UNIT 12

NITROGEN METABOLISM

Structure

12.1 INTRODUCTION

You have studied about the role of nitrogen as a macronutrient in Unit 3. Nitrogen is considered to be the fourth most abundant nutrient element after carbon, hydrogen and oxygen in plants. This element occurs in both inorganic and organic forms. Nitrogen is an essential constituent of amino acids, proteins, enzymes, hormones, nucleic acids, alkaloids, chlorophyll, vitamins, glycosides and other important primary and secondary plant constituents. Needless to say protoplasm, the physical basis of life consists of a major portion of proteins.

Although molecular nitrogen (N_2 or dinitrogen) constitutes 78% by volume of the atmosphere, this odorless, colorless gas cannot readily diffuse into the plant and get utilized directly. This is partly due to the exceptionally stable $N \equiv N$ bond. No such enzyme is present in higher plants that can reduce this triple covalent bond. Thus, these plants need to be supplied with nitrogen in a *usable* or *combined* form for the biosynthesis of the above-mentioned substances.

Objectives

After studying this unit, you should be able to:

- describe the steps involved in assimilatory reduction of nitrate and the requirements of the process;
- explain the significance of organelle specific or tissue specific distribution of *nitrate reductase* and *nitrite reductase*;
- explain the significance of induction, repression and control of activity of *nitrate reductase*;
- list the requirements of assimilation of ammonia into organic form; and
- discuss the metabolic interrelation of nitrogen, carbon and sulfur.

12.2 NITRATE ASSIMILATION

The higher green plants can utilize nitrogen from soil as nitrates (NO_3^-), nitrites (NO_2^-), ammonium salts (NH_4^+) and organic nitrogenous compounds.

The higher plants depend on prokaryotic organisms in soil for conversion of dinitrogen into this usable forms. Only certain microbes are 'gifted' to convert the atmospheric nitrogen into utilizable form. You will read about the biological nitrogen fixation in the next Unit 13.

Atmospheric nitrogen fixation by some microbes (discussed in Unit 13) accounts for nearly half of nitrate assimilation by green plants, which is estimated to be about 2×10^4 Mt/year.

Interestingly, nitrogen is often a limiting nutrient for plants, placed after water, since the plants and soil microorganisms compete with each other for this element which is limited in the soil pool.

12.2.1 Sources of Nitrogen

Atmosphere and soil are the main sources of nitrogen. The molecular form of nitrogen (dinitrogen) is prevalent in the atmosphere while both inorganic and organic forms of nitrogen are present in soil. Inorganic forms of nitrogen in the soil are, **nitrite nitrogen**, **nitrate nitrogen** and **ammonical nitrogen**. The organic forms of nitrogen in soil are in the form of amides, amino acids and urea.

Remains of dead animals and plants, excretions and nitrogenous wastes in soil are first acted upon by saprophytic microorganismswhich hydrolyze the proteins into amino acids. The later are then deaminated to release ammonia. This process is called **ammonification**. The liberated ammonia immediately combines with H^+ of water to form NH_4^+ ion.

$$\text{Proteins} + \text{Water} \xrightarrow[\text{hydrolysis}]{\textit{Bacillus}\text{ sp}, \textit{Actinomyces}\text{ sp.}} n\ \underset{\text{amino acids}}{\text{RH. NH}_2\text{. COOH}}$$

$$RH.\ NH_2.\ COOH + Water \xrightarrow{deamination} R\ HOH.\ COOH + NH_3$$

Amino acid Organic acid

$$NH_3 + H^+ \rightarrow NH_4^+$$

Ammonium ion

Some of these ammonium ions can be absorbed by roots of some plants. Remaining ammonium ions get converted into nitrate nitrogen by the microbial activity. The process is called **nitrification** which occurs in two steps.

i) **Nitrite Formation** : The nitrite microorganisms like *Nitrosomonas*, *Nitrosococcus* and *Aspergillus* oxidize ammonical nitrogen into nitrites.

$$NH_4^+ + 2O_2 \xrightarrow{nitrite\ \ microbes} NO_2^- + 2H_2O + Energy$$

The energy is utilized by chemosynthetic bacteria for their carbon assimilation.

ii) **Nitrate Formation** : Both biological as well as non-biological oxidation of nitrite is possible. Biological nitrate formation is carried out by *Aspergillus*, *Nitrobacter*,*Nitrocystis* and *Penicillium*.

Energy released in the process is used for carbon assimilation by the chemosynthetic nitrifying bacteria.

$$2\ NO_2^- + O_2 \xrightarrow{nitrate\ \ microbes} 2\ NO_3^- + Energy$$

The nitrates are now available to plants for absorption and assimilation.

