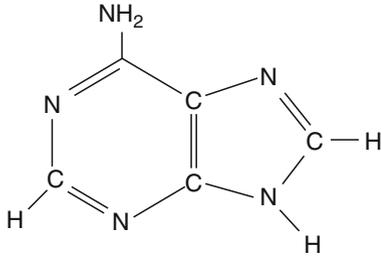


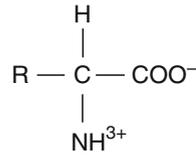
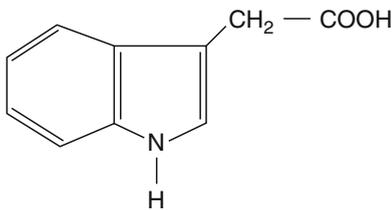
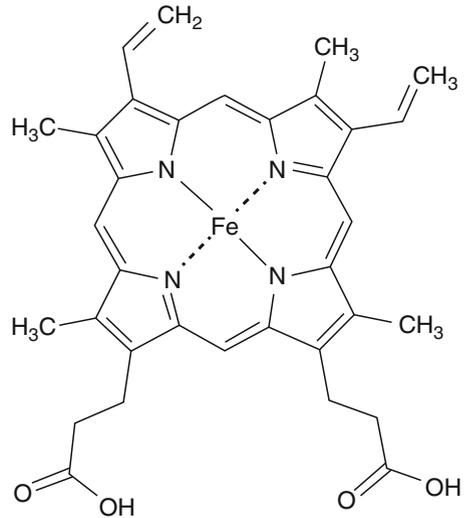


Manju A. Lal

Nitrogen is one of the most important macronutrients required by the plant. All proteins consist of nitrogen-containing amino acids. Heme component of the chlorophylls, nitrogenous bases of DNA and RNA, and **phenylpropanoids** (such as **flavonoids**) are all nitrogen-containing biomolecules (Fig. 11.1). Plants absorb carbon in the form of CO₂ fixed by photosynthetic process; hydrogen and oxygen are taken up in the form of H₂O. Oxygen is also absorbed from the air. In contrast, plants are not able to utilize molecular nitrogen directly. In spite of the fact that nitrogen constitutes almost 80% of the air, it needs to be provided to plants in the form of fertilizers. There are some prokaryotes, however, which have the ability to fix molecular nitrogen by means of biological fixation leading to enhancement of soil nitrogen as well. Thus, it becomes necessary to study the forms of nitrogen which plants can absorb, mechanisms of their uptake, biological nitrogen fixation, and also the mechanisms involved in their further metabolism. Plants absorb nitrogen through their roots either in the form of ammonium ions or nitrates which are available in the soil. Nitrate uptake can take place through leaves, only in case of epiphytes or only through foliar spray of fertilizers. Molecular nitrogen can be fixed directly by the nitrogen-fixing prokaryotes (*diazotrophs*), which are either free living or growing in symbiotic associations with plants. When uptake of nitrogen by plants is in the form of nitrate, it needs to be reduced to ammonium before further assimilation for amino acid biosynthesis, followed by biosynthesis of purines and pyrimidines. Within plants nitrogen is transported in the form of nitrates, amino acids, amines, and ureides. This chapter shall focus on nitrogen uptake in the form of ammonium and nitrate ions, mechanisms and the enzymes involved in their reduction, physiology and biochemistry of molecular nitrogen fixation, ammonium assimilation, transport of the assimilated nitrogen, and finally amino acid biosynthesis (Fig. 11.2).



Adenine (a nitrogenous base)

 α - Amino acidIndole-3-acetic acid
(a plant hormone)

Heme (a cofactor of various hemoproteins)

Fig. 11.1 Some of the nitrogenous biomolecules

11.1 Biogeochemical Cycle of Nitrogen

Nitrogen occurs in different forms in nature. It is present as molecular nitrogen in the air, and among living organisms, it is present in various organic forms. In soil, inorganic nitrogen is present as nitrates and ammonium ions, which are mostly derived from supplementation of fertilizers to the soil. Organic nitrogen in soil originates either from dead animals or plants or is derived from the excreta of the animals. Its source can also be the fertilizers. Some prokaryotic organisms also contribute to the nitrogen pool of the soil. These organisms are either free living or are growing in symbiotic associations with certain plants. These organic and inorganic forms of nitrogen are interconvertible which is facilitated by a number of processes responsible for the geochemical and biochemical cycle of nitrogen (Box 11.1).

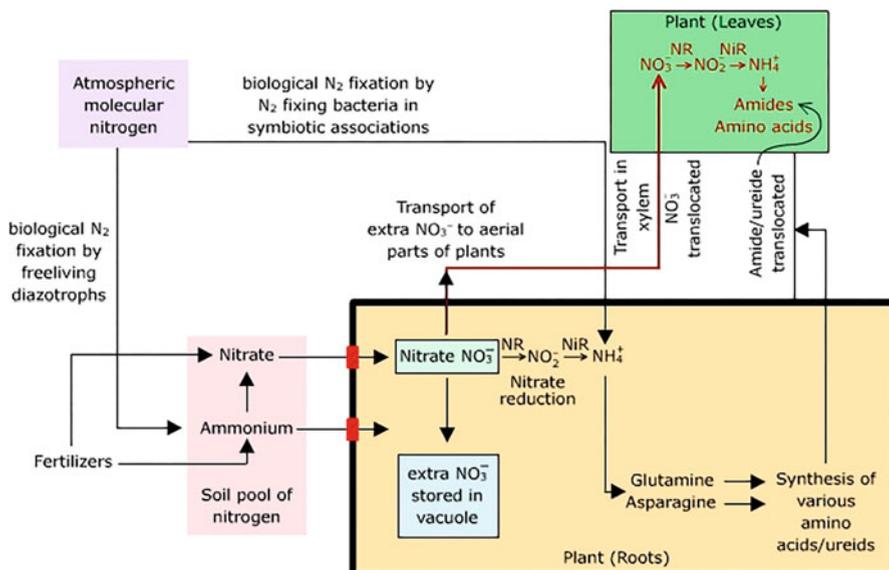


Fig. 11.2 An overview of nitrogen metabolism

Box 11.1: Oxidation States of Nitrogen

Oxidation state of an element (often called oxidation number) refers to the state of the element in a compound when it has either gained or lost electrons. Since, in free state, the number of protons is equal to number of electrons, oxidation state of the element will be zero. In bound forms, oxidation state of the element can be calculated by subtracting the number of electrons from the number of protons of the element. The number refers to number of lost or gained electrons, in which loss of an electron raises the oxidation state of the element by one. In similar way, addition of an electron lowers the oxidation state by number one and is known as reduction. In compound form, nitrogen can have the oxidation levels ranging from -3 (in ammonia) to $+5$ (in nitric oxide). In the following table, some of the nitrogenous compounds are listed along with the oxidation levels of nitrogen present in them.

Molecule/compounds	Name of the molecule/compounds	Oxidation level of nitrogen in the compounds
NH_3	Ammonia	-3
NH_2NH_2	Hydrazine	-2

(continued)

Box 11.1 (continued)

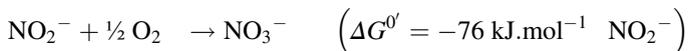
Molecule/compounds	Name of the molecule/compounds	Oxidation level of nitrogen in the compounds
NH ₂ OH	Hydroxylamine	-1
N ₂	Dinitrogen	0
N ₂ O	Dinitrogen monoxide	+1
NO	Nitrogen monoxide	+2
HNO ₂	Nitrous acid	+3
NO ₂	Nitrogen dioxide	+4
HNO ₃	Nitric acid	+5

11.1.1 Ammonification

It is the process by which organic nitrogen in the soil is broken down to ammonium ions. In soil, organic nitrogen is present as amino acids, nitrogenous bases, urea, uric acid, or in various other forms of biomolecules. Dead animals, plants, and the excretory products of animals are the major sources of organic forms of nitrogen. Proteins are hydrolyzed by **proteases** released from microorganisms in the soil, resulting in the release of amino acids by the process known as **proteolysis**. At the time of ammonification, amino acids undergo **oxidative deamination**, and organic nitrogen is released as ammonia. Microorganisms such as *Clostridium* sp., *Proteus* sp., *Micrococcus* sp. etc. are responsible for deamination. The process occurs under aerobic conditions. Ammonia produced in this process gets dissolved in water resulting in the formation of ammonium ions. In alkaline soil, H⁺ are not available, so NH₃ formed, which leaks out of the soil resulting in loss of soil nitrogen.

**11.1.2 Nitrification**

In acidic soils, ammonia exists as ammonium ions (NH₄⁺). Ammonium ions do not leach out of the soil because these are positively charged and are held by the negatively charged clay particles. Certain plants are capable of absorbing NH₄⁺. However, in most cases ammonium ions are converted to nitrates by a process known as nitrification. The nitrifying bacteria are **chemoautotrophs** which oxidize ammonium ions. Oxidation of ammonium ions is an exergonic reaction and the released energy is utilized by chemoautotrophs for biosynthetic reactions. Oxidation of ammonium ions occurs in two steps:



These two reactions are carried out by autotrophic bacteria which include *Nitrosomonas* sp. and *Nitrobacter*, respectively, which exist in well-aerated soil. The two reactions occur simultaneously so that there is no accumulation of nitrite in the soil. These reactions are the source of energy for bacteria to form ATP, which is used by them for fixation of CO_2 . The reactions are complex and are catalyzed by various enzymes such as **ammonia monooxygenase, hydroxylamine oxidoreductase, and nitrite oxidoreductase** present in these bacteria. The first two enzymes are present in *Nitrosomonas* and are required for oxidation of ammonium ions, while nitrite oxidoreductase required for the oxidation of nitrite to nitrate is present on the inner side of the cell membrane of *Nitrobacter*. In some microorganisms NO_3^- serves as the electron acceptor in place of O_2 during oxidation of the substrate which gets reduced to NH_4^+ in a process known as anaerobic nitrate ammonification or **nitrate respiration**.



Many bacteria do not reduce nitrogen for their own sake. Rather, there is a need to eliminate reducing equivalents produced during respiration. So, in place of O_2 , nitrate is used. This process is different from assimilatory nitrate reduction.

11.1.3 Denitrification

In some microorganisms, nitrate is degraded to molecular nitrogen under anaerobic conditions. NO_3^- serves as the electron acceptor in place of oxygen during oxidation of the substrates. This process is known as denitrification, and it occurs in the soil especially under anaerobic conditions. Nitrogen so formed escapes in the air.



In waterlogged soils, high nitrate concentration causes a great loss of soil nitrogen. Some of the denitrifying bacteria are *Thiobacillus denitrificans*, *Micrococcus denitrificans*, etc.

11.1.4 Nitrogen Fixation

Nitrogen pool of the soil is enriched from different sources. Atmospheric molecular nitrogen can be fixed by physical phenomenon, such as electrical discharge during

lightning. Fixed nitrogen reaches soil in the form of nitrates. Molecular nitrogen is also fixed by some organisms by a process known as biological nitrogen fixation. Biological nitrogen fixation is of considerable importance and is carried out by various prokaryotes, which are either free living or are growing in symbiotic association with the plants (Fig. 11.3).

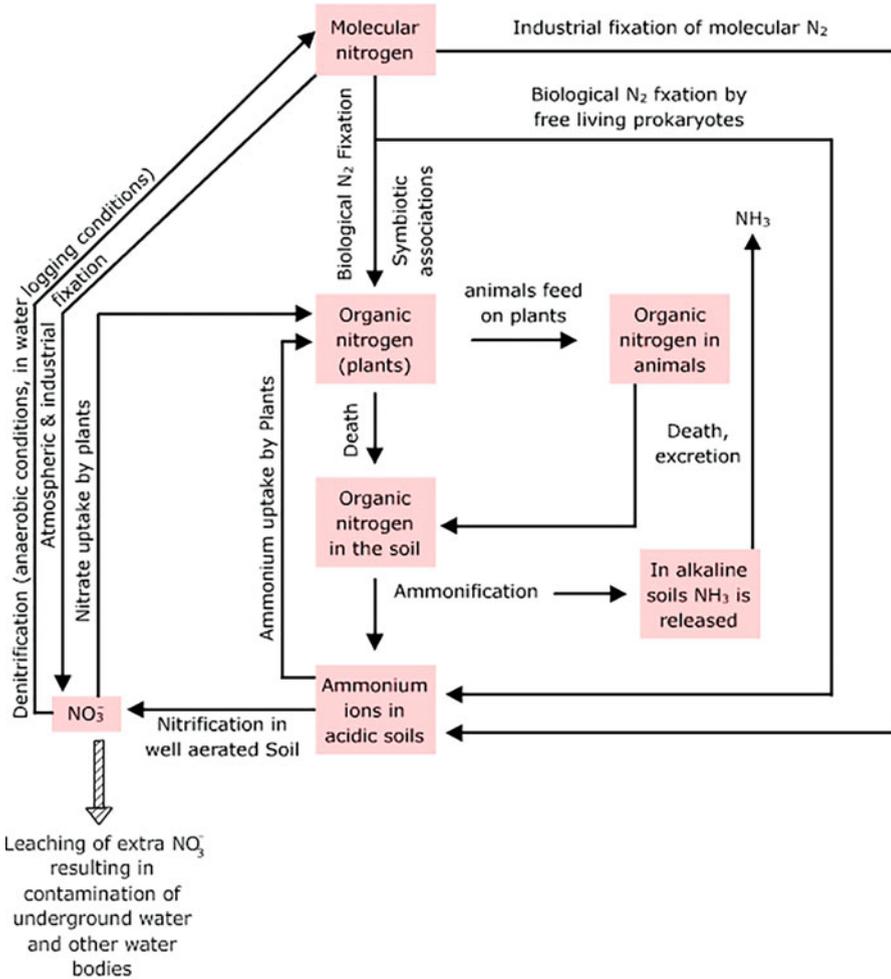


Fig. 11.3 Nitrogen cycle

11.2 Nitrogen Nutrition for the Plants

As stated in the previous section, animals obtain their nitrogen supply through food, whereas plants have to depend solely upon the supply of fixed nitrogen present in the **rhizosphere** as ammonium or nitrate ions. Mostly these ions are absorbed through roots except in case of nitrogen fertilizer which are applied as the foliar spray. It is only in prokaryotic organisms growing in associations (symbiotic or asymbiotic) with higher plants that free nitrogen can be fixed and made available to plants. In the following section, various forms of nitrogen available to plants and their uptake mechanisms are being discussed.

11.2.1 Ammonium Ions

Under waterlogged conditions and in acidic soils, nitrogen is available in the form of ammonium ions because of low rates of nitrification. Unlike nitrates, ammonium ions do not easily leach out from the soil. Since they are positively charged (NH_4^+), they bind to the soil particles. Plant roots can absorb ammonium form of nitrogen into the symplasm via ammonium transporters located on the plasma membrane. High ammonium concentrations suppress the expression of the genes encoding for nitrate transporters. There are two classes of ammonium transporters (AMT) in the plants—low-affinity transporters, which are not saturated, and high-affinity saturable transporters. In the low-affinity non-saturable transporters, there is a possibility of the involvement of the aquaporins or nonselective ion channels. Proteins of low-affinity ammonium transporters, however, have not been identified so far. High-affinity transporters include transporters encoded by the AMT/Rh gene family. K_m values of ammonium transporters range from 10 to 70 micromoles. Plant AMTs function as NH_4^+ uniporters. These transporters have multiple membrane-spanning domains. Five members of the gene family encoding AMT1 have been identified in *Arabidopsis*. All of them are expressed in roots, while two are expressed in shoots. NH_4^+ diffuses from soil to the symplasm of the root cells down the electrochemical gradient through the AMT1 (Fig. 11.4). Once inside the cytosol, some of NH_4^+ may be converted to NH_3 due to alkaline cytosolic pH, and it may enter the vacuoles since the membranes are permeable to ammonia. Ammonia may also enter the vacuoles through aquaporins called as **tonoplast intrinsic proteins (TIPs)**, located in the tonoplast membrane. Since NH_3 can bind with H^+ and is converted to NH_4^+ ions, it is trapped inside the vacuoles in ammonium form. NH_3 in the gaseous form can diffuse through the cell membranes and dissipate the proton gradient across the membranes, which is required for the transport of substances across the membrane. It may also be responsible for the dissipation of proton gradient created across the thylakoids by the photosynthetic electron transport or the proton gradient across inner mitochondrial membrane created during oxidative electron transport. The proton gradient across membranes is essential for ATP synthesis and transport of ions and other biomolecules. Since excess of ammonia/ammonium ions is toxic for the cells, animals have developed an aversion for ammonia (Box 11.2).

