutamic acid synthesis from ammonia are described in the following paragraphs:

Reductive amination of 2-oxo-glutarate (GDH pathway): This reversible reaction, is catalysed by the enzyme glutamate dehydrogenase,

2- xo-glutarate + NH_4^+ + NAD(P)H $\Longleftrightarrow$ glutamate + NAD(P) + H_2O

The enzyme is present in large quantities in plant tissues and uses either NADH or NADPH as coenzymes. The enzyme also catalyses the reversible reaction i.e. hydrolysis of glutamate to 2-oxo-glutarate and ammonia and uses either NAD or NADP as coenzymes. Various studies have shown that enzyme prefers NADH over NADPH and NAD over NADP by various degrees. The enzyme is thought to be a hexameric metalloprotein having molecular weight in the range of 208,000 to 270,000. The metallic component is zinc.

The role of glutamate dehydrogenase in the synthesis of glutamic acid has been doubted by various workers, because it catalyses the hydrolysis of glutamic acid as well and because its affinity for ammonia is very low. However, it appears that the enzyme plays an active role in the assimilation of ammonia under stress conditions, such as dark starvation, high temperature, salinity, water stress etc

Via Glutamine Synthesis (GS/GOGAT pathway).

The other reaction by which ammonia is converted to glutamic acid by two consecutive reactions, is through the synthesis of amide glutamine:

$$\text{Glutamic acid} + NH_4^+ + ATP \xrightleftharpoons[\text{Glutamine synthetase (GS)}]{Mg^{2+}} \text{glutamine} + ADP + Pi + H^+$$

$$\text{Glutamine} + \text{2-oxo-glutarate} + \text{NADPH/Fd red} + H^+ \xrightarrow{\text{Glutamate synthtase (GOGAT)}} 2 \text{ glutamic acid} + \text{NADP/Fd}$$

The first reaction in the series is catalysed by the enzyme glutamine synthetase while the second one is catalysed by glutamate synthase. Synthesis of glutamine from glutamate and ammonia occurs in both roots and leaves. The enzyme glutamine synthetase has much more affinity for ammonia than glutamate dehydrogenase. However, a amount of glutamate isalways necessary (as a reactant) to drive this reaction.

Have carbohydrate metabolism is presented in figure 8.6.

The second enzyme, glutamate synthase catalyses the transfer of amide group of glutamine to 2-oxo-glutarate producing two molecules of glutamate. It uses either reduced ferredoxin or NADP)H as reductant.

The glutamic acid arising from nitrate assimilation is converted into many other amino acids. It can also be incorporated into proteins. As we seen, the process of nitrate assimilation is heavily dependent upon carbohydrate metabolism for the supply of reducing powers (electron donors) and organic acids for accepting the reduced form (ammonia) of nitrogen. A scheme, summarising the relationship between nitrate assimilation and carbohydrate metabolism is presented in figure 8.6

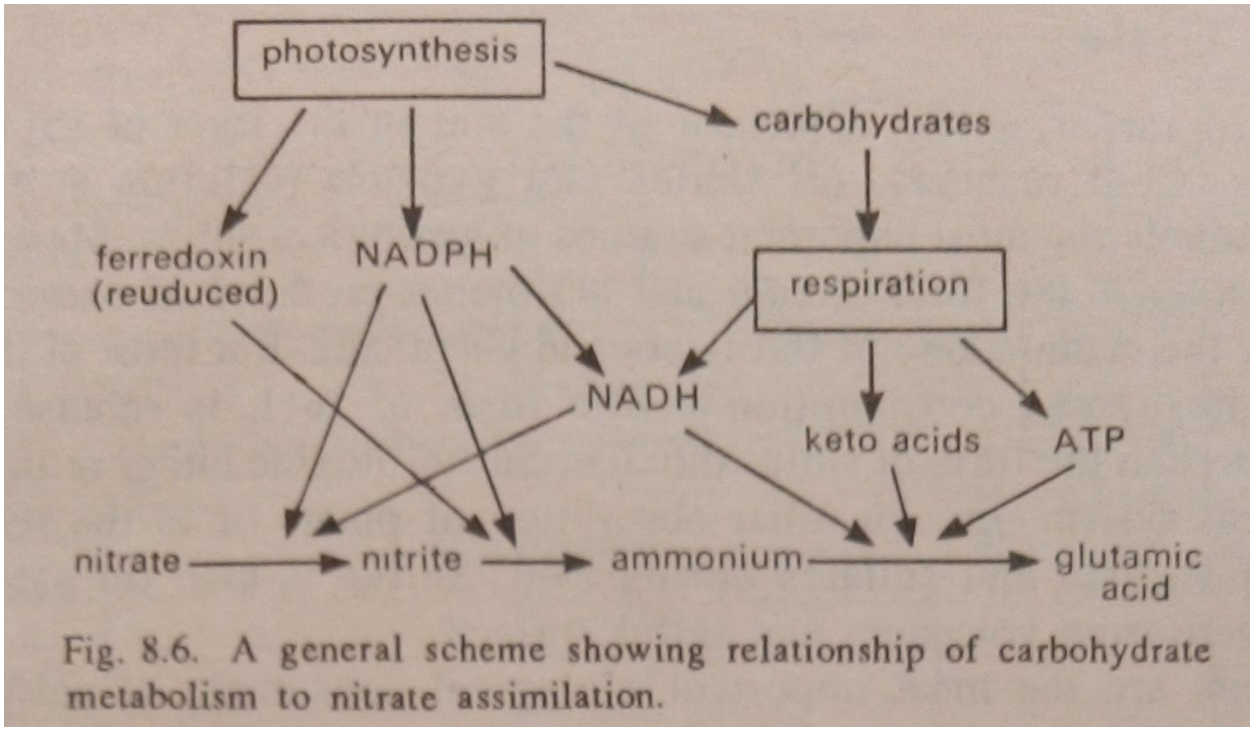


Fig. 8.6. A general scheme showing relationship of carbohydrate metabolism to nitrate assimilation.

Urea Metabolism

Urea is often supplied to the plants as nitrogenous fertilizer either through soil or foliar application. In many plants, foliar application of urea has proved to be a very effective method of relieving nitrogen shortage. It can be taken up both by roots as well as leaves. The first step in the urea assimilation is its rapid hydrolysis by the enzyme urease, to yield ammonia and carbon dioxide,

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

Urea

The hydrolysis of urea may take place either in the soil itself, or in the plant tissue after its absorption. The ammonia thus produced; is absorbed by water to form ammonium ion, which is assimilated by the usual pathways of ammonium assimilation,

$$NH_3 + H_2O \longrightarrow NH_4^+ + OH^-$$

However, the enzyme urease, has not been detected in Chlorella species and it has been suggested that in these plants, urea is assimilated directly without prior hydrolysis to ammonia and CO_2.