Most of the plants absorb nitrogen in the form of nitrates. The mechanism of absorption of nitrate ions is essentially similar to that of other ions. A small amount of nitrites can also be absorbed. Interestingly, plants complete with the denitrifiers in soil like *Thiobacillus denitrificans* which readily reduce nitrate into dinitrogen, returning it into the atmosphere. Denitrification itself accounts for a loss of 93-190 million metric tonnes of nitrogen per year! Thus, in order to maintain a steady state of the supply, demand, turnover, recycling and growth of plants, there must be an element of a “nitrogen economy” in the biosphere which can balance the nitrogen recycling. A relationship between inorganic and organic nitrogen metabolism is illustrated in Fig. 12.1.

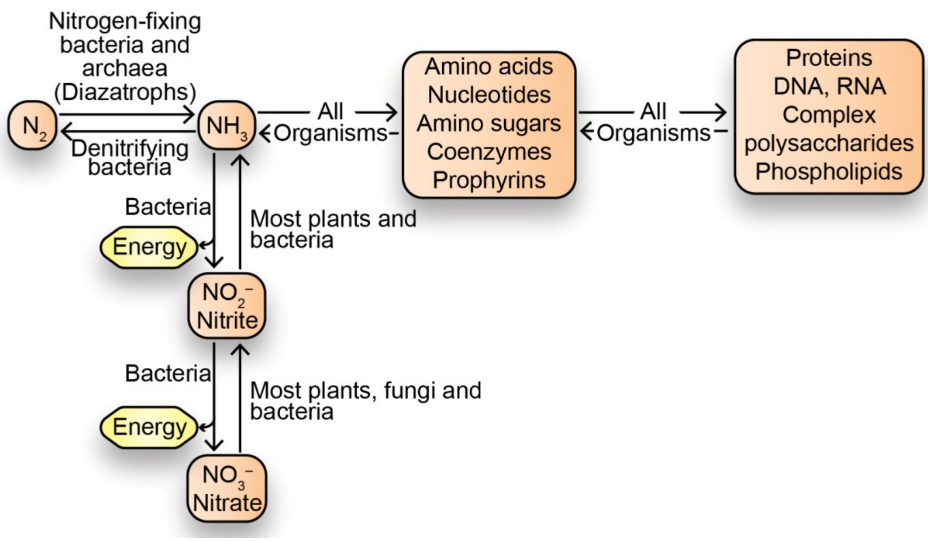

Fig. 12.1: Diagram depicting relationships between inorganic and organic nitrogen metabolism (From Appling et al).

12.2.2 Biochemistry of Nitrate Assimilation

As you have studied in subsection 12.2.1, nitrate is the most readily available and preferred source of nitrogen for plant growth. Once inside the roots and after being transported to leaves through the transpiration stream, the nitrates are converted into ammonia. This assimilatory reduction of NO_3^- to NH_3 occurs in **two steps** as shown below :

i) $NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$

Nitrate Nitrite

ii) $NO_2^- + 7H^+ + 6e^- \longrightarrow NH_3^- + H_2O$

Nitrite Ammonia

Recent evidence suggests that this conversion may involve many more steps as indicated below:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow HNO^- \longrightarrow NH_2OH \longrightarrow NH_3$$

Nitrate Nitrite Nitroxyl Hydroxylamine Ammonia

The first step is catalyzed by ***nitrate reductase*** **(NR)** in the cytosol which reduces NO_3^- to NO_2^- at the expense of two electrons. The second step is catalysed by ***nitrite reductase*** which converts NO_2^- into NH_3 at the expense of six electrons. *Nitrate reductase* is a Mo-enzyme like *dinitrogenase* and *nitrite reductase* is a Fe-protein.

The physiological source of reductant for the two reductive processes could be reduced ferredoxin –$Fd_{(red)}$ or reduced pyridine nucleotides (NADH or NADPH) depending upon the system. It is important to point out here that NO_3^- is also known to undergo dissimilation process in which it is reduced to N_2 gas. Such a process of nitrate metabolism is called **nitrate respiration** or **denitrification** and occurs exclusively in certain bacterial forms under anaerobic conditions. The enzymes of denitrification of nitrate are called *dissimilatory nitrate reductases*.

Assimilatory *Nitrate Reductase* and *Nitrite Reductase*

There are two kinds of *nitrate reductases* depending upon their specificity to reductant. *Nitrate reductase* of cyanobacterial system requires reduced ferredoxin (Fd-dependent) to catalyse the reaction while *nitrate reductase* in plants and fungi requires reduced pyridine nucleotide (NADH or NADPH – dependent) to carry out the reaction.

In general, most pyridine nucleotide *nitrate reductases* are capable of using both NADH and NADPH as source of reductant. The enzyme from photosynthetic organisms like higher plants and algae show preference for NADH but those from fungi show preference for NADPH. This suggests inherent difference between cyanobacterial *nitrate reductase* and eukaryotic *nitrate reductase* in respect to reductant requirement. The two types of reductant dependent nitrate reduction reactions are show below (Fig. 12.2).