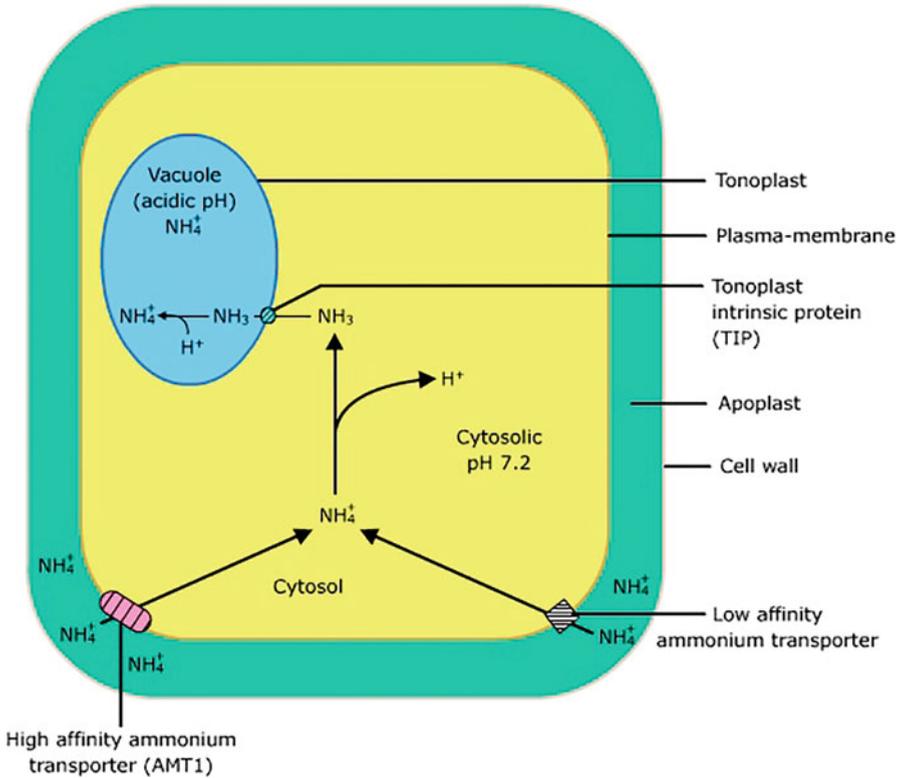


Fig. 11.4 Ammonium ion transport in the cell

Box 11.2: Toxicity of Ammonia

Ammonia is a polar molecule. It gets readily dissolved in water to form ammonium hydroxide, which is dissociated to form ammonium (NH_4^+) ions. It is the ammonia which is toxic and not the ammonium ions because the cell membrane is permeable to ammonia and it can combine with H^+ ion to form NH_4^+ and disturb the proton gradient across the membranes. Among animals, ammonia gets converted to lesser toxic forms such as urea and uric acid before it is excreted out.

11.2.2 Nitrate Uptake

Ammonium ions are short-lived in the soil as nitrifying bacteria convert them into nitrate ions. Nitrate is the most common form in which nitrogen is absorbed by plants from the soil. Generally, epidermal and cortical cells of the root absorb nitrate

Box 11.3: Toxicity Due to Excess Nitrates in Plants

Excess nitrate ions accumulated in plants may be dangerous to the humans or other animals feeding upon them. Nitrate may be reduced to nitrite, nitric oxide, or nitrosamines by the liver. Nitrosamines are potent carcinogens. Nitric oxide is a biosignaling molecule and is involved in many physiological processes, such as widening of blood vessels. Nitrite ions (NO_2^-), upon combining with hemoglobin, render it incapable of binding with oxygen, resulting in a disease known as methemoglobinemia. High nitrate concentration in plants is deterrent to herbivorous animals.

from the soil solution, although it can also be taken up from leaves during foliar application of fertilizers. Nitrate concentration in the soil may vary from micromolar to millimolar range. Nitrate may be derived in the soil from the nitrification of the organic forms or from fertilizer application. In spite of energy consumed for reduction of nitrate to ammonium in the plant, nitrate is still the preferred form of nitrogen because, unlike ammonium, excess nitrate can either be safely stored in the vacuoles up to a concentration $\geq 20\text{--}70$ mM or it can be transported to the leaves through xylem, where again it may be stored or metabolized (Box 11.3). If plants are supplied with ammonium nitrate as fertilizer, after plants have been subjected to nitrogen deficiency, accumulation of glutamine in cells will downregulate the transporter for ammonium ions, AMT1. Once inside the epidermal, cortical, or endodermal cells, further transport of nitrate into the root cells is mediated by two classes of symporters, located in plasma membrane and tonoplast, and an antiporter localized only in the tonoplast. In order to deal with varying concentrations of nitrates in soil and in tissues, plant cells possess nitrate transporters having different affinities for nitrates. On the basis of their affinity with nitrate, nitrate transporters are classified as high-affinity transporter systems (HATS) and low-affinity transporter systems (LATS). These two classes of transporters function at micromolar to millimolar nitrate concentrations. HATS function at 0.2–0.5 mM nitrate concentration, while LATS function above 0.5 mM and do not display any saturating effect for nitrate. HATS can either be inducible (iHATS) or constitutive (cHATS). Constitutive HATS are expressed even in the absence of nitrate, while inducible HATS are expressed only in the presence of nitrates. These transporters are expressed differently in different tissues and at various stages of development. Nitrate transporters consist of 12 transmembrane domains which are distributed in two sets of six helices in each, which are separated by a hydrophilic domain extending into the cytosol.

The gene families encoding the symporters that have been discovered so far are NRT1 and NRT2. NRT2 encodes for the inducible type of HATS nitrate transporters and is conserved in algae, fungi, and plants. NRT2 is downregulated by several reduced forms of nitrogen such as ammonium and glutamine. This helps the plant to regulate gene expression for transporters in response to changing needs of the plant. NRT2 is expressed mainly in roots. There are seven members in NRT2 gene family

in *Arabidopsis* genome. NRT1 gene family encodes both constitutive and inducible nitrate transporters and is more complex. Fifty-three members of this gene family have been identified in *Arabidopsis* genome so far. These are mainly expressed in roots and encode either low-affinity or high-affinity nitrate transporters. Nitrate uptake, mediated by symporters present in plasma membrane, is driven by H^+ gradient. It is a $2H^+/NO_3^-$ symport. H^+ gradient is created across the plasma membrane by the ATPase-mediated H^+ pumps. Flow of H^+ in response to their gradient is coupled with nitrate uptake. NO_3^- concentration in the cytosol is maintained by translocating excess nitrate through xylem or by storing it in vacuoles. One class of nitrate transporters present in tonoplast belongs to **chloride channel family (CLC)** (Fig. 11.5). CLC can be either H^+ gated anion channels or $2NO_3^-/H^+$ antiporter. It functions to transport nitrate across tonoplast. CLC can have dual-affinity system. On being phosphorylated at threonine (at position 101), it functions

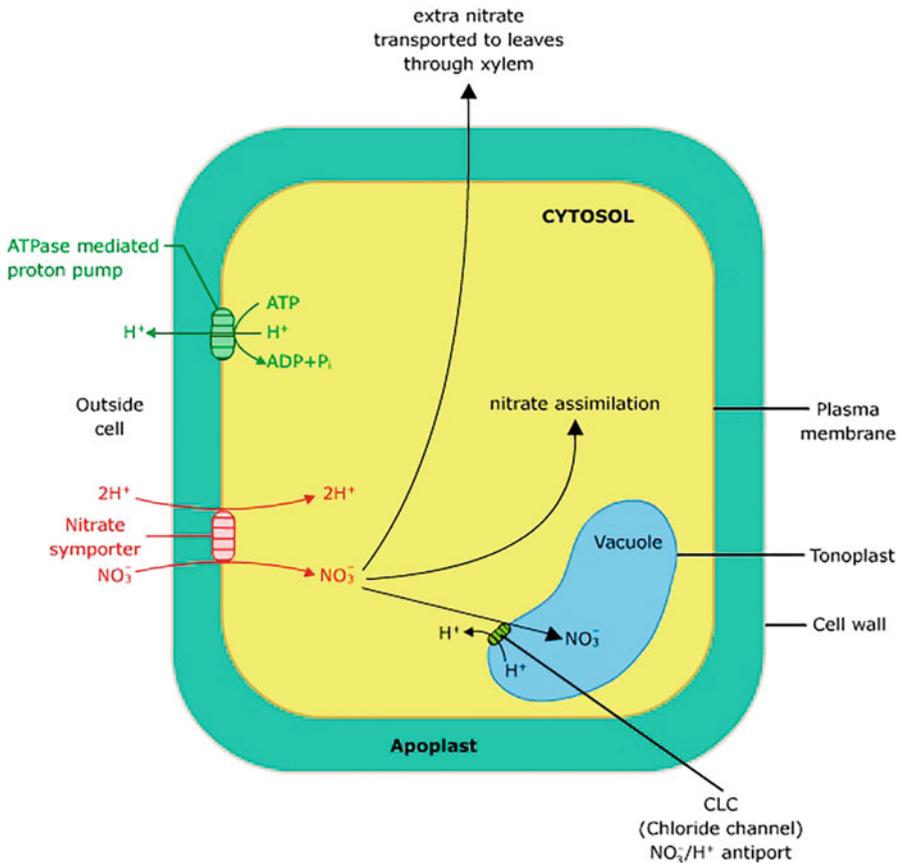


Fig. 11.5 Nitrate uptake by a root cell

as high-affinity system, while its dephosphorylation makes it a low-affinity transporter. Homeostasis of nitrate in cytosol is determined by the balance between nitrate uptake, nitrate efflux, reduction of nitrate, or storage of nitrate in the vacuoles.

11.2.3 Nitrate Assimilation

Nitrate reduction occurs throughout in all parts of the plant. However, some species, such as white clover (*Trifolium repens*) or cranberry (*Vaccinium macrocarpon*), exhibit nitrate reduction in roots only. In cocklebur (*Xanthium* sp.), it occurs in leaves only. Distribution of NR activity within plant is also governed by nitrate concentrations of available nitrate. At low concentrations (up to 1 mM), nitrate is directly assimilated in the roots of temperate legumes. If NO_3^- concentration is higher than 1 mM, its transportation to shoot becomes significant. NO_3^- transport occurs via xylem sap. In tropical and subtropical cereals and grain legumes, nitrate assimilation occurs in shoot irrespective of the external concentration of nitrate. In maize, nitrate reduction occurs in mesophyll cells (and not in bundle sheath cell) since mesophyll cells have greater capacity for generating reductant [NADPH], which is required for nitrate reduction.

Nitrate assimilation occurs in two steps (Fig. 11.6). First step requires reduction of nitrate to nitrite. It occurs in the cytosol of the cells and is catalyzed by the enzyme nitrate reductase (NR). However, nitrite reduction to ammonium occurs in plastids since the enzyme nitrite reductase (NiR) is located in the plastids.



Nitrate Reductase (NR) Reduction of nitrate to nitrite requires transfer of two electrons from the electron donor to NO_3^- and consumption of one proton. Reaction is coupled with oxidation of NADH or NADPH, which serves as electron donor for the reaction in most of the plants. NADH specific reduction of NO_3^- occurs in land plants and algae, while NADPH is used as the electron donor in fungi. However, some NR forms in land plants and algae are able to use either NADH or NADPH as the reductant in the reaction. The reaction occurs in cytosol of the cell. Active form of NR is a **homodimer**. It has the molecular weight of about 100–114 kDa. Each monomeric unit consists of about 1000 amino acids. It has three domains associated with cofactors. The three cofactors are molybdenum cofactor (MoCo), heme Fe, and FAD. N-terminal region of each monomeric unit is associated with MoCo, while the C-terminal region of each monomeric unit is associated with the cofactor FAD. Central region of each polypeptide unit is complexed with heme Fe. MoCo center is the molybdenum complexed with organic molecule **pterin** (Box 11.4). Heme Fe contains cytochrome b_5 . FAD-binding domain consists of 260–265 amino acids;

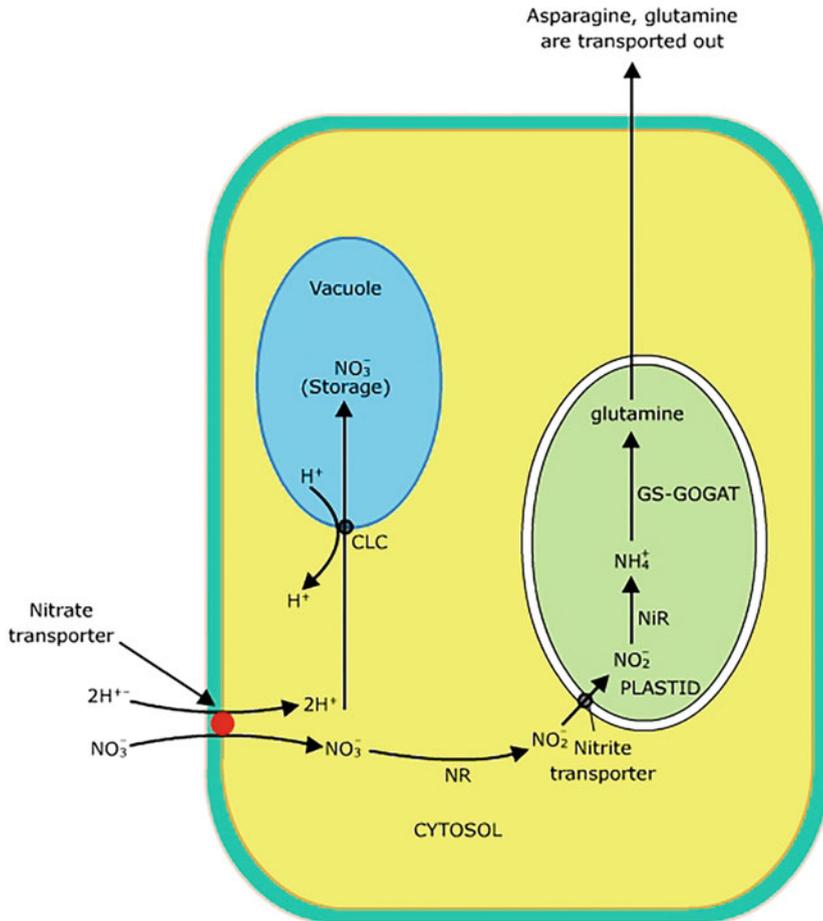


Fig. 11.6 Fate of nitrate in a plant cell. Nitrate is assimilated by nitrate reductase (NR) in cytosol and nitrite reductase (NiR) in plastids. Excess nitrate is stored in vacuoles. Ammonium (NH_4^+) ions are assimilated in plastids

central **heme** domain consists of 75–80 residues, while MoCo-binding domain of enzyme protein is large and consists of 360–370 amino acids. Each domain of the enzyme is an independent unit and is believed to belong to a distinct protein family. These three domains of each monomeric unit of the homodimeric enzyme are associated with hinge (hI and hII) regions, hI being present between MoCo center and heme domain of the polypeptide, while hII is present between heme domain and the domain complexed with FAD. hI region exhibits a regulatory role in maintaining enzyme activity (Fig. 11.7).