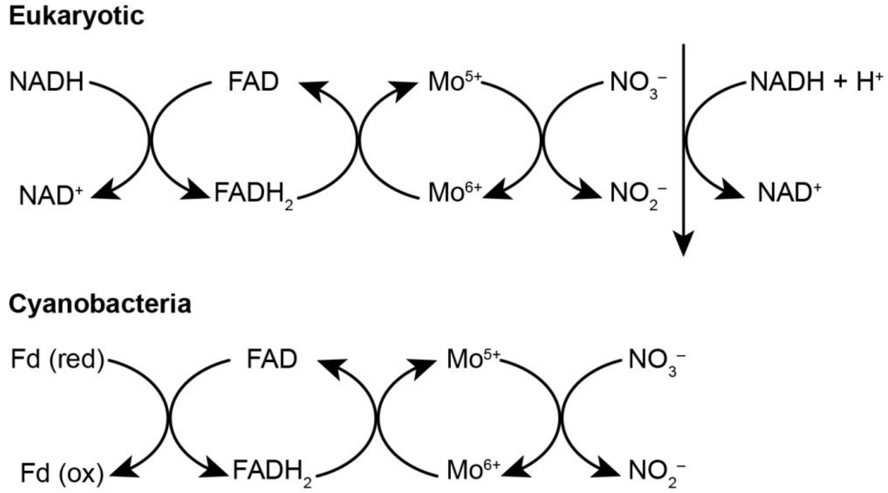

Fig. 12.2: Two major types of reductant dependent nitrate reduction reactions in eukaryotes and cyanobacteria.

The overall reaction of eukaryotic *nitrate reductase* is summarized as:

$$NO_3^- + NAD(P)H + H^+ \longrightarrow NO_2^- + NAD(P)^+ + H_2O$$

In comparison to the Fd-dependent enzyme which contains only molybdenum as prosthetic group, the eukaryotic *nitrate reductase* is made up of two identical sub-units (dimer), each containing one molecule of FAD heme, and a Mo atom as prosthetic group complexed with an organic molecule called **Pterin**. Functionally, while the Fd-dependent enzyme catalyses only reduction of nitrate to nitrite, the NAD (P)H-dependent enzyme catalyses two independent activities, one called ***diaphorase*** activity in which NAD(P)H is oxidized and one electron acceptor like cytochrome $_C$ or flavin mono-nucleotide (FMN) is reduced, and the other called ***terminal nitrate reductase*** activity which is NAD(P)H independent and in which nitrate is reduced at the expense of reduced flavins ($FMNH_2$) or viologens. *In vivo* both activities participate jointly and sequentially in the transfer of electrons from reduced pyridine nucleotide to nitrate as shown in reaction (Fig. 12.3). *Nitrate reductase* in cyanobacteria lacks *diaphorase* activity and contains only Mo as prosthetic group.

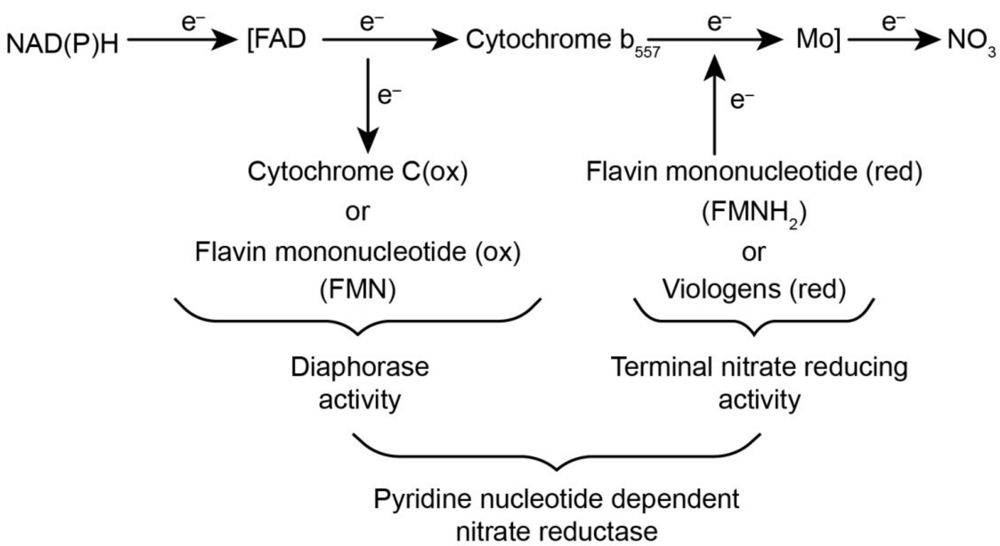

Fig. 12.3: Activities of NAD$^+$(P)-dependent *nitrate reductase* i) *diaphorase* activity; ii) terminal *nitrate reductase* activity.

Reduction of nitrite to ammonia occurs in chloroplasts. The enzyme *nitrite reductase* in cyanobacteria or chloroplasts requires reduced ferredoxin for reduction. *Nitrite reductase* of fungi requires NADPH to carry out the reductive function. The two reactions are shown below (Fig. 12.4).

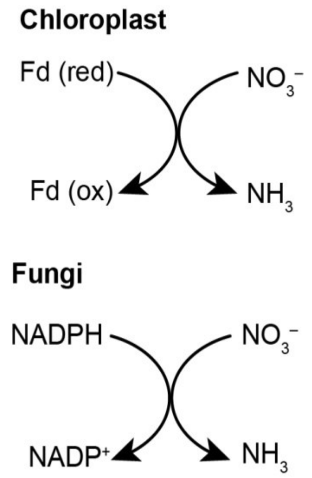

Fig. 12.4: *Nirtite reductase* activity in the chloroplast and in fungi.

Since NO_2^- is highly reactive and does not accumulate in plant tissue, it is immediately transported from the cytosol to the chloroplast in leaves and plastids in roots for reduction. Nitrite is reduced to ammonia by the enzyme *nitrite reductase*. This involves the transfer of six electrons:

$$NO_2^- + 6Fd_{red} + 8H^+ \longrightarrow NH_4^+ + 6Fd_{ox} + 2H_2O$$

Ferredoxins involved in this reaction are obtained from the electron transport system of chloroplasts (see Unit 5).

Nitrate Uptake

Cells accumulate NO_3^- against concentration gradient and this accumulation is a result of the presence of active NO_3^- transport system in the cell membrane. Nitrate uptake and nitrate reduction are independent processes because organisms genetically deficient in *nitrate reductase* activity contain normal transport activity.

12.2.3 Regulation of Nitrate Assimilation

There are two levels of regulation of nitrate assimilation. One is long-term and another is short-term. The long-term regulation operates at the level of enzyme synthesis (**transcription level**) and the short-term regulation operates at the level of enzyme activity (**translation level**).

Enzyme Synthesis

Nitrate assimilating systems in general are known to show an increase in nitrate uptake system and *nitrate reductase* in the presence of nitrate. In other words, nitrate assimilatory system is induced by the presence of nitrate. Similarly, cells or organisms assimilating NH_3 as nitrogen source show lack of nitrate assimilatory system. Such control of nitrate is called **repression control**. Thus, nitrate is an inducer while ammonia is repressor of nitrate assimilatory system.

Red light is known to enhance synthesis of nitrate assimilatory system which is mediated by well-known photomorphogenic pigment called **phytochrome** about which you will learn in detail in Unit 15 of this course.

Enzyme-activity Control

Availability of the substrate, NADH and nitrate would be an important determinant of the rate of nitrate assimilation. In addition, there are number of substances which are known to cause reversible inactivation of *nitrate reductase* under reducing conditions (Fig. 12.5).

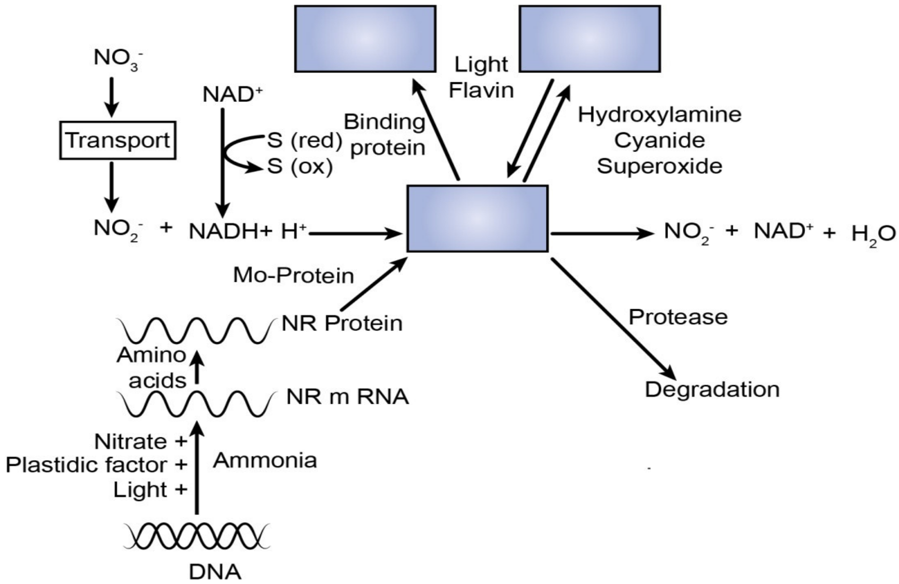

Fig. 12.5: Regulation of assimilatory *nitrate reductase* (NR). Reversible inactivation of NR by combination with hydroxylamine, cyanide, or superoxide and reversal of this inactivation by blue light in the presence of flavin; inactivation by combination with specific binding proteins or by limited proteolysis. Enzyme synthesis is regulated by positive effector-nitrate, plastidic factor, light and negative effectors derived from ammonia.

Cyanide: Plants generate cyanide from cyanogenic glycides and histidines. Ethylene biosynthesis is also accompanied by small amount of cyanide production. *Nitrate reductase* can occur in reduced form following its interaction with NADH in the absence of nitrate. The reduced form of the enzyme has the ability to combine with cyanide forming enzyme-CN complex which is enzymatically inactive. Nitrate, oxygen or blue light oxidise the enzyme-CN complex releasing cyanide and making cyanide - free enzyme active.