Box 11.4: Molybdopterin

Molybdenum is a transition element. It is catalytically active when complexed with the cofactor pterin (molybdopterin). Molybdenum cofactor is known as MoCo. Pterin-bound Mo is the cofactor for many plant enzymes involved in redox reactions, e.g., xanthine dehydrogenase, sulfite oxidase, nitrate reductase, etc. Sulfite oxidase has sequence quite similar to NR. It has a cytochrome b_5 , heme domain attached to it, but contrary to NR (where heme terminal is C-terminal to MoCo domain), heme domain of sulfite oxidase is N-terminal to MoCo domain.

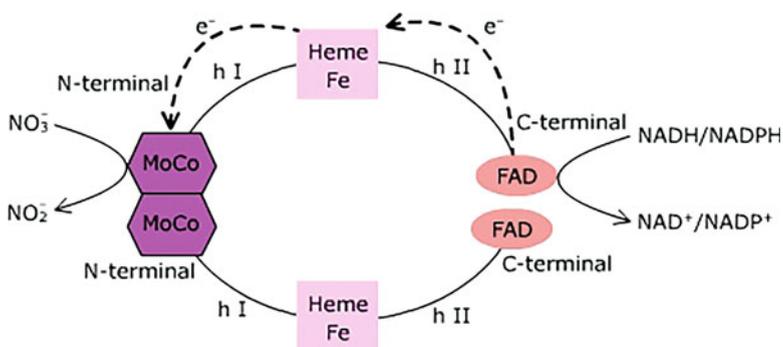
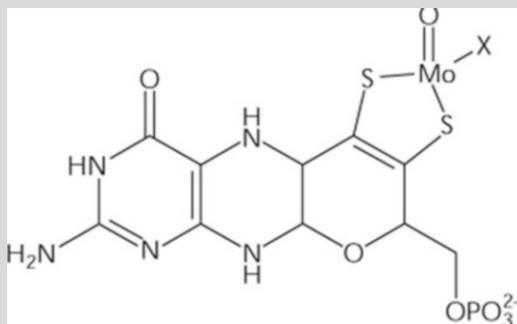
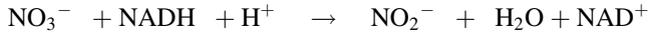


Fig. 11.7 Nitrate reductase (NR) is a homodimer. Each monomeric unit has three domains associated with FAD at the C-terminal, MoCo (molybdenum cofactor) with N-terminal, and central region complexed with Heme Fe. The three domains of each monomeric unit is associated with hinge regions hI and hII. Electron transfer from NADH/NADPH at the C-terminus is facilitated by the thiol group of cysteine of the polypeptide. MoCo facilitates transfer of electron to NO_3^- at the active site present at the N-terminal of the protein

Reduction of NO_3^- by NR requires transfer of two electrons and one proton from NADH/NADPH; another proton is obtained from the medium.



Midpoint potentials of the three centers are -272 mV for FAD, -160 mV for heme, and -10 mV for MoCo center. Thiol group of cysteine present at the C-terminus of the enzyme has been found to facilitate transfer of electrons between NADH/NADPH and FAD (the cofactor complexed with the enzyme protein at the C-terminus). However, the presence of cysteine at the C-terminus of the enzyme has not been found to be mandatory. In case cysteine is substituted with serine, even then the enzyme is able to catalyze electron transfer but with lesser affinity. FAD-binding domain is believed to belong to the **ferredoxin-NADP reductase (FNR)** family of oxidoreductases. Reduction of FAD cofactor is followed by reduction of heme, the central domain of enzyme belonging to the **cytochrome b_5 family** of proteins, which further passes electrons to MoCo center. The domain containing MoCo factor belongs to the family of enzymes which includes xanthine oxidase and sulfite oxidase. Reduced molybdenum passes the electrons to NO_3^- at the active site of the enzyme. NR functions as mini electron transport chain oxidizing NADH/NADPH and reducing the terminal electron acceptor NO_3^- (Fig. 11.8).

Regulation of NR Activity NR activity is highly regulated. Regulation may be at the transcription level of gene NIA, a nuclear gene which encodes NR. Regulation at the transcription level is time requiring (may be hours or days), while at the post-translational level, enzyme activity is regulated within minutes. In 1957, Tang and Wu had demonstrated that NR is a substrate-inducible enzyme. NO_3^- induces not only NR synthesis but also regulates the transcription of many other genes. Genes involved for synthesis of nitrate transporters, Fd, enzymes of pentose phosphate pathway, and PEP carboxylase are upregulated by nitrates. Genes, which are involved in the synthesis of enzymes required for the storage of carbohydrates, are

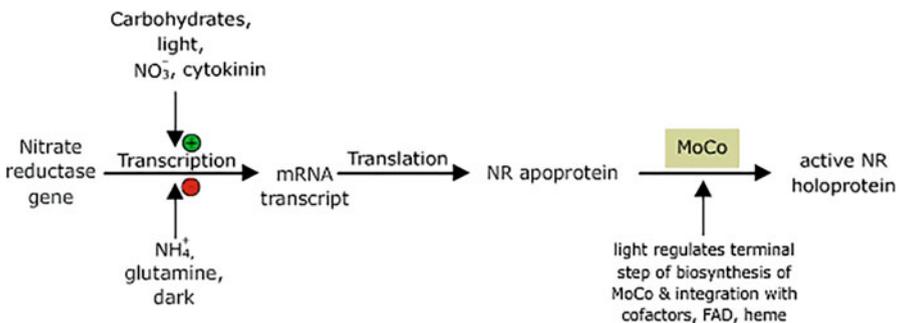


Fig. 11.8 Regulation of biosynthesis of nitrate reductase (NR). Red circle represents inhibition and green circle represents promoting effect on gene transcription

downregulated, while genes involved in the synthesis of amino acids and organic acids are upregulated. Thus, NR is responsible for redirecting the carbon from storage as carbohydrates to synthesis of amino acids. In *Arabidopsis*, NO_3^- induces the synthesis of MoCo factor as well. NO_3^- also regulates the synthesis for NiR, so that NO_2^- generated in nitrate reduction does not accumulate. Since nitrite is toxic for the cell it must be assimilated fast. NR activity is highly regulated. Regulation may be at the transcription level of gene NIA, a nuclear gene which encodes NR. Regulation at the transcription level is time requiring (may be hours or days), while at the post-translational level, enzyme activity is regulated within minutes. In 1957, Tang and Wu had demonstrated that NR is a substrate-inducible enzyme. NO_3^- induces not only NR synthesis, but also regulates the transcription of many other genes. Genes involved for synthesis of nitrate transporters, Fd, enzymes of pentose phosphate pathway and PEP carboxylase are upregulated by nitrates. Genes, which are involved in synthesis of enzymes required for the storage of carbohydrates are downregulated while genes involved in synthesis of amino acids and organic acids are upregulated. Thus, NR is responsible for redirecting the carbon from storage as carbohydrates to synthesis of amino acids. In *Arabidopsis*, NO_3^- induces the synthesis of MoCo factor as well. NO_3^- also regulates the synthesis for NiR, so that NO_2^- generated in nitrate reduction does not accumulate. Since nitrite is toxic for the cell, it must be assimilated fast. Light stimulates transcription of NIA. In some of the cases, light requirement can be replaced by providing sucrose in the dark. Besides generation of reductants and photosynthates, light may also be acting through signaling pathways of photomorphogenesis. Once transcription has been initiated, mRNA transcript exhibits diurnal fluctuations which shows possible involvement of phytochrome. At the end of the dark period, there is decrease in mRNA transcript of NR, which may be because of inhibition of transcription of NR gene due to accumulation of glutamine. Post-translational regulation of NR is very quick and occurs within minutes. It occurs through phosphorylation and dephosphorylation of the enzyme protein. The phosphorylated enzyme protein is inactive, while dephosphorylated form of the enzyme protein is active. ATP-dependent phosphorylation occurs at one specific conserved serine residue present in hI region (in spinach it is serine₅₄₃) and is catalyzed by Ca^{2+} -dependent NR kinase. Activity of NR kinase is inhibited by light, triose phosphates, and hexose phosphates. Post-translational regulation of the enzyme activity is mediated by a highly conserved 14-3-3 class of regulatory proteins. These proteins (14-3-3) are inactivator proteins, and the name 14-3-3 comes from the separation pattern of the proteins during purification (Box 11.5). Binding of 14-3-3 proteins with phosphorylated serine present in hI region of the enzyme interferes with the electron transfer in between the heme and MoCo cofactor of the enzyme. NR/14-3-3 complex formation is promoted in dark due to accumulation of Mg^{2+} , while it is broken down in light due to low Mg^{2+} concentrations. This NR/14-3-3 complex is sensitive to attack by cytosolic proteases. Dephosphorylated NR protein prevents 14-3-3 regulatory proteins from binding with the enzyme protein. Dephosphorylation of NR is catalyzed by a protein phosphatase (PP2A). Dephosphorylation of the

Box 11.5: 14-3-3 Proteins

14-3-3 proteins belong to a class of conserved multifunctional regulatory proteins. These proteins are present in animals and plants. These specifically bind to proteins, which have got a specific binding site of six amino acids with phosphorylated serine being present at position 6. The name 14-3-3 refers to particular elution fraction number (14) and band position (3.3) while purifying the protein from bovine brain homogenate on DEAE cellulose chromatography. A large number of gene families of 14-3-3 proteins have been identified. These are involved in diverse functions in plants, including regulation of plasma H^+ -ATP pumps, transmembrane receptors, sucrose synthetase, and nitrate reductase. They have a potential role in regulating plant development in response to abiotic and biotic stress conditions as these regulatory proteins bind to various protein kinases. One of the examples for the role played by 14-3-3 class of proteins has been studied with reference to attack on plant by the fungus *Fusicoccum* spp. the substance produced by the fungus is fusicoccin which specifically binds to 14-3-3 binding sites of various proteins, thereby interfering with the regulatory functions of 14-3-3 proteins. This results in the disruption of the metabolism of the plant to such an extent that the plant does not survive.

enzyme protein is also promoted by AMP and iP. This reversible post-translational regulation of enzyme activity occurs within minutes of exposing plants to light or darkness or stress conditions. (Fig. 11.9).

Nitrite Reductase Nitrite produced as a result of nitrate reduction is immediately transported from the cytosol into the plastids, chloroplasts in green tissues, and plastids in nongreen tissues where it is further reduced. Efforts are underway for the identification of nitrite transporters in higher plants. Initially diffusion of nitrite was proposed. However, very low cytosolic nitrite concentration does not justify its diffusion across inner chloroplast membrane. Furthermore, saturation kinetics of nitrite transport suggests the presence of a nitrite transporter in the thylakoid membranes of the chloroplasts. In cucumber, the existence of transporter—Nitr1—belonging to **proton-dependent oligopeptide transporters (POT)**, has been suggested. However, it has been suggested that in *Arabidopsis* a member of **CLC family** (chloride channel) is involved.

Reduction of nitrite occurs in a single step, catalyzed by the enzyme nitrite reductase (NiR). Nitrite reduction requires transfer of six electrons. Reduced ferredoxin is used as the donor for electrons, and eight protons are obtained from the medium.

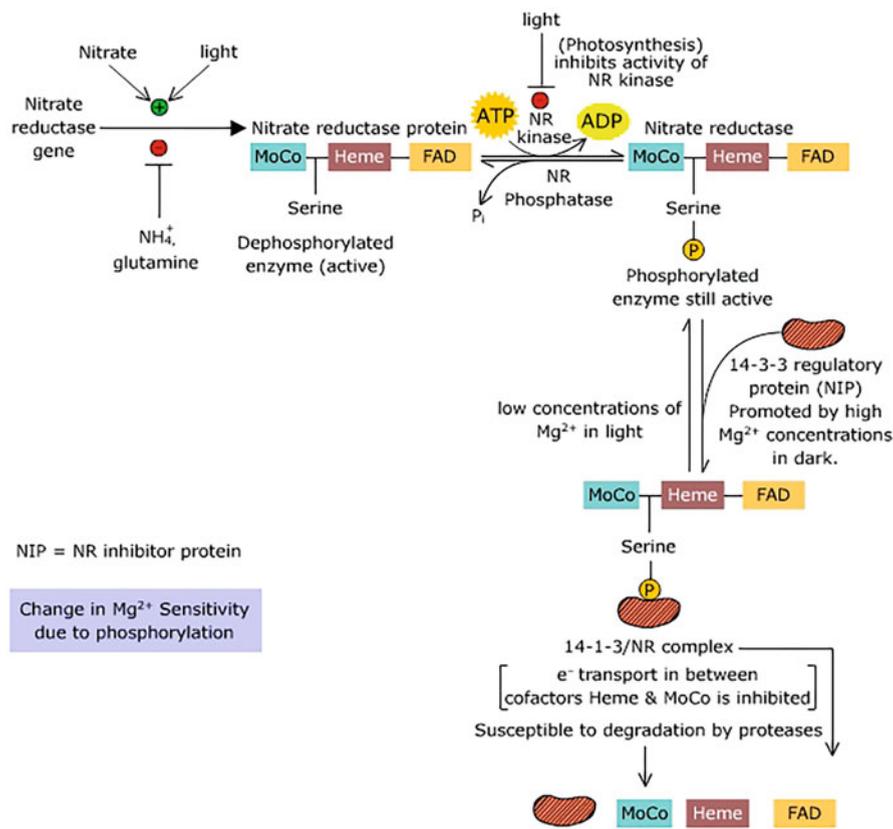
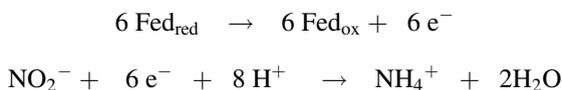


Fig. 11.9 Post-translational regulation of NR. Phosphorylation of NR enables binding of 14-3-3 regulatory protein which makes the NR protein susceptible to degradation by proteases



The reduced ferredoxin used in NO_2^{-} reduction is generated in chloroplasts during light reaction of photosynthesis. In nongreen tissues, such as roots, reduced ferredoxin is produced from NADPH, which is generated during oxidative pentose phosphate pathway. Ferredoxin in non-photosynthetic tissues has been found to be more electropositive than ferredoxin of the chloroplasts. Reduction of ferredoxin by NADPH in nongreen tissues is facilitated by the enzyme **ferredoxin-NADP reductase**. Since nitrite is toxic, it should not accumulate in the cells. That is why activity of NiR (expressed as per fresh gram weight basis) is several times higher than the activity of NR. This ensures no accumulation of nitrite in the cells. NiR is a constitutive enzyme. In some plants NiR synthesis is also induced by nitrate. NiR is encoded in nuclear genome, and the transcribed mRNA is translated by cytosolic

ribosomes. Cytosolic NiR proteins carry a **transit polypeptide** sequence at the N-terminal, which is identified at the plastid surface allowing the entry to the NiR protein. The transit sequence present at the N-terminal is cleaved while the polypeptide enters through the membranes of the plastid. NiR consists of single polypeptide having molecular weight of about 60–70 kDa. It consists of two domains. One domain present at the N-terminal of the polypeptide is the ferredoxin-binding domain. There are three lysine residues present at the N-terminal domain of the polypeptide, which are required for binding with ferredoxin. The other domain is at the C-terminal of the polypeptide. It has a single cluster of [4Fe-4S] and a **siroheme** as the prosthetic group. These two cofactors are in close proximity bridged by sulfur ligand. There are four cysteine molecules associated in two clusters on the polypeptide, which provide the bridging ligand and sulfur ligands for the 4Fe-4S cluster. Siroheme serves as the binding site for NO_2^- . In plants, reduced Fd is used as the reductant, but in bacteria and fungi, NADPH is used instead (Fig. 11.10).