Hydroxyl amine or superoxide inactivates the enzyme which on exposure to blue light gets converted to active form. It is observed that plants grown in blue light are higher in protein contents.

Inhibitor proteins: One kind of inhibitor protein found in higher plants is an *endopeptidase* which degrades *nitrate reductase* thus causing irreversible loss of the enzyme. The other kind includes binding proteins that specifically bind to *nitrate reductase* leading to permanent inactivation of enzyme. Such inactivator proteins have been isolated from rice seedings and spinach leaves. These reactions are explained in Fig.12.6. You will see that the transcription of enzyme is mediated by light. It is a unique enzyme in this respect. On the other hand, darkness and Mg^{2+} stimulate a protein *kinase* that phosphorylates serine residues which interact with an inhibitor to inactivate *nitrite reductase*. This type of regulation is shown to be a much quicker one.

12.2.4 Interaction of Nitrogen and Carbon Assimilation

Application of nitrogenous fertilizers brings about dramatic effects on thegrowth and performance of the plant. One of the most important consequences is the effect on utilisation of carbohydrates in plants. It is known that the level of carbohydrates in the plants goes down with increase in the level of N_2 supplied to them.The form in which the fertilizers are supplied are NO_3^-, and NH_3.In the plant NO_3^- is reduced to NH_3 and assimilation of NH_3 requires carbon-skeleton like α-ketoglutaric acid, an intermediate of TCA cycle for its incorporation into organic form. Naturally, the rate of NH_3 assimilation into organic form would depend on the rate at which TCA cycle supplies the carbon skeleton. The C-skeleton removed during the assimilation of NH_3 must be replenished through the catabolism of carbohydrates. Consequently, the carbohydrate content of the plant decreases in proportion to the amount of organic N_2 produced.

The other places of nitrate assimilation in the plant as you have already seen in photosynthetic tissue and the reductants are reduced ferredoxin and pyridine nucleotide. Since such reductants are also required for carbon assimilation, theproduction of carbohydrates in photosynthesis is bound to go down in proportion tothe increase in rate of reductive assimilation of NO_3^-. In other words; the level of NO_3^- utilisation is inversely related to the level of carbohydrate in the plant.

We will describe how the practical applications of this knowledge is used in raising crops with desired protein and carbohydrate content. Take the example of celery eaten as salad; the stalks of this plant are most edible when soft and this softness is the result of lower availability of carbohydrates. Now, to raise a celery crop with soft stalks one uses nitrate fertilizers which divert a major part of carbohydrate in the synthesis of amino acids and proteins. Another example is sugarcane, a crop of tropical region. It requires a period of ten months from the time of planting to the period of harvest. Here, the growers apply nitrogen fertilizers in the beginning and not near the time of harvest.Similar practices have resulted in production of beet roots highly rich in sugar concentration. The reason for rise in sugar in the beet root is again due to withholding of N_2-fertilizer near the harvest time, so that the C-skeleton is not utilized for producing amino acids and proteins.

SAQ 1

a) Match the characteristics listed below of Column I with enzymes given in Column II.

Column I	Column II
i) *Nitrate reductase*	a) Non-heme Fe-protein
ii) *Nitrite reductase*	b) Present in chloroplast
	c) Present in cytoplasm
	d) Molybdo-flavo protein
	e) *Diaphorase* activity

b) Choose the alternate correct word given in parenthesis in the following statements:

i) Nitrate assimilation in higher plants occurs mainly in (roots/leaves).

ii) Number of electrons used when NO_3^- is reduced to NO_2^- (1/2).

iii) Number of electrons used when NO_3^- is reduced to ammonia (2/6).

iv) In cyanobacteria *nitrate reductase* is located in the (photosynthetic membranes/plasma membrane).

v) In higher plants *nitrate reductase* is in (cytoplasm/chloroplast) and *nitrite reductase* is in (cytoplasm/chloroplast).

vi) In C_4 plants nitrate assimilation occurs in (bundle sheath/mesophyll cells) and CO_2 assimilation occurs in (bundle sheath/mesophyll cells).

vii) *Nitrate reductase* activity is induced in the presence of (NO_3^- / NH_4^+).

12.3 AMMONIUM ASSIMILATION AND ITS REGULATION

Atmospheric nitrogen (N_2) and NO_3 are the most common available source of inorganic nitrogen. Both are enzymatically reduced to ammonia because it is only ammonia that is incorporated into organic form. The major product of ammonia assimilation is usually considered to be amino nitrogen. Ammonium generated during nitrite assimilation (as discussed in Unit 12.2.2) and during photorespiration (Unit 7) has to be rapidly converted as its accumulation is toxic to a plant. Molecular nitrogen fixation has been discussed in Unit 13.2.4.