11.2.4 Fixation of Molecular Nitrogen

Molecular nitrogen is an inert gas since the two atoms of nitrogen are linked to each other by three covalent bonds. For industrial production of fertilizers, chemical fixation of molecular nitrogen is required by Haber Bosch process in which NH_3 is synthesized from N_2 and H_2 in presence of iron catalyst at a temperature of 400–650 °C and at a pressure of about 150 to 400 atm. However, nitrogen-fixing bacteria can fix the molecular nitrogen to ammonia at ambient temperature and pressure. Nitrogen fixation by the prokaryotes is known as biological nitrogen fixation. Almost 80–90% of the nitrogen available to the plants originates through biological nitrogen fixation. Of the biologically fixed nitrogen, a major fraction is fixed through symbiotic associations. Process of nitrogen fixation is limited to prokaryotes only. The organisms which fix molecular nitrogen are known as

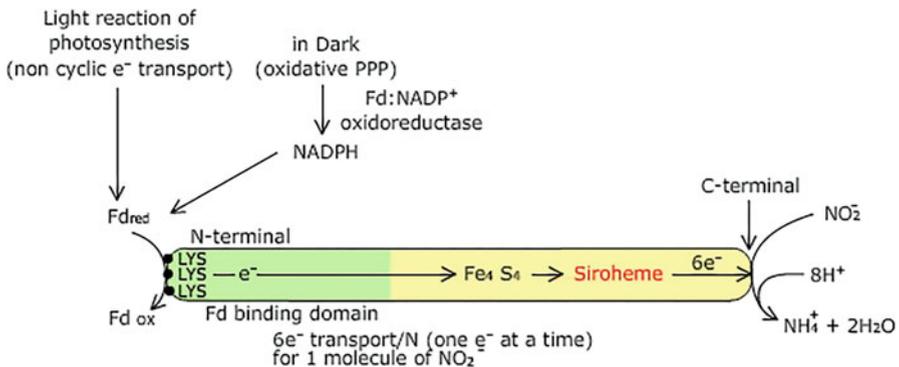


Fig. 11.10 Nitrite reductase (NiR) structure. NiR (Fd nitrite oxidoreductase) has two functional domains, Fd-binding domain at N-terminal and nitrite-binding domain at C-terminal

diazotrophs (diazo, molecular nitrogen; trophs, eater). Free-living diazotrophs include prokaryotes in the soil without any type of associations with plants. Aerobic nitrogen-fixing bacteria, such as *Azotobacter*, maintain low partial pressure of oxygen at the site of nitrogen fixation by maintaining high respiration rate and in the process also provide ATP as the energy source. *Gleotheca* evolve photosynthetic oxygen during day while fix nitrogen during night. Cyanobacteria such as *Anabaena* and *Nostoc* grow in flooded rice fields and can fix nitrogen in anaerobic conditions. Anaerobic conditions can also be created in the heterocystous forms of cyanobacteria. Heterocysts are the specialized cells of these organisms since photosystem II is not present in them and there is no photosynthetic oxygen evolution in these cells. Thus, anaerobic conditions prevail which are required for nitrogen fixation. Diazotrophs also include facultative anaerobes (e.g., *Klebsiella*) which can fix nitrogen only under anaerobic conditions. However, there are obligate anaerobes, which grow and fix nitrogen only under anaerobic conditions. These anaerobes can be photosynthetic (e.g., *Rhodospirillum* sp.) or non-photosynthetic (e.g., *Clostridium* sp.).

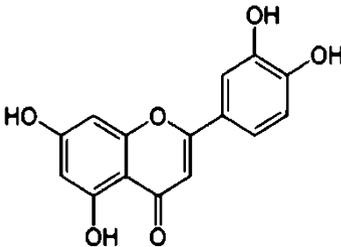
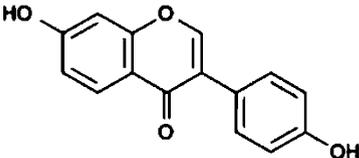
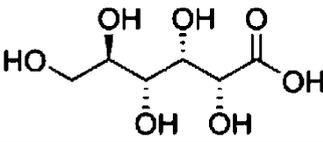
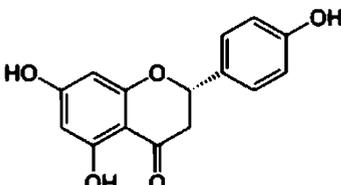
Diazotrophs also fix nitrogen in symbiotic associations with plants and in turn are benefitted from the host plant in getting the nutrients, carbon skeletons, and the energy source required for their growth and nitrogen fixation. These are classified under three categories. The first category includes symbiotic association between cyanobacteria (e.g., *Anabaena*) and diverse group of plants. This includes liverworts (such as *Anthoceros*), coralloid roots of cycads, *Azolla* (a water fern in which *Anabaena* grow in the leaf cavities), and one angiosperm genus, i.e., *Gunnera* in which *Anabaena* is present in the glands found at the base of leaf petioles. *Azolla* meets the nitrogen requirement of the rice plants when grown along with them. Second category includes the actinorhizal symbiosis. It is the symbiotic association of *Frankia* species with various dicots. *Frankia* are the gram-positive actinomycetes, which grow in symbiotic associations with as many as 20 genera of dicots belonging to 8 or more families. These include *Alnus*, *Casuarina*, *Ceanothus*, and many other species. These plants are known as actinorhizal plants. This symbiotic association plays an important role in maintaining the nitrogen content of soil in forests. The third category includes the symbiotic association of rhizobia with plants. Unlike *Frankia* spp. rhizobia are gram-negative bacteria, which in most cases form symbiotic associations with legumes (Fabaceae). One non-leguminous plant (*Parasponia* spp. family Ulmaceae) also forms symbiotic associations with rhizobia. In both actinorhizal and rhizobial symbiotic associations, root nodules are formed due to division of cortical and hypodermal cells. In grasses as well, symbiotic associations develop but without any nodule development. In this case nitrogen-fixing organism either anchors on the root surface near the elongation zone or it sticks on the root hairs. These may also colonize the plant tissues as **endophytes**. This category of nitrogen fixers includes *Acetobacter diazotrophicus* and *Herbaspirillum* spp. Nitrogen-fixing bacteria colonize in the apoplasmic regions in the stem tissues of sugarcane. These diazotrophs in symbiotic association meet 30% of the nitrogen requirement of the sugarcane plant (Table 11.1).

Table 11.1 Diversity of diazotrophs

Diazotrophs	Types of diazotrophs	Examples
<i>Free-living diazotrophs</i>		
	Cyanobacteria	
	Heterocystous forms	<i>Anabaena</i> sp., <i>Nostoc</i> sp.
	Non-heterocystous forms	<i>Gloeotheca</i> sp.
	Other bacteria	
	Obligate aerobic	<i>Azotobacter vinelandii</i>
	Facultative aerobic	<i>Klebsiella pneumoniae</i>
	Anaerobic	<i>Clostridium pasteurianum</i>
	Photosynthetic bacteria	<i>Rhodospirillum</i> sp.
<i>Diazotrophs in symbiotic associations</i>		
	Endosymbiotic associations (nodule formation)	
	Legumes	<i>Rhizobium</i> , <i>Azorhizobium</i> , <i>Bradyrhizobium</i> , <i>Mesorhizobium</i>
	Non-legumes symbiosis	
	<i>Parasponia</i> sp.	<i>Bradyrhizobium</i> sp.
	Actinorhizal (<i>Alnus</i> sp.)	<i>Frankia</i> sp.
	Endosymbiotic (no nodules formed)	
	<i>Gunnera</i> sp. (Angiosperm)	<i>Nostoc</i> sp.
	<i>Azolla</i> (Pteridophyte)	<i>Anabaena</i> sp.
	Associative symbiosis	
	Sugarcane	<i>Acetobacter</i> sp.

Legume Rhizobia Symbiosis This is the most common type of symbiosis, which occurs between the rhizobia (includes species of *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) and leguminous plants. Symbiosis is not obligatory as either member of the symbiotic association can survive independently. However, both partners are benefitted from each other. Rhizobia are free-living bacteria in the soil and can multiply without the host plant, but they fix nitrogen only after forming symbiotic association with the host plant. Legumes develop nodules to encase the rhizobia, which develop well only when they are grown in nitrogen-poor soils. This symbiotic association is of commercial significance since it contributes nitrogen in the form of proteins to the seeds, such as soybean, lentil, peanut, and also to the forage crops such as alfalfa (*Medicago sativa*) and clover (*Trifolium* sp.) Nodulation process begins with identification of the specific host plant by a particular rhizobial species. The symbiotic association is very specific since one particular species of rhizobia (also called biovar) infects either only specific host plant or its range of the host plants is very small. This specificity is because of interaction of certain chemical signals between the bacteria and the host plant, which causes changes in gene expression in both the symbiotic partners resulting in effective nodulation.

Table 11.2 Some of the *Rhizobium* strains and their specific hosts releasing chemotactic compound

Rhizobial strain	Host plants	Nod factor inducer released by the plant
<i>Sinorhizobium meliloti</i>	<i>Medicago truncatula</i>	Luteolin 
<i>Bradyrhizobium japonicum</i>	<i>Glycine</i> (soybean) <i>Vigna</i> (cowpea)	Daidzein 
<i>Mesorhizobium loti</i>	<i>Lotus japonicus</i> , <i>Lupinus</i>	Aldonic acid 
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>	<i>Vicia</i> , <i>Pisum</i>	Flavanone 

Steps of Nodulation

1. The first step of identification begins with the release of specific signals by the host plant which generally are flavonoids and secondary metabolites derived from **phenylpropanoid pathway**. Specific flavonoids, released by the prospective host plant, are recognized by compatible rhizobia (Table 11.2).
2. These flavonoids are recognized by the specific rhizobial spp. followed by synthesis of nodulation factors (Nod) by the bacteria. Synthesis of Nod factors is under the regulation of nodulation (*nod*) genes of the bacteria (Box 11.6).
3. Nod factors produced by rhizobia are recognized by the receptors present in the membrane of root hairs of the host plant. After recognition of Nod factors, various

changes in plant-specific proteins (nodulins) are produced within the host plant which trigger physiological responses associated with nodulation process. First step is the curling of the root hair causing trapping of the bacteria inside the curl.

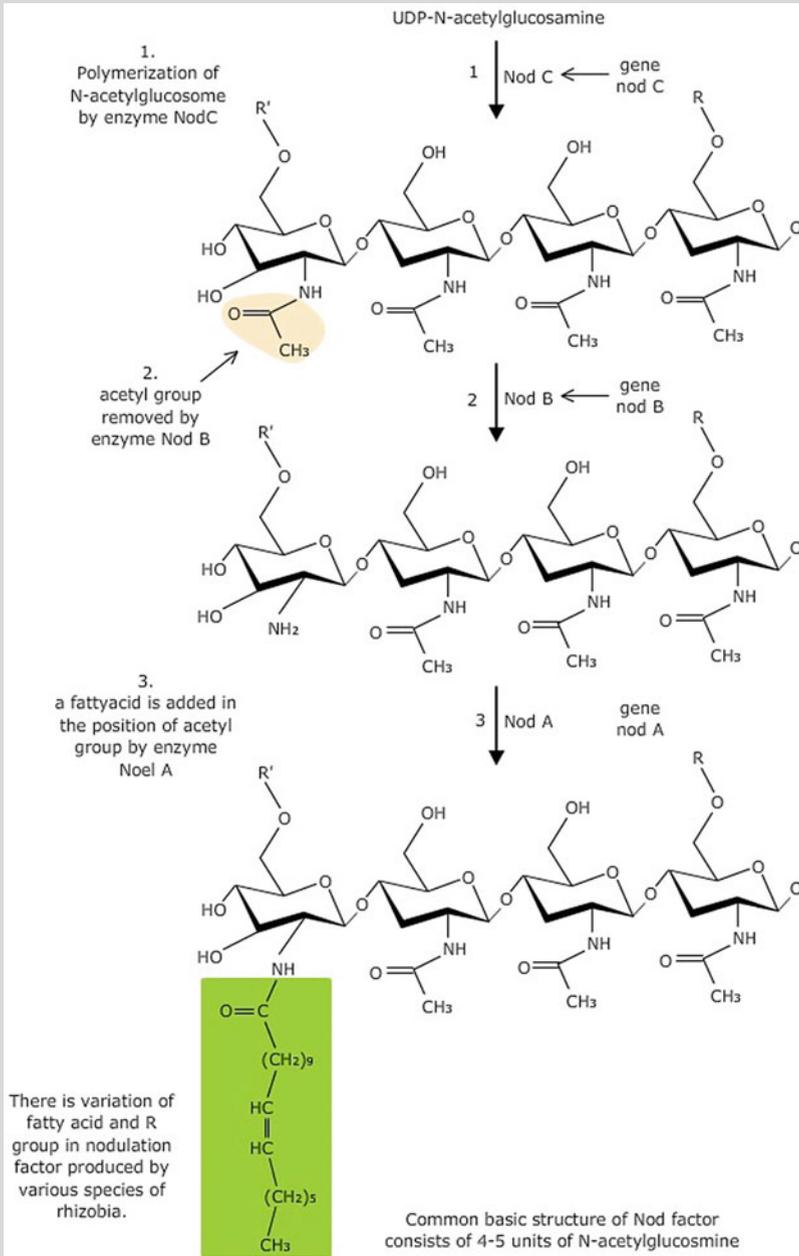
4. Bacteria enter the root hair through infection thread which is formed as a result of invagination of the plasma membrane of the root hair cell. Formation of the infection thread is followed by triggering of cell division in the cortical or cambial cells of the root, causing nodule development.
5. Bacteria reach these dividing cells, which later on develop into nodules, through the growth of infection thread and are released while still enclosed by the cell membrane of the host plant. These bacteria are differentiated into bacteroids inside **symbiosome**. Bacteroids do not divide further and are capable to fix nitrogen.
6. Nodule-specific proteins (nodulins) are synthesized as a result of expression of the nodulin genes of the host plant (Fig. 11.11).

Box 11.6: Nod Factors

The core structure of Nod factor consists of a backbone of three to four N-acetyl-D-glucosamine which are linked by β -1,4 linkages. It is linked to a fatty acid on the terminal sugar. Synthesis of core Nod factor is under the control of the products of three common *nod* genes, i.e., *nodA*, *nodB*, and *nodC* (NodA, NodB, and NodC, respectively). NodC catalyzes polymerization of N-acetyl-D-glucosamine. NodB removes the acetyl group from the end glucosamine residue followed by addition of the fatty acyl group to the free amino group of the oligomer by NodA. Further alterations are done in the core structure of this polysaccharide by the products of the host-specific *nod* genes of the bacteria. The alterations include changes in the length and saturation of the fatty acyl group or addition of the groups which determine the host specificity. The host-specific genes *nodE* and *nodF* encode the enzymes required for the synthesis of factors NodE and NodF. These enzymes catalyze the synthesis of an 18:4 and 16:2 fatty acyl groups, respectively. The enzyme product of *nodL* catalyzes the synthesis of factor NodL through addition of specific groups at the reducing and nonreducing N-acetylglucosamine residues of the Nod factors. This includes C6 modification on the reducing and nonreducing ends. This may include N-methylation and O-substitution of the inner N-acetyl-D-glucosamine residues or other modifications. These modifications help the bacteria to determine its host specificity. The rhizobia infecting broad range of the hosts produce multiple Nod factors.

(continued)

Box 11.6 (continued)



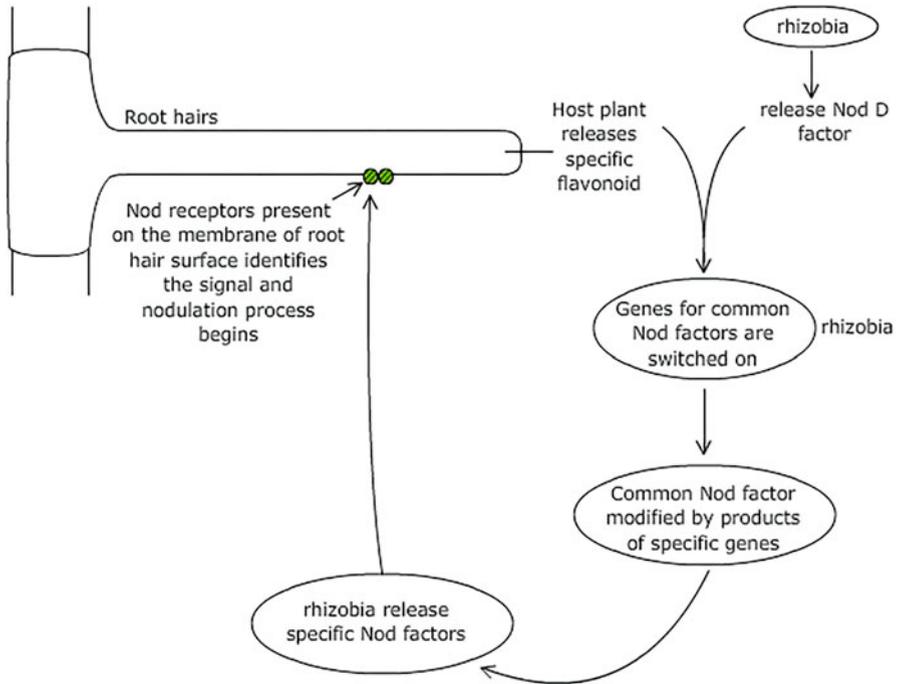


Fig. 11.11 Nodulation process triggered by flavonoid synthesis by the host plant

Only about 1–5% of root hairs become infected, and about 20% of these infections result in nodules. Plants produce chitinase that breaks down Nod factors of incompatible rhizobia sp. Chitinase produced later during the infection process also acts against rhizobia to control further nodulation. The biochemical signal molecules released by the prospective host plant induce gene expression in rhizobia, which results in the synthesis of Nod factors. Nod factors are **lipochitooligosaccharides (LCO)**. Rhizobial genes responsible for the synthesis of Nod factors are called *nod* genes. Expression of *nod* genes in bacteria depends on the interaction of a transcription inducer NodD in bacteria and chemical signals released by the prospective host plant. The gene responsible for the expression of the regulatory factor NodD is *nodD*, which is expressed constitutively. It regulates the transcription of other *nod* genes, which are of two types, the common *nod* genes and host-specific *nod* genes. Common *nod* genes include *nodA*, *nodB*, and *nodC*. These common nod genes are present in all rhizobial strains and encode enzymes required for the synthesis of basic structure of the Nod factor. The host-specific nod genes include *nodP*, *nodQ*, and *nodH* or *nodF*, *nodE*, and *nodL*. These host-specific genes differ in rhizobial sp. These are involved in the modification of the basic structure of

Nod factors. These modified Nod factors are recognized by the legume receptors present in plasma membrane of root hairs of specific host. Besides these, the invading bacteria may also require some extracellular polysaccharides (EPS) for their recognition by the host plant for nodule formation. For example, **succinoglycan** (EPS-I) produced by *Sinorhizobium meliloti* is required for the invasion of its host plant *Medicago* sp.

Nodulins Nodulins are the proteins synthesized by the host plant in response to bacterial infection. These are required for effective nodulation of host plants and for establishing symbiotic associations with the invading bacteria. The host specificity is determined by the interaction of specific Nod factors with plant receptors present on the plasma membrane of root hairs. The very first response of the host to specific rhizobial sp. is expressed as root hair curling. The proteins, which are essential for the initial identification of the Nod factor, have been identified in *Lotus* mutants which fail to nodulate in the presence of *Mesorhizobium loti*. These are a type of **pattern recognition receptors (PRRs)**. These receptors are transmembrane kinases, which have extracellular region-bearing LysM domains, a transmembrane domain, and a cytosolic domain. These include “entry” receptors and “signaling” receptors, which are encoded by specific genes. These receptors resemble other LysM receptors which are involved in identifying the pathogenic bacteria (Box 11.7). Mutations in any of the genes encoding these plant membrane receptors can cause severe nodulation defects, and plant is unable to nodulate. Extracellular domain of the receptors may be interacting with the Nod factors. If extracellular domains of the receptor proteins from the two different host plants are swapped, there will be change in their specificity to infection by the compatible species of rhizobia. The cytosolic

Box 11.7: LysM Motif and LRR Domains

It is a peptidoglycan-binding module. LysM motif of the proteins consists of 44–65 amino acid residues. It binds to peptidoglycan and chitin non-covalently. It serves as a module which mediates recognition of different N-acetylglucosamine-containing ligands. The glucans (e.g., Nod factor oligosaccharides consisting of N-acetyl-D-glucosamine residues) probably might have served as the immunogenic patterns, and this class of proteins might have evolved as the sensor for these kinds of ligands. So, LysM protein receptor-mediated immune system might have been developed to stop microbial infection. It has been identified in plant proteins that are also involved in detection of fungus. LRR domain of the protein refers to leucin-rich repeat domain. There are 2–45 motifs of 20–30 amino acids in each, which are rich in hydrophobic amino acid leucine. These motifs are folded in the form of a horseshoe-like structure. The LRR domain has been found in the proteins of diverse functions. These comprise the largest subfamily of transmembrane receptor-like kinases (LRR-RKs) in plants. These LRR domains are particularly suitable for protein-protein interactions.

domain of “entry receptor” exhibits typical kinase function. It has a phosphorylation loop, a catalytic domain, and an activation loop. Upon phosphorylation, cytosolic domain changes its conformation and is responsible for initiating a signaling cascade by phosphorylating the target proteins. Target proteins might be the proteins involved in Ca^{2+} channel regulation. On the contrary, P-loop and activation loop are missing in the cytosolic domain of the “signaling receptor.” Signal receptor might be involved in transmitting the signal to the proteins via a kinase activity. The “entry receptor” is required for infection, while the “signaling receptor” is required for the host response to the nodulation factor. Besides these receptors, there is another class of receptors present in the membrane of the host plant, i.e., **symbiosis receptor kinase (SymRK)**, which is involved in protein recognition. This receptor is required both for symbiosis with bacteria as well as for establishing symbiosis with mycorrhizal fungi which help plants in acquisition of minerals. This receptor has three extracellular LRR (leucine-rich repeats) domains in a large N-terminal segment of the receptor (Fig. 11.12). SYMRK gene is responsible for the expression of extracellular LRR domain, a membrane-spanning domain, and a cytoplasmic intracellular kinase. The kinase domain is capable of **autophosphorylation** and can act as a kinase for the target proteins in vitro.

Nodulation Once Nod factors are identified by the receptors present on the root hair surface, and the signal is transduced, calcium ion oscillations are activated in the nuclear and perinuclear regions of the root hair cell. This causes periodic **calcium spiking**, i.e., a transient increase or decrease in Ca^{2+} levels. The reservoir for the release of Ca^{2+} is believed to be the lumen of nuclear membrane. Ca^{2+} spiking requires a channel for the release of Ca^{2+} from the reservoir and an energy-requiring pump for uptake of Ca^{2+} into the reservoir. Oscillations in the calcium ion concentrations are recognized by the **calcium- and calmodulin-dependent protein kinase (CCaMK)**. CCaMK is required for subsequent nodule development. Kinase activity at the C-terminal of protein is inhibited when N-terminal of the protein is autophosphorylated in response to Ca^{2+} . When autoinhibitory N-terminal domain of CCaMK is deleted or mutated, nodule development occurs in the absence of bacteria. This indicates that CCaMK acts as a central coordinating switch in early nodule development. Another protein required by the CCaMK for its action is a nuclear protein, i.e., **CYCLOPS**. CYCLOPS is a DNA-binding transcriptional activator and is a direct phosphorylation substrate of CCaMK. It induces nodule organogenesis. CCaMK and CYCLOPS are the key regulators in interpreting Ca^{2+} spiking in the nucleus and nodule development (Fig. 11.13). Proteins, which are expressed in plants early during the infection, are called **early nodulins** and are encoded by **ENOD** genes. By using Nod factors in absence of bacteria, synthesis of these proteins can be triggered. However, further development of the nodules will require infection with live bacteria. Proteins synthesized by the plants during the later phase of infection process are called late nodulins. After the recognition of Nod factors by the receptors present on root hair and epidermal cells of the host plant, there is signaling for the development of the nodules. It brings about curling of the

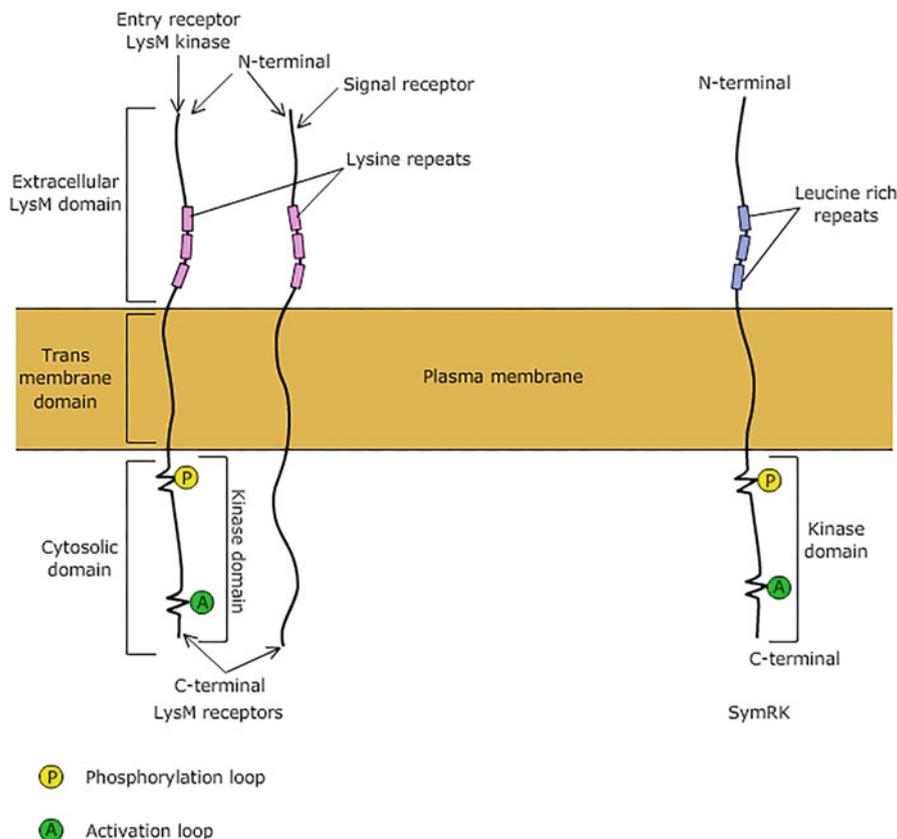


Fig. 11.12 Nodulation factor receptors present on root hair surface of the host plant. Each of these proteins has membrane-spanning domain, an extracellular domain, and cytosolic domain. Proteins, bearing LysM domains in extracellular region, are Nod factor receptors, which are “Entry receptors” or “Signal receptors.” “Entry receptor” is required for infection, and “signaling factor” is required for host response to nodulation factors. Cytosolic domain of “Entry receptor” has a phosphorylation loop (P) and an activation loop (A). It exhibits a typical kinase function. P-loop and A-loop are missing in cytosolic domain of “signaling receptor.” SymRK (symbiosis receptor kinase) has three extracellular leucine-rich repeats (LRR) and is required both for symbiosis with rhizobia and establishing symbiosis with broad host range mycorrhizal fungi that enhance nutrient uptake

tip of the root hair in which bacteria are trapped. Cell wall of the root hair adjacent to bacteria partially degrades followed by invagination of plasma membrane, forming a tunnel-like structure. Bacteria enter into this, and it develops into an infection thread. Infection thread grows through the cell by coalescing with Golgi vesicles at the tip (Fig. 11.14). Matrix of the infection thread is rich in products of bacterial origin and the one synthesized by the host plant. These include various extracellular products, such as protein of plant origin, **arabinogalactan** protein (of the **extensin** family). On reaching the cortical cells, bacteria are released in the apoplastic region followed by

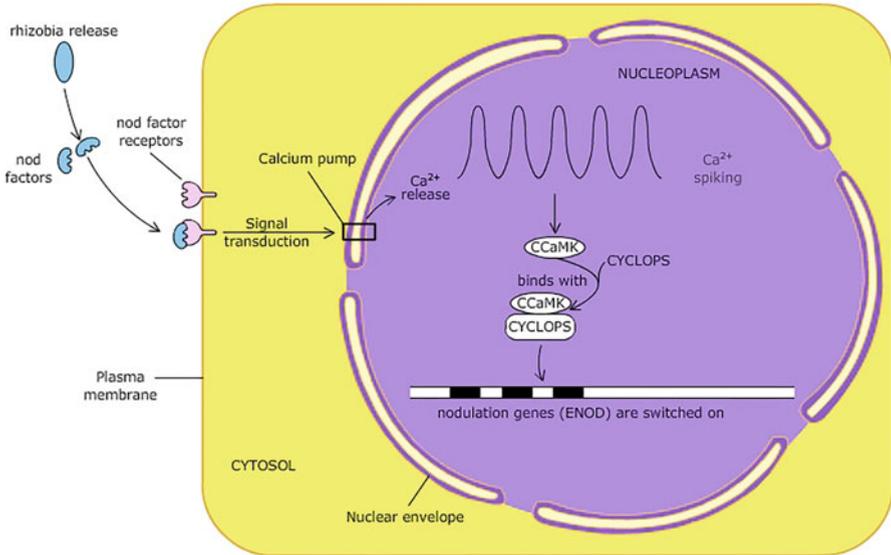


Fig. 11.13 Calcium spiking is induced in the nucleus of the rhizobia-infected root cells of legume. Calcium spiking is recognized by CCaMK (calcium-calmodulin-activated protein kinase). CYCLOPS (a DNA-binding transcriptional activator) is a direct phosphorylation substrate of CCaMP. CYCLOPS is required for subsequent nodule development. ENOD (genes encoding early nodulins)

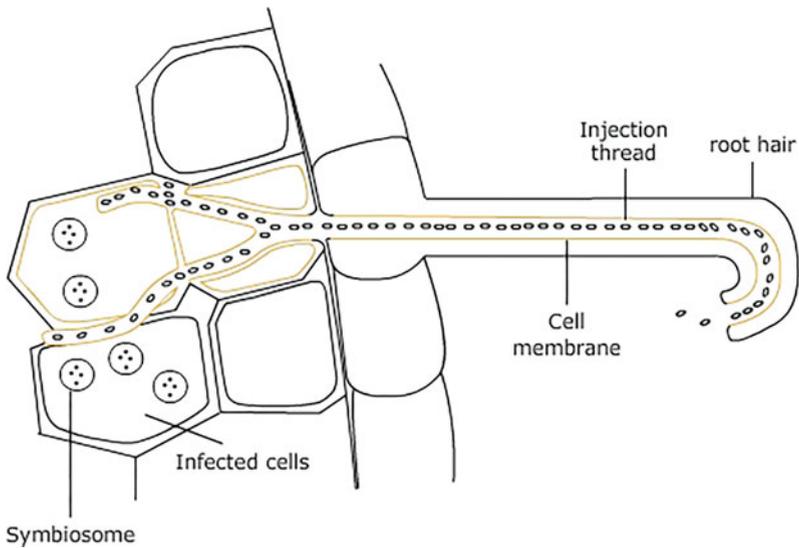


Fig. 11.14 Formation of infection thread and nodule development

Table 11.3 Characteristic features of determinate and indeterminate type of nodules

Characteristics	Determinate	Indeterminate
Nodule initiation	In outer cortex	In inner cortex
Meristem	Meristem does not persist in mature nodules; meristem is active for days	Meristem persists throughout nodule growth; meristem is active for months
Shape	Spherical	Elongate, cylindrical
Infected cells	Non-vacuolate	Vacuolate
Nod genes inducer	Isoflavones	Flavones, isoflavones
Bacteroids in symbiosomes	Each symbiosome contains several smaller rod-shaped bacteroids; number of bacteroids per symbiosome may be up to 20	Each symbiosome contains single enlarged pleomorphic bacteroid
Nitrogen transported from the nodules	In the form of ureides, presence of ureides in the xylem sap is indicative of nitrogen being fixed by the plants	In the form of amides (glutamine and asparagine), which can be found in xylem even when nitrogen is not being fixed by the plants. Presence of amides in xylem sap is not marker for nitrogen fixation by the plants
Geographical origin	Tropical	Temperate
Examples	<i>Vigna unguiculata</i> (cow pea), <i>Glycine max</i> (soybean)	<i>Trifolium</i> sp., <i>Pisum</i> sp.

further invagination of the plasma membrane of cortical cells. As the infection thread is established, there is dedifferentiation of the cortical cells near the xylem, which enter cell cycle, triggering cell division and forming a distinct area of dividing cells, called **nodule meristem**. This results in the development of protuberances on the roots. These protuberances, which are formed due to cell division, develop into nodules. Depending upon the type of meristem, nodules can be **determinate** (spherical nodules with the limited growth potential, e.g., soybean, *Lotus japonicus*) or **indeterminate** (continually growing which are cylindrical, e.g., in *Medicago* sp.) type. Determinate nodule cells mature synchronously so that no meristematic cells are present in mature nodules. In the indeterminate type of nodules, newly formed cells are constantly formed (Table 11.3). The tip of the infection thread is pinched off, and bacteria are released in the cells in the form of vesicles, surrounded by plasma membrane of the host plant. Bacteria stop dividing thereafter and enlarge. These are differentiated into nitrogen-fixing forms called **bacteroids**. Bacteroids have diminished ability to grow in culture medium. They may be greatly enlarged and possess various shapes. Vesicles are surrounded by the plasma membrane of the host plant, known as **peribacteroid membrane (PBM)**. One of the reasons for this effective symbiotic association of the host plant with rhizobia is that the bacteria do not trigger plant defense mechanism. The polysaccharides, which are present within the vesicles around bacteria, may be the possible reason for this. Some of the

Bradyrhizobium sp., which lack Nod factors, may adopt an alternate mechanism of infection. These may enter through the cracks in the epidermis and invade through the short infection thread. Possibly these invade the host cells by endocytosis-like mechanism and establish symbiosis.