12.3.1 Biochemistry of Ammonium Assimilation

Ammonia resulting from N_2- fixation or nitrate reduction or supplied exogenously is assimilated in plants by the following two primary pathways, i) **Reductive amination/*glutamate dehydrogenase* (GDH) pathway**, and ii) ***glutamine synthetase(GS)-glutamate synthase*** also called (***Glutamine oxoglutarate aminotransferase-GOGAT*) pathway.**

i) **Reductive Amination (High cellular ammonia Pathway):**

$$\mathrm{NH_3 + \alpha - Ketoglutarate + NADPH + H^+ \xrightarrow{GDH} Glutamate + NADP^+ + H_2O}$$

ii) **GS-GOGAT (Low cellular ammonia Pathway): (**NADH-GOGAT occurs in diatoms, fungi and in the nucleus of vascular plants). Fd-GOGAT is prevalent in photosynthetic eukaryotes and cyanobacteria. NADPH-GOGAT occurs in mitochondria, chloroplasts, archaea and non-photosynthetic bacteria.

$$\text{Glutamate} + NH_3 + \text{ATP} \xrightarrow{\text{Glutamate Synthetase}} \text{Glutamine} + \text{ADP} + \text{Pi}$$

$$\underset{}{\text{Glutamine}} + \alpha - \text{Ketoglutarate} + \underset{(\text{or}Fd_{(red)})}{\text{NADPH} + H} + \xrightarrow[\text{synthase}]{\text{Glutamate}}$$

$$2\text{Glutamate} + \underset{(\text{or } Fd_{(ox)})}{NADP^+} + H_2O$$

As you may note, both the pathways generate the same amino acid, **glutamate** as end product of the reaction. The two pathways may operate simultaneously as in higher plants and eukaryotic algae or alternatively as in certain heterotrophic enterobacteria.In cyanobacteria ***glutamine synthetase-glutamate synthetase*** **(*GS*)** is the main primary pathway of ammonia assimilation.

The two enzymes involved in ammonia assimilation differ significantly in their affinity for ammonia which is very high for *glutamine synthetase* and significantly low for*glutamate dehydrogenase*. In other words, *glutamate dehydrogenase* - mediated pathway functions under conditions of high cellular ammonia and *glutamine synthetase*-mediated pathway under conditions of low cellular ammonia.

The *GS-glutamate synthase* pathway is characteristically cyclic in nature in which glutamate acts as both the acceptor and product of ammonia assimilation. This pathway of ammonia assimilation is called ***glutamate synthase*** **cycle** (Fig. 12.6)***.***

12.3.2 *Glutamate Synthase* (GOGAT) Route

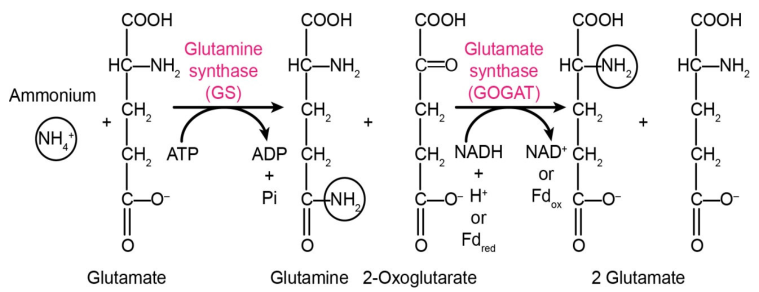

Fig.12.6 : *Glutamine Synthase* (GS) Route.

Two classes of GS exist in plants:

1. Cytosolic GS expresses itself in vascular bundles of roots and shoots to produce glutamine for intracellular nitrogen transport.

2. GS present in root plastids or shoot chloroplasts generate amides and catalyze reassimilation of photorespiratory NH_4^+ respectively.

When there is an enhanced concentration of glutamine in the plastids, the enzyme *glutamate synthase* (also called *glutamine: 2-oxoglutarate aminotransferase, or GOGAT*) getsactivated. The amide group of glutamine is transferred to 2-oxoglutarate, finally yielding two molecules of glutamate (Fig. 12.6).

There are two types of GOGAT. One accepts electrons from NADH and the other (Fd).

i) $\text{Glutamine} + 2 - \text{oxoglutarate} + \text{NADH} + \text{H}^{+} \rightarrow 2\ \text{glutamate} + 2\text{NAD}^{+}$

ii) $\text{Glutamine} + 2 - \text{oxoglutarate} + \text{Fd}_{\text{red}} \rightarrow 2\ \text{glutamate} + \text{Fd}_{\text{ox}}$

The NADH type of enzyme (***NADH-GOGAT***) is

- Located in plastids of non-photosynthetic tissues e.g., roots and vascular bundles of developing leaves.
- NADH-GOGAT helps in the assimilation of NH_4^+ absorbed from the rhizosphere and also assimilates glutamine which has been translocated from the senescing leaves or roots.

The Fd-dependent type (***Fd-GOGAT)*** is

- Located in the chloroplasts and help in photorespiratory nitrogen metabolism.
- Helps to incorporate glutamine generated during nitrate assimilation.