Role of Phytohormones in the Nodule Development During early stage of the nodule formation, an increase in the cytokinin activity is reported, while auxin transport in the nodules is decreased. Nodule formation can be inhibited in mutants of cytokinin receptors, which indicates that cytokinin has an important role in the formation of nodule primordia. Cytokinins act downstream of the Nod factor signaling pathway. Auxin transport is inhibited in developing nodules. Use of an inhibitor of the auxin transport has, in fact, been found to induce nodule development. However, auxin may be required in the later part of nodule development. Ethylene diminishes the sensitivity of the host plant to the Nod factors released by the bacteria. With ethylene available, host plants require higher amount of Nod factors for the nodulation responses (calcium spiking or synthesis of early nodulin transcription factors). Ethylene mutants exhibit higher degree of nodulation.

Symbiosomes Symbiosomes are the vesicular structures formed in the infected cortical cells of nodules of the host plant. When infection thread reaches the cells, which later on develop into nodules, there is an endocytosis-like process. By pinching of infection thread, rhizobia are released in the form of vesicles still surrounded by the host cell membrane. These vesicles are called symbiosomes, which refer to the new organelle-like structures formed as a result of symbiosis. They contain one or more bacteria (Fig. 11.15). Membrane of symbiosome is derived from the host plant and is known as peribacteroid membrane. It is thought to mediate the flow of the nutrients between bacteria and host plant. Various intrinsic proteins are present in the peribacteroid membrane including **nodulin 26-like intrinsic protein (NIP)**, which transports water, glycerol, and ammonia. The space between the peribacteroid membrane and bacteroid membrane is known as peribacteroid space. Some of the plant proteins, which are uniquely expressed only in the infected cells, are synthesized in the cytosol of the infected cells and are transported across symbiosome membrane. These proteins carry a distinct targeting peptide which facilitates their entry into the symbiosome. It indicates symbiosome to be a unique cellular compartment. Symbiosome provides the microaerobic nodule environment, which is suitable for N_2 fixation by the bacteroids. Inside the symbiosomes, bacteria develop nitrogen-fixing enzyme dinitrogenase and are differentiated into bacteroids. These bacteroids appear to be terminally differentiated. There may be single enlarged pleomorphic bacteroid in a vesicle, or there may be several (up to 20) smaller, rod-shaped bacteroids. Nitrogen fixation is an energy (ATP)-requiring process. Requirement of ATP is met through aerobic respiration by the bacteria. At the same time, nitrogenase requires reducing environment since it is highly sensitive to oxygen. Thus, it becomes challenging for the plant to provide suitable conditions for the efficient functioning of the enzyme and

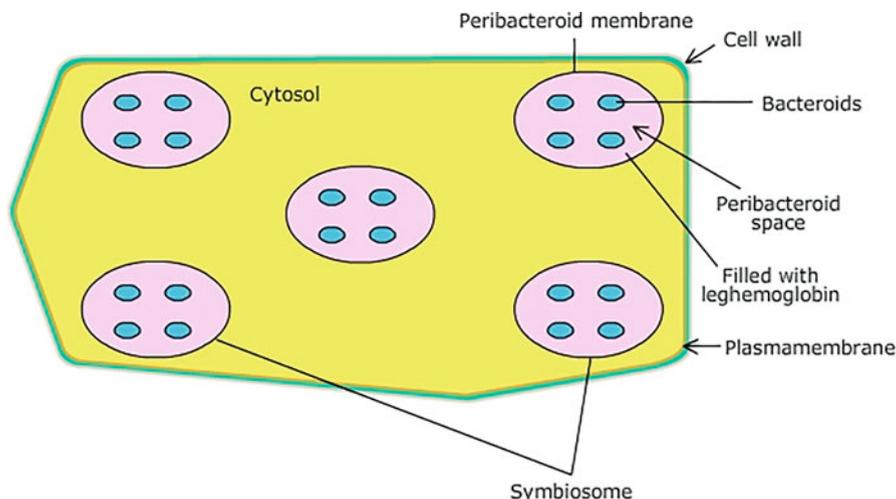


Fig. 11.15 Infected cell of a nodule. Vesicles are formed as a result of pinching of infection thread containing rhizobia. Rhizobia divide inside vesicles and get transformed into nitrogen-fixing bacteroids. Bacteroids are surrounded by membrane derived from the host plant plasma membrane (peribacteroid membrane) and a polysaccharide matrix. The structure so developed is known as symbiosome

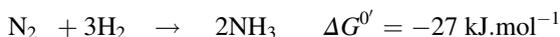
simultaneously to be able to meet the high ATP demand for the nitrogen fixation. This requirement is met through by the following factors:

1. High respiration rate in bacteria serves as sink for the oxygen. K_m value of the cytochrome oxidase of the bacteroids is much lower than that of free-living bacteria, which indicates the efficiency of the bacteria to utilize oxygen more efficiently. K_m value of cytochrome oxidase of the bacteroids is 8 nM, while that of free-living bacteria is 50 nM. K_m for plant mitochondrial **cytochrome oxidase** is near 100 nM.
2. There is some control on the permeability for the entry of oxygen in the nodule parenchyma, which can change depending upon the external O_2 availability.
3. Presence of **leghemoglobin (Lb)** in the space of symbiosome surrounding the bacteroids. Although it is well established that apoprotein parts of the leghemoglobin are synthesized by the host plant, the source for heme moiety is uncertain (plant or bacteria!). In nodules, it is present in millimolar concentration. Nodules need to be kept under low oxygen concentration in order to prevent nitrogenase enzyme from getting inactivated by oxygen. Lb has an active role in delivering O_2 to the actively respiring nodules and in regulating the amount of O_2 near the site of nitrogen fixation. Lb acts like a buffer to regulate changes in concentration of oxygen which might arise due to changes in the rate of respiration in the bacteria. Leghemoglobin reduces the availability of free oxygen but increases the flux of oxygen to the respiring bacteroids.

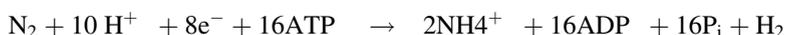
Non-legume Symbiosis with Rhizobia Symbiosis between a rhizobium sp. and a non-legume species *Parasponia* (family Ulmaceae) was reported in the highlands of Papua New Guinea in 1970. The infecting nitrogen-fixing bacteria were identified as *Bradyrhizobium*. Symbiosis was as effective as that of legume and rhizobia. Though it was found to be generally ineffective in nodulating legumes, *Bradyrhizobium* results in nodule formation in the roots of *Parasponia*. Multiple-branched nodules are formed. They resemble actinorhizal nodules. Each nodule contains an apical meristem with a single vascular bundle surrounded by nodule cortex. Bacteria-infected cells of nodules are surrounded by the uninfected cells. Unlike the nodules formed in legume-rhizobia symbiosis, nodule formation in *Parasponia* occurs in pericycle region and not in the cortex. Hemoglobin is present in the cytoplasm of the infected cells. A similarity between the hemoglobin found in *Parasponia*-rhizobia symbiosis and legume-rhizobia symbiosis exists. Understanding the process of symbiosis in between *Parasponia* and *Bradyrhizobium*, including the role played by the plant genes in establishing the symbiosis, will help in increasing the host range of the bacteria, and it can help in transferring this trait to other non-nodulating species.

Frankia-Actinorhizal Symbiosis In these symbiotic associations also, nodules are formed in the roots of host plant for nitrogen fixation. The contribution of this type of nitrogen fixation to the soil nitrogen is as important as by rhizobia and legume symbiosis, especially in forests and other natural ecosystems. *Frankia* sp., a gram-positive bacterium belonging to family Frankiaceae (Actinomycetales), are involved in nitrogen fixation in symbiotic associations with some of the woody plants which include trees or woody shrubs predominantly of temperate zones, for example, *Alnus*, *Casuarina*, *Ceanothus*, and *Myrica* sp. *Casuarina* is more prevalent in tropical climates. The term actinorhizal is given to the nodules formed by *Frankia*. Actinorhizal plants are dicotyledon angiosperms characterized by their symbiotic associations with nitrogen-fixing actinobacteria of *Frankia* sp. *Frankia alni* is the only named species in the genus *Frankia*. Unlike rhizobia, *Frankia* are filamentous gram-positive bacteria. Their **nitrogenase** enzyme is also sensitive to oxygen similar to nitrogenase in legume-rhizobial symbiosis. *Nif* genes in both cases share sequence homology. *Frankia* also infects host plants through root hairs. In case of *Frankia*-actinorhizal symbiosis, prenodules are formed which consist of both infected and uninfected cells. Hemoglobin is synthesized in most of the actinorhizal plants. Hemoglobin present in actinorhizal symbiosis shares sequence homology with the one present in *Parasponia*-rhizobia symbiosis but is quite different from that of soybean. Unlike rhizobia, common *nod* genes, *nodA*, *nodB*, *nodC*, and *nod*, are located on the resident chromosome of *Frankia* sp., while in rhizobia these are located on megaplasmid. Mutations in any of these genes (*nod*⁻) will result in a phenotype, which is not able to nodulate. The actinorhizal spp. are the pioneer species which are able to grow in nitrogen-poor soils and are able to enrich the soil with nitrogen because of their ability to form symbiotic associations with N₂-fixing *Frankia* sp.

Biochemistry of Nitrogen Fixation Reduction of nitrogen to ammonia is an exergonic reaction:



However, molecular nitrogen in the atmosphere is an inert gas since the two atoms of nitrogen are triple bonded to each other. Bond energy of the nitrogen molecule is $930 \text{ kJ}\cdot\text{mol}^{-1}$. Breaking of these bonds requires quite a high amount of activation energy. However, biological nitrogen fixation can occur at 0.8 atm and at normal temperature of the cell. Reduction of molecular nitrogen to two molecules of ammonia requires transfer of 8 electrons and 16 ATPs. The reaction that is catalyzed by the enzyme nitrogenase is



Reduction of nitrogen by nitrogenase is coupled with the reduction of two protons resulting in the release of hydrogen. Production of H_2 during reduction of nitrogen by nitrogenase is obligatory. The biological role of the same has not been understood.

Nitrogenase enzyme is a highly complex and conserved protein. It consists of two components, component I and component II. These components are also called as dinitrogenase and dinitrogenase reductase, respectively. Active site for binding with molecular nitrogen is present in the dinitrogenase, while reduction of dinitrogenase is catalyzed by dinitrogenase reductase. Dinitrogenase reductase (component II) is an iron protein with a molecular weight of 62–64 kDa. It has two identical subunits of 30–32 kDa each. The protein is associated with single metal center [4Fe-4S]. Each of the two subunits has a site for binding with Mg.ATP. MoFe protein (component I), a heterotetramer. It is also known as dinitrogenase. It has a molecular weight of 220 kDa and consists of four polypeptide subunits of two types α and β ($\alpha_2\beta_2$). Each α subunit has the molecular weight of approx 56 kDa, while molecular weight of each of β subunit is about 60 kDa. Binding site for nitrogen reduction is present in this component. Component I consists of two units. Each unit is a dimer consisting of one α and one β polypeptides. Each dimer ($\alpha\beta$) is associated with two metal centers called P cluster and M cluster. P cluster has a pair of [4Fe-4S] centers. Two [4Fe-4S] centers share one sulfur atom, so the stoichiometry will be 8Fe-7S. P cluster is bound to $\alpha\beta$ dimer through covalent bond with cysteine residue. P cluster is also linked to the dimer through O-side chain of serine and amide from the peptide backbone. P cluster accepts electrons from Fe protein. Each dimer is also associated with another cofactor FeMo cofactor (FeMoCo). It is also known as M cluster, which is composed of Mo, Fe, S, carbide, and an organic molecule homocitrate, with an overall stoichiometry [1Mo-7Fe-9S-1C-homocitrate]. Organic homocitrate is bound to Mo of the cofactor on one side and to the protein on the other side through covalent bond with cysteine and histidine (Fig. 11.16).

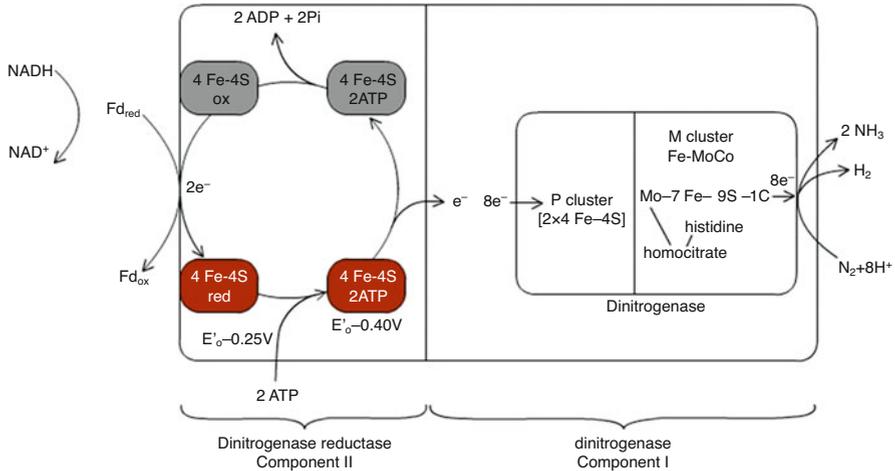


Fig. 11.16 Nitrogenase complex consists of dinitrogenase and dinitrogenase reductase. Dinitrogenase reductase is a homodimer which is associated with single metal center (4Fe-4S). Each of the two subunit has binding site with Mg.ATP. Dinitrogenase is a heterotetramer which consists of two units. Each unit of dinitrogenase is dimer consisting of two types of polypeptide subunits (α, β). Each dimer is associated with two metal centers, called P cluster and M cluster. P cluster has a pair of [4Fe-4S] centers. M cluster is composed of Mo, Fe, S, carbide (C), and an organic molecule homocitrate with overall stoichiometry 1Mo-7Fe-9S-1C-homocitrate. Homocitrate is bound to Mo on one side and histidine of the protein on the other side. Reduction of N_2 requires eight electron transfers from dinitrogenase reductase to dinitrogenase, one at a time. MoFe protein must accumulate eight electrons for N_2 reduction. Both ATP binding and ATP hydrolysis brings about conformational changes in dinitrogenase reductase which help in overcoming high activation energy of nitrogen reduction

N_2 reduction to NH_3 by nitrogenase In most of the cases, it is the reduced ferredoxin, which reduces dinitrogenase reductase (component II). Reduction is accompanied with transfer of single electron from reduced Fd to dinitrogenase reductase. This is accompanied with binding of two ATP molecules at their binding sites bringing about the conformational change. As a result, reduction potential of the enzyme shifts from -300 mV to -420 mV, enhancing the reduction potential of the enzyme. 4Fe-4S center of the reductase comes closer to the P center of dinitrogenase which facilitates transfer of electron between dinitrogenase reductase and dinitrogenase accompanied with ATP hydrolysis. In oxidized state, Fe protein (component II) gets dissociated, and the other reduced Fe protein will take its place. Fe protein gets dissociated at least eight times during the transfer of eight electrons. MoFe protein must accumulate a minimum of eight electrons to complete the nitrogen reduction cycle. In the initial stage, possibly $2H^+$ might bind with the active site, resulting in the release of H_2 . Release of H_2 is inevitable, causing wastage of reducing energy. Almost 30–60% of the energy supplied to nitrogenase gets wasted due to release of hydrogen. Dinitrogenase can possibly exist in different oxidation states depending upon number of electrons received by it. It is only after the

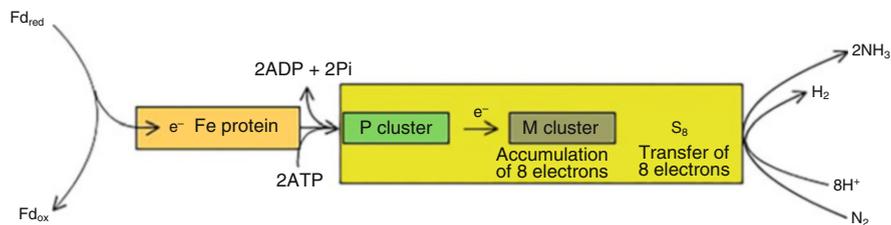


Fig. 11.17 Molecular nitrogen reduction by dinitrogenase. N_2 binding site is present in M cluster which should accumulate eight electrons (S_8) before N_2 is reduced to NH_3 . In the initial stage, possibly two H^+ might bind with the active site resulting in release of H_2

accumulation of eight electrons that the enzyme can possibly reduce molecular nitrogen to two molecules of ammonium ions (Fig. 11.17). Besides dinitrogenase, which has molybdenum as a part of the structure, other nitrogenases have also been reported, which are called **alternate nitrogenases**. These contain vanadium or Fe in place of Mo, but not much information is available on this aspect. Reduction carried out by nitrogenase is a slow process, which is compensated by large amount of nitrogenase synthesized by the bacteria. Nitrogenase can amount up to 20% of the total soluble protein in the bacteria.

Endosymbiont-Host Plant Partnership Host plant provides ecological niche to bacteria by providing suitable conditions for nitrogen fixation, such as low partial oxygen pressure, besides providing the source for ATP. Since nitrogen fixation is an energy-requiring process, it consumes up to 20% of the total photosynthates generated in plants. According to one of the calculations, for every mole of nitrogen fixed, plant will require 9.3 moles of CO_2 . Carbon skeleton provided by the host plant is mainly in the form of dicarboxylic acid—malate. Source of malate is sucrose produced in the leaves during photosynthesis. It is metabolized through glycolysis, resulting in the formation of phosphoenolpyruvate, which is carboxylated to produce oxaloacetate. Oxaloacetate is reduced to malate, which is taken up by the bacteria. Malate is metabolized in bacteria through TCA cycle to provide reducing equivalents for generation of ATP, which is required for nitrogen fixation. Peribacteroid membrane allows transport of malate. Nitrogen fixation by nitrogenase requires anaerobic conditions. Most of the available oxygen is only allowed to pass through the nodule apex. As a result, a longitudinal gradient of oxygen concentration is created. Oxygen concentration is less than 50 nM in the center of nodule, where most of the nitrogen fixation takes place. Leghemoglobin present in peribacteroid space of the symbiosome regulates the supply of oxygen to rhizobia. Oxygen is required for active respiration in bacteria to meet the demand for ATP during nitrogen fixation. Bacteroids have developed a cytochrome oxidase, which has got higher affinity for oxygen, and it operates at maximal rate at lower oxygen concentration than the free-living rhizobia (Fig. 11.18). Rhizobia provides nitrogen to the host plant in the form ammonia, which is converted to ammonium ions after combining with protons once it diffuses into the peribacteroid space. Ammonium

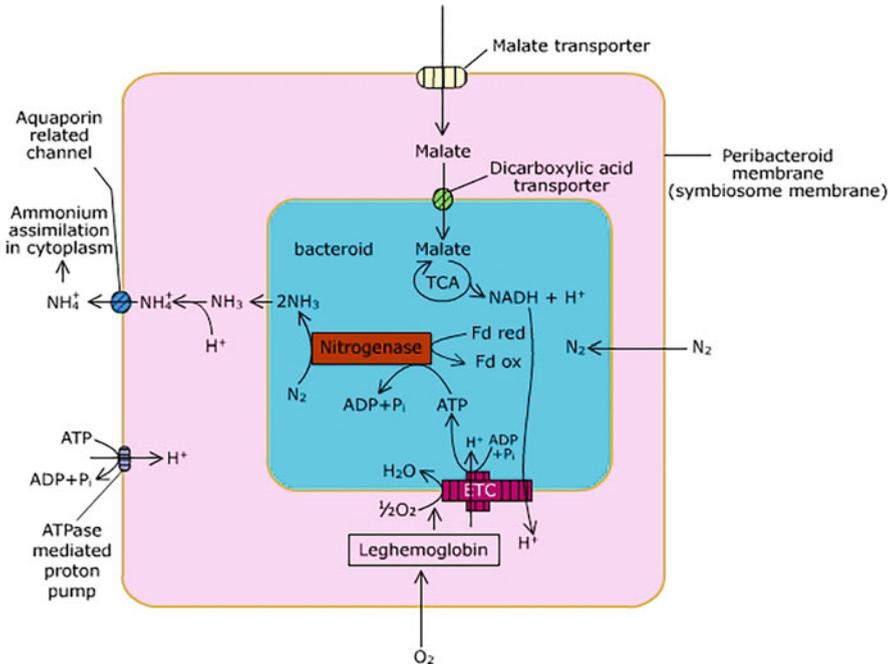


Fig. 11.18 Host-bacteria partnership: Bacteroid metabolism in symbiosome. Bacteroids receive malate as energy source from the host which is then metabolized to generate ATP. NH_3 traverse through symbiosome membrane as NH_4^+ by facilitated diffusion, mediated by the NOD-26 aquaporin-related channel

ions are released into the cytosol of the infected cell of the host from symbiosomes through the channels present in the peribacteroid membrane. The host plant immediately assimilates ammonium ions in the form of **amides** (glutamine or asparagine) or **ureides**, either in the cytosol or in the organelles of the infected host cell. Host plant provides dicarboxylic acids, ATP, and reducing equivalents for assimilation of ammonium ions. Amides/ureides are then transported through xylem to rest of the plant.

Genetics of Nitrogen Fixation Genes involved in the effective symbiotic relationship in between the bacteria and the host plant can be considered under two categories. One set of genes is required to develop effective symbiotic relationship. This includes the *nod* genes present in the plasmid of bacteria (Sym). Mutant strains of bacteria, which are not able to form the symbiotic relationship with the host are called *nod*⁻ bacteria. Second set of genes is required for the synthesis of nitrogen fixation apparatus and bacteroid metabolism. In nitrogen-fixing bacteria, these can be grouped into two categories: 1. The *nif* genes are required for nitrogen fixation by the symbiotic bacteria and by free-living nitrogen fixers, while 2. *fix* genes are essential for nitrogen fixation in symbiotic nitrogen fixers but do not have

counterparts in free-living forms. *fix* genes are involved in the development and metabolism of bacteroids. *nifD* and *nifK* encode for alpha and beta subunits of nitrogenase, while the monomeric Fe protein is encoded by *nifH*. Ferredoxin is encoded by *nifF*. In *Rhizobium meliloti*, *nifHDK* genes are organized in an operon along with *nifE*. Over 20 *nif* genes are known. Synthesis of FeMo cofactor requires the products of *nifE*, *nifN*, and *nifB*. However, exact biochemical functions of these proteins are not known. Host plant can contribute to the formation of nitrogenase by providing homocitrate, a component of nitrogenase, to the bacteria. Expression of the *nif* genes is regulated by the availability of nitrogen and oxygen. Some of the *nif* genes are involved in regulatory functions. Transcription of *nif* genes is activated by the product of *nifA*, which is expressed in the absence of oxygen. FixK is a protein in symbiotic bacteria, as in rhizobia, which regulates the expression of *fix* group of nitrogen-fixing genes. Expression of *fixK* and *nifA* is regulated by FixL, which is an oxygen-sensitive heme-containing protein kinase. This protein is anchored to the bacterial plasma membrane. In the absence of O₂, this protein is phosphorylated and, in turn, regulates the activity of FixJ by phosphorylating it, which regulates expression of *nifA* and *fixL* genes. These genes, in turn, will control the expression of other *nif* and *fix* genes. *fix* genes are present only in those bacteria which are in symbiotic relationship. One of the *fix* genes is involved in the synthesis of specific ferredoxins, and others may be involved in the synthesis of high-affinity cytochromes. Host plant genes required for the nodulation process encode nodulins. Plant proteins, which are formed early before any infection by bacteria has taken place, are called “early nodulins.” They are encoded by *ENOD* genes. Late nodulins are the proteins, which are synthesized later in the symbiotic process. Leghemoglobin is an example of late nodulin. Other late nodulins include proteins required for metabolizing carbon and nitrogen and in facilitating transport of compounds across symbiosome membrane. Some of the nodulins may be involved in transducing signal for cell differentiation in plants and bacteria to accomplish nitrogen fixation.

Transport of Ammonium from Nodules Ammonia formed in the bacteroids is released in the peribacteroid space, where it combines with proton ions and is converted to ammonium ions. Protons are pumped into the peribacteroid space by ATPase-mediated proton pumps localized in the symbiosome membrane and also by the electron transport chain present in the bacteroid membrane. Ammonia is transported as ammonium ions to the cytosol of the infected cell of the nodules via monovalent cation channels. Glutamine synthetase (GS) and GOGAT (glutamine oxoglutarate aminotransferase) enzymes are expressed in the cytosol of the infected nodule cells, which assimilate ammonium ions firstly to glutamine and then to glutamate. Fixed nitrogen is transported out of the nodule either in the form of amides or ureides via xylem. In the legumes of temperate origin, nitrogen is transported in the form of amides, i.e., glutamine or asparagine. These products are generally synthesized in the nodules with indeterminate apical meristem. Examples of the amide transporter legumes are pea (*Pisum sp.*), broad beam (*Vicia sp.*), clover (*Trifolium sp.*), etc. In the legumes of tropical origin, e.g., soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), peanut (*Arachis hypogea*),

etc., fixed nitrogen is transported out of the nodules in the form of ureides. Possibly ureides are sparingly soluble in temperate conditions, so it is the tropical legumes in which fixed nitrogen travels in the form of ureides. Major ureides transported are allantoin, allantoic acid, and citrulline. Allantoin is synthesized from uric acid in the peroxisomes of the infected cells of the nodules, while allantoic acid is synthesized from allantoin in the endoplasmic reticulum of the cell. The site for synthesis of citrulline is not known. It is the determinate type of nodules which serve as ureide exporter. However, all the three ureides are released in xylem and are transported to the shoot. In shoot, these compounds (ureides) are converted to ammonium ions, which are further assimilated by the GS/GOGAT system (Fig. 11.22).

11.3 Ammonia Assimilation

Ammonia assimilation in plants is a complex process since it is produced in plants from different sources. These sources can be classified as the primary or secondary. Primary source refers to the ammonia which is produced from the inorganic nitrogen, such as ammonium ions absorbed by the plants and ammonia generated due to nitrite reduction or due to nitrogen fixation by the nitrogen-fixing bacteria in symbiotic association with plants. Secondary sources refer to the ammonia generated from the organic compounds during metabolism. These include ammonia generated due to (i) oxidation of glycine to serine during photorespiration; (ii) degradation of nitrogenous compounds, such as asparagine, arginine, and ureides; and (iii) protein degradation and deamination of amino acids. Since ammonia is produced at different sites in plants, the enzymes catalyzing ammonia assimilation function in different cellular conditions. As a result, various isozymes of the enzymes are involved in assimilation of ammonia.

11.3.1 Ammonia Assimilation by GS/GOGAT

Ammonia produced in nodules by the bacteroids is released through the bacteroid membrane. Since ammonia is nonpolar, it can easily diffuse out of the membrane. Once in the peribacteroid space, ammonia accepts up proton and is converted to NH_4^+ . Ammonium ions are transported to the cytosol of the infected cell of the nodule through the channels present in the peribacteroid membrane of the symbiosome by facilitated diffusion. NH_4^+ transport is mediated by an aquaporin-related channel (NOD-26) across the peribacteroid membrane in soybean nodules. NH_4^+ is assimilated in the cytosol of the infected cells of the host plant. Two enzymes responsible for the initial assimilation of NH_4^+ are **glutamine synthetase** (GS) and **glutamate synthase** (which is also known as **GOGAT**, **glutamine 2-oxoglutarate aminotransferase**) (Fig. 11.19).

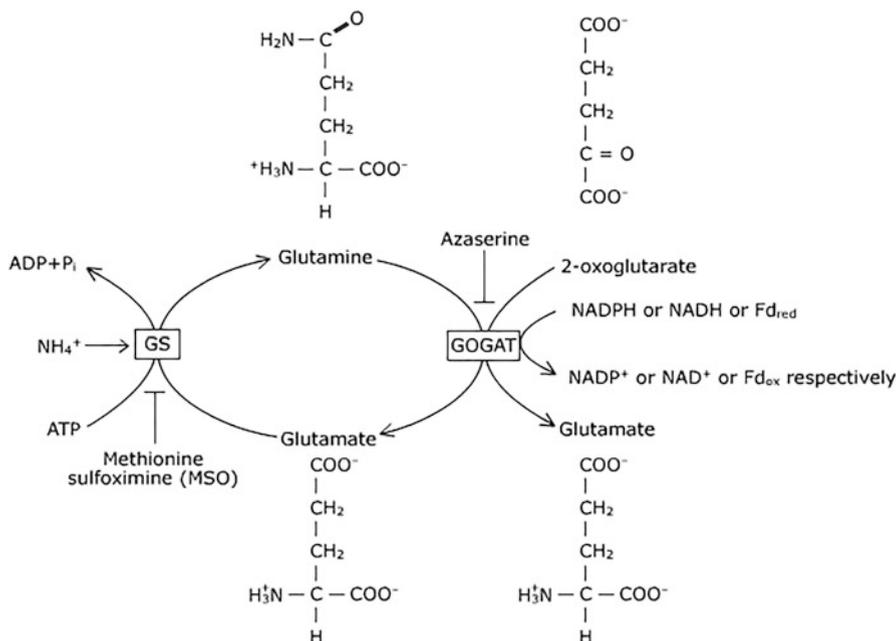


Fig. 11.19 GS/GOGAT reaction. Methionine sulfoximine is inhibitor of glutamine synthetase. Azaserine is substrate analogue of glutamate and is inhibitor of GOGAT (also known as glutamate synthase). GS, glutamine synthetase; GOGAT, glutamine 2-oxoglutarate aminotransferase

Glutamine synthetase (GS) catalyzes the synthesis of glutamine from NH_4^+ and glutamate. (Box 11.8). This ATP-requiring reaction is



A divalent cation such as Mg^{2+} or Mn^{2+} or Co^{2+} is also required as the cofactor for the enzyme. There are two classes of glutamine synthetase (GS) in plants: cytosolic (GS1) and plastidial (GS2). GS1 is also present in leaves in low concentrations, while it is present in higher concentrations in roots. Role of GS1 is important in primary assimilation of NH_4^+ . GS1 is expressed in germinating seeds or in vascular bundles of roots or shoots and generates glutamine for intercellular transport. GS2 is responsible for assimilating NH_4^+ produced during photorespiration. GS2 is the primary enzyme of glutamine synthesis in the leaves. Gene encoding GS2 is expressed in mesophyll cells, while gene for GS1 is expressed in phloem, indicating its primary role in producing glutamine for long-distance transport. Mutants of GS2 do not survive in conditions which favor photorespiration, while they survive in conditions which suppress photorespiration. Glutamate synthase transfers amino group from glutamine to 2-oxoglutarate, resulting in the synthesis of two molecules of glutamate. One of the glutamate molecules serves as the substrate for GS, while the other one is available for further metabolism. The combined reaction for ammonium metabolism is known as **GS/GOGAT** reaction.