12.3.3 *Glutamate Dehydrogenase* Pathway

An alternate pathway involving ***Glutamate dehydrogenase***(***GDH***) can also assimilate ammonium ions in rice and under anaerobic conditions (Fig. 12.7), particularly when ammonia concentration is high. This NADH-dependent enzyme is present in mitochondria whereas its NADPH-dependent enzyme is present in the chloroplasts of photosynthetic parts. This mechanism of ammonia assimilation is less expensive than the GS-GOGAT Pathway.

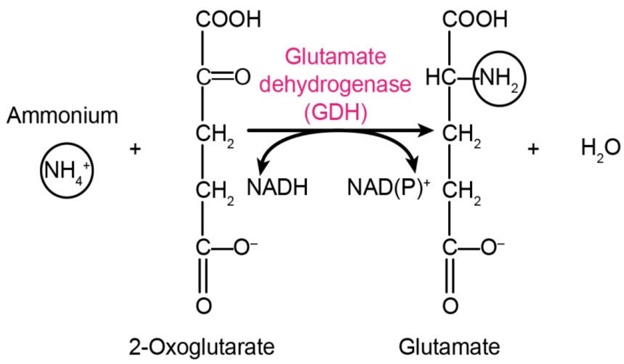

Fig. 12.7 : *Glutamate Dehydrogenase* Route.

Nutrient assimilation consumes a considerable amount of energy. The reduction of nitrate to nitrite and subsequently to ammonium involves the transfer of about 10 electrons. This amounts to nearly 25% of the total energy expenditure of both roots and shoots. Interestingly, nitrogen accounts for only 2% of the total dry weight of the plant, yet requires nearly one fourth of energy for its assimilation.

As most of these assimilation reactions occur in the chloroplast stroma, and utilize the reducing agents of the photosynthetic electron transport chain, the coupling of the two processes viz., nutrient assimilation and photosynthetic electron transport chain, is called **Photoassimilation**. The processes involved in the assimilation of mineral nitrogen and the energy expenditure is depicted in Fig. 12.8.

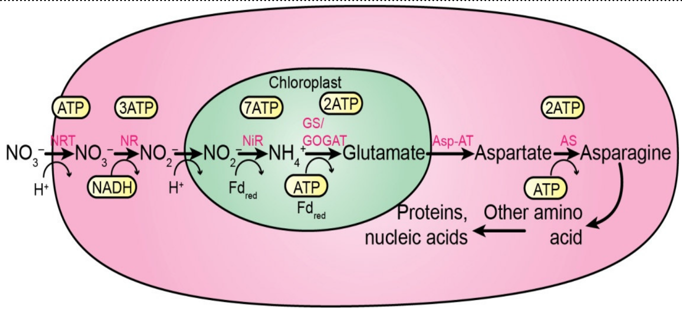

Fig. 12.8: A generalized diagram depicting the processes involved in the assimilation of mineral nitrogen in the leaf (From Taiz & Zeiger).

Since enhanced CO_2 levels inhibit nitrate inhibition in both C_3 and bundle sheath of C_4 plants, this phenomenon is bound to affect the plant nutrient reactions significantly by the end of this century, when the atmospheric CO_2 levels are expected to rise further.

12.3.4 Uptake of Ammonia

Ammonia (NH_3) diffuses freely across biological membranes according to its concentration gradient. However, ammonium (NH_4^+) ion requires a specific transport system to cross the biological membrane.

12.3.5 Ammonia Assimilation and its Regulation

Heterotrophic bacteria like *Escherichia coli* and *Klebsiella aerogenes* induce theoperation of *GS-glutamate synthase* pathway of ammonia assimilation underconditions of low ammonia and of *GDH*-pathway under conditions of high ammonia.Thus the two pathways are mutually exclusive and the level of cellular ammonia determines which pathway of ammonia assimilation is likely to operate.Cyanobacteria like systems have only GS-*glutamate synthase* pathway functioning under low or high cellular ammonia. Information about the role of ammonia in metabolic regulation of its two assimilatory pathways in higher plants is not clearly understood.

12.3.6 Metabolic Interrelation of Nitrogen, Carbon and Sulphur

Plants grow and develop into characteristic individuals as a result of a series of well programmed and regulated biochemical and morphological events. The main elements that enter into the composition of an individual plant are carbon, hydrogen, oxygen, nitrogen and sulphur. The three elements are taken by plants in the oxidized form CO_2, NO_3^-, SO_4^{2-}, and thus need to be reduced for assimilation. Assimilation of nitrate and sulphate, like carbon dioxide, requires ATP and reductants. It seems that plants must have evolved mechanisms to integrate the three assimilatory reductions with light harvesting photosynthetic reactions. Eukaryotic algae and higher plants have achieved this integration by localizing most of these assimilatory reactions within the chloroplast.

Photosynthetically produced ATP and reductant (ferredoxin or NADPH) together constitute what is called assimilatory power of the plants. Light reactions of photosynthesis produce ATP and reductants and dark reactions of photosynthesis are related to the reductive assimilation of carbon dioxide, nitrate and sulphate.

In plants, since carbon, nitrogen and sulphur also occur together in sulphur containing amino acids there must be regulatory mechanisms controlling integrated assimilation of carbon dioxide, nitrate and sulphate into organic forms. The results of scientific studies also support this view. Inhibition of photosynthetic carbon dioxide assimilation is known to simultaneously inhibit nitrate assimilation. This is partly because the carbon skeleton for compounds like α-ketoglutarate is ultimately produced from photosynthetically generated organic carbon from carbon dioxide. Assimilation of N_2 by nodulated legumes also depends upon the extent and amount of photosynthate like organic carbon reaching them. There is evidence which suggests that sulphur also controls nitrogen metabolism. Protein synthesis is known to decline under sulphate limitation and comes to a halt after sulphate exhaustion. During sulphur starvation, nitrate or ammonia is assimilated mainly into free amino acids and not in proteins. Because, in the absence of sulphur, the sulphur containing amino acids (cysteine and methionine) cannot be synthesized and the disulphide bridges cannot be formed in proteins. Some of the amino acids preferentially synthesized under these conditions are arginine, glutamine and alanine.

The major regulatory mechanisms controlling interdependence of inorganic carbon, nitrogen and sulphur metabolism are to be studied and understood in detail as they hold the key to the desired production of major plant products at commercial scale.

SAQ 2

a) i) Name the two pathways of ammonia assimilation in plants.

ii) Which of the two pathways operates under high concentration of cellular ammonia?

iii) Which of the two pathways is costly in respect of energy and material needs?

b) i) Which of the source among NH_4^+, NO_3^- and N_2 is preferred by plants?

ii) What signal in plant causes repression of both NO_3^- assimilation and N_2-fixation?

12.4 SUMMARY

- Nitrate assimilation occurs in two enzymatic steps. Nitrate reduction to nitrite is catalysed by *nitrate reductase* and nitrite reduction to NH_3 is catalysed by *nitrite reductase.* NADH is the reductant for *nitrate reductase* in plants, NADPH is the reductant in fungi and reduced ferredoxin in cyanobacteria. Reductant for *nitrite reductase* in fungi is NADPH and in plants and cyanobacteria is reduced ferredoxin.

- *Nitrate reductase* is predominantly localized in cytoplasm while *nitrite reductase* is localized exclusively in chloroplast.

- Nitrate assimilation is regulated by inducer and repressor control at synthetic level and activity control involving reversible inhibition of enzyme activity by cyanide, hydroxyl amine, superoxide radical and reactivation by blue light.
- In C_4 plants nitrate assimilatory pathway remains localized within mesophyll cell and CO_2 assimilation in bundle sheath cells.
- Plants prefer ammonia as a source of nitrogen over NO_3^- or N_2 and this is achieved by repression control of nitrate assimilation and N_2 fixation.
- *Glutamate dehydrogenase* and *glutamine synthatase/synthase* are the two primary enzymes of ammonia assimilation.
- Sulphate like NO_3^- is also assimilated reductively. Unlike NO_3^-, it is required to undergo ATP dependent activation before entering into pathway of assimilatory reduction.
- A clear understanding of metabolic interrelationship of N,C and S nutrition are a must from agricultural point of view.

12.5 TERMINAL QUESTIONS

1. Trace the steps of nitrification.
2. Distinguish between the eukaryotic and bacterial *nitrate reductase* enzyme.
3. What are the three basic modes of NO_3^- reduction?
4. Where are the *nitrate reductase* and *nitrite reductase* enzymes present in higher plants (C_3)?
5. Explain the control of *nitrate reductase* by various regulatory factors.
6. Explain the two major pathways for ammonium assimilation.
7. How expensive is the nitrogen uptake?
8. How are the sulphates, phosphates and cations assimilated?

12.6 ANSWERS

Self-Assessment Questions

1. a) i) c, d and e;

   ii) a and b

   b) i) leaves

   ii) 2

   iii) 6

   iv) photosynthetic membranes

v) cytoplasm, chloroplast

vi) mesophyll cells, bundle sheath cells

vii) NO_3^-

2. a) i) *Glutamate dehydrogenase* pathway and *glutamine synthetase-glutamate synthetase* pathway;

ii) *Glutamate dehydrogenase* pathway

iii) *GS-glutamate synthase*.

b) i) NH_4^+;

ii) high ratio of glutamine to α-ketoglutarate

Terminal Questions

1. Refer to Subsection 12.2.1.
2. Refer to Subsection 12.2.2.
3. Refer to Subsection 12.2.3.
4. Cytoplasm and chloroplast respectively.
5. Refer to Subsection 12.2.4.
6. Refer to Subsection 12.3.1.
7. Refer to Subsection 12.3.3.
8. Refer to Subsection 12.3.7.