Box 11.8: Glutamine Synthetase (A Molecular Computer)

Glutamine synthetase (GS) is an important enzyme of nitrogen metabolism since it is the entry point for assimilation of ammonia. The products of the ammonia assimilation are responsible for regulation of the GS activity. Thus, GS monitors nitrogen requirement of the plant. The enzyme exists as multi-subunit complex consisting of similar subunits. The number of subunits varies from 8 to 12 depending upon the source. Bacterial GS consists of 12 subunits, while in eukaryotes, including plants, it has 10 subunits. Structure of the enzyme is stabilized due to non-covalent interactions between the subunits. Regulation of GS activity is both due to allosteric and covalent modulation. Covalent binding of Tyr³⁹⁷ residue (present near the active site of the enzyme subunits) with AMP increases the sensitivity of subunits to allosteric inhibition by the end products of glutamine metabolism, such as alanine, glycine, and histidine. Binding of subunits with one of the inhibitors partially decreases GS activity, and the effect of multiple inhibitors is additive, thereby completely shutting down its activity. Thus, GS monitors the nitrogenous status of the plant and is active accordingly. That is why the enzyme may also be called “molecular computer.”

There are two types of GOGAT in plants. One of them accepts electrons from NADH (NADH-GOGAT), while the other type accepts electrons from reduced ferredoxin (Fd-GOGAT).



Unlike GS, which has both cytosolic and plastidic isoforms, GOGAT is present only in the plastids. NADH-GOGAT is present in non-photosynthetic tissues such as roots or in vascular bundles of developing leaves. NADH-GOGAT, present in roots, is involved in glutamine assimilation which is produced due to NH_4^+ assimilation in the roots, either absorbed from the rhizosphere or has been fixed by the bacteria in the symbiotic association (Fig. 11.20). The NADH-GOGAT present in vascular bundles is responsible for assimilation of glutamine, which is destined to be translocated either from roots or senescing leaves. The other type, Fd-GOGAT, is present in chloroplasts. It catalyzes metabolism of glutamine, produced from assimilation of NH_4^+ generated during photorespiration. Glutamine utilization by the Fd-GOGAT accounts for 95–97% of the total GOGAT activity in leaves. This shows its predominant role in leaves in metabolism of glutamine, which is produced due to ammonia assimilation generated both from primary sources (produced as a result of NO_3^- reduction) or from secondary sources (produced due to photorespiration) (Fig. 11.21). Most plants have been found to contain a single gene for

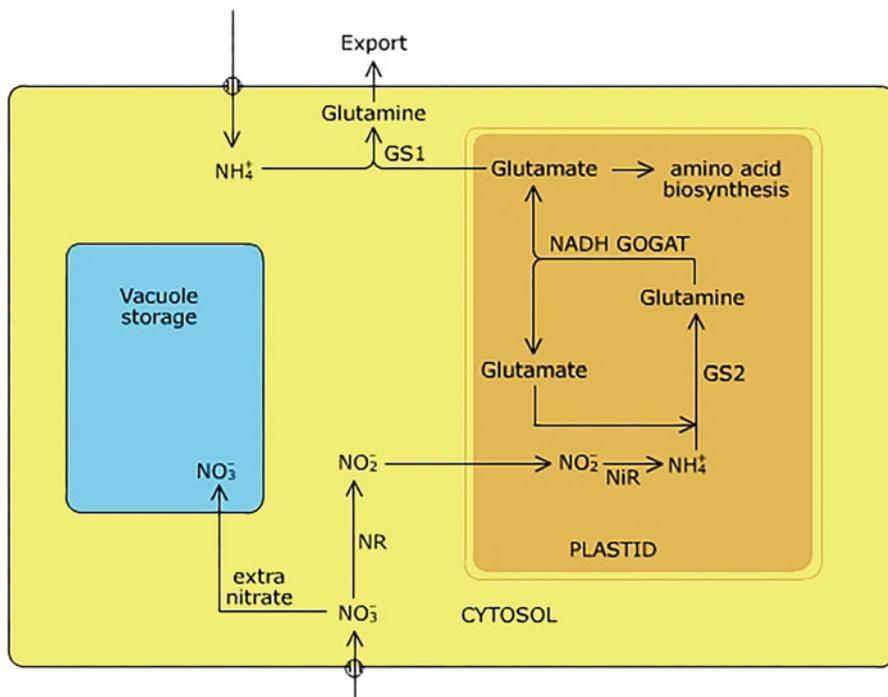


Fig. 11.20 Ammonium assimilation in roots. Isozymes of glutamine synthetase, GS 1 and GS 2, are present in cytosol and plastids, respectively

Fd-GOGAT. However, in *Arabidopsis* Fd-GOGAT is encoded by two genes (GLU1 and GLU2), while NADH-GOGAT is encoded by a single gene (GLT). Both Fd-GOGAT and NADH-GOGAT exist as monomeric proteins (Fig. 11.22).

11.3.2 Ammonia Assimilation by Reductive Amination

Reductive amination was thought earlier to play primary role in ammonia assimilation. The enzyme **glutamate dehydrogenase (GDH)** catalyzes the reaction in which ammonia, in the form of ammonium ions, is assimilated and α -ketoglutarate is aminated to form glutamate. Two types of GDH are present in plants. One class of GDH, NADH-dependent GDH, functions in mitochondria and requires NADH as the reductant for the reaction. The other is NADPH-dependent GDH found in chloroplasts. The reaction is reversible as GDH catalyzes both reductive amination, in which 2-oxoglutarate is converted to glutamate, and **oxidative deamination** in which glutamate is deaminated to generate 2-oxoglutarate. In reductive amination, reaction is coupled with oxidation of NADH/NADPH to $\text{NAD}^+/\text{NADP}^+$,

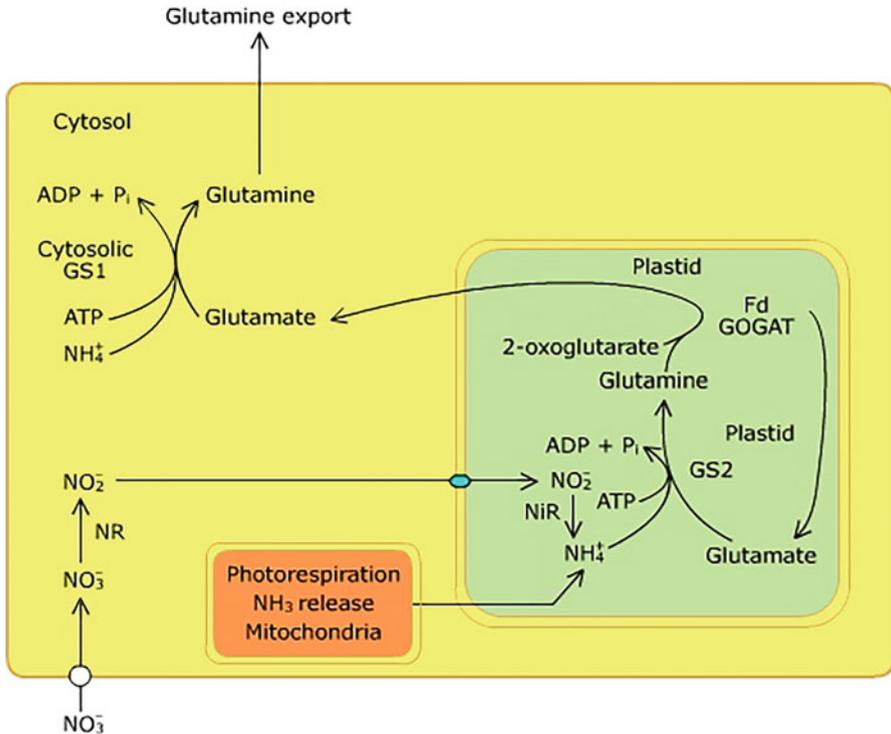


Fig. 11.21 Ammonium assimilation in leaves

respectively. On the contrary, reducing power is generated during oxidative deamination (Fig. 11.23). Ammonium assimilation by GDH was earlier considered to be of primary importance. But currently its role has been found to be more significant in the catabolism of glutamate. K_m value of GDH for ammonium ions is significantly higher (10–80 mM), while ammonium concentration in the cell has been found to be very low (0.2–1.0 mM). On the contrary, GS has high affinity for ammonium ions (K_m 3–5 micromoles) and can operate effectively at cellular concentrations. Studies carried out with $^{15}\text{NH}_4^+$ have provided evidence that labeled nitrogen first appears in the amide group of glutamine and then in the glutamate. Use of inhibitors of GS (**MSO**, **methionine sulfoximine**) leads to inhibition of NH_4^+ incorporation into glutamate even in presence of GDH and higher concentrations of ammonium. GDH might possibly be involved in assimilation of higher amount of ammonium ions (generated from photorespiration) in mitochondria. GDH activity increases in presence of high ammonium concentrations, indicating a possible role of GDH in detoxification. GDH may be important in glutamate catabolism generating 2-oxoglutarate which might be used as the TCA intermediate for energy generation in dark, in germinating seeds, or in senescing leaves. The reaction catalyzed by

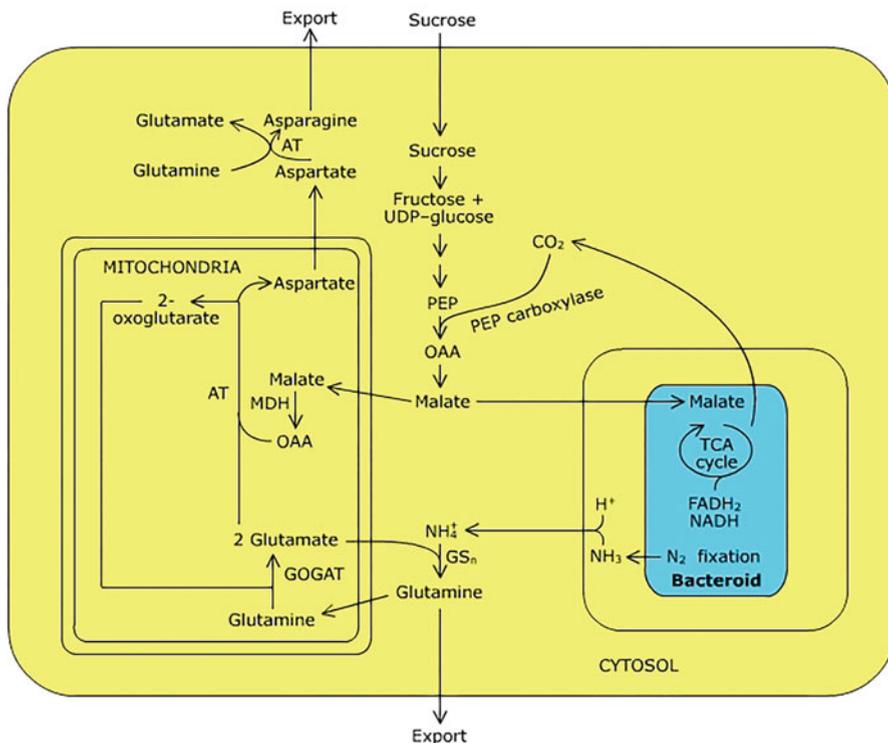


Fig. 11.22 Nitrogen metabolism in the infected cells of root nodules. GS_n , nodule-specific cytosolic GS isoenzyme, *AT* aminotransferase, *MDH* malate dehydrogenase

GS/GOGAT requires ATP and is irreversible. On the contrary, there is no ATP requirement by *GDH* for the formation of glutamate. Reaction catalyzed by *GDH* is reversible, and the equilibrium is unfavorable for glutamate formation. In plants *GDH* is encoded by two genes, *GDH1* and *GDH2*. *GDH* has a **hexameric** structure, and seven isoforms have been reported in plants depending upon the products of these two genes.

11.4 Nitrogenous Compounds for Storage and Transport

The amino group of the glutamate is transferred to oxaloacetate, producing aspartate and 2-oxoglutarate. Regeneration of 2-oxoglutarate is required for the continuation of ammonium metabolism by *GS/GOGAT*. Amino group transfer is catalyzed by an **aminotransferase**, i.e., **aspartate aminotransferase (AspAT)**, which is also known as **glutamate-oxaloacetate aminotransferase (GOT)**. It

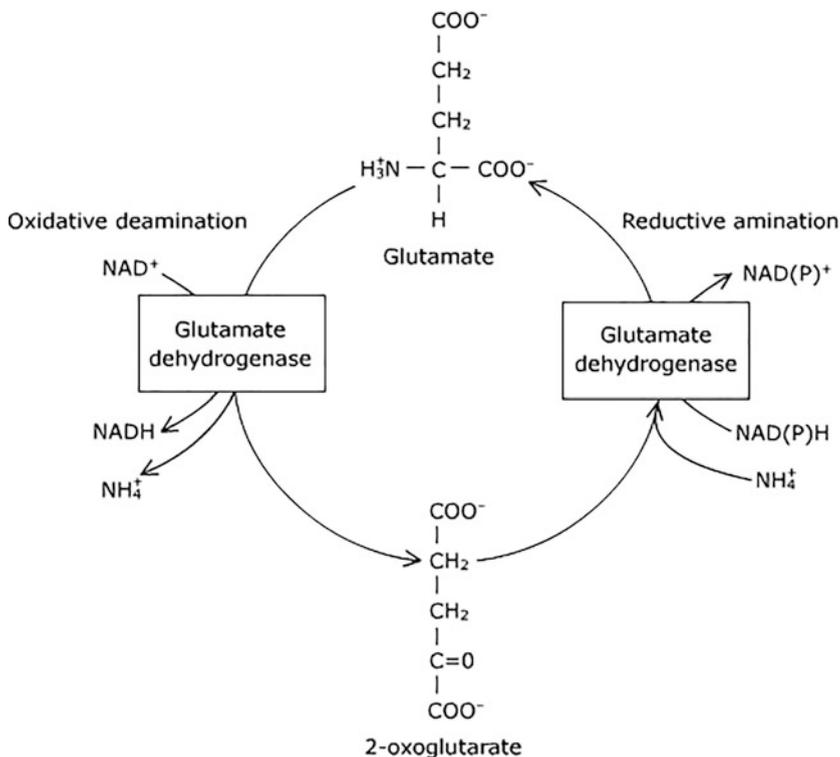
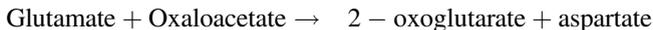


Fig. 11.23 Reductive amination/oxidative deamination reactions

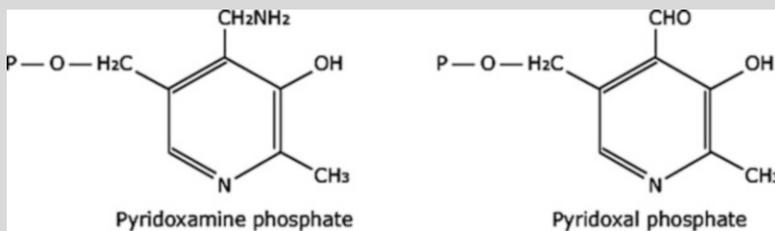
requires pyridoxal phosphate as a cofactor like all other aminotransferases (Box 11.9). The reaction is known as transamination reaction.



In addition to other compounds, **asparagine** is also used for storage and transport of nitrogen by the plants. High stability of asparagine and high N/C ratio of the molecule (it is 2:4 in case of asparagine, while in case of glutamine, it is 2:5) make it suitable for storage and transport of nitrogen. [*Asparagine was the first amino acid to be isolated almost 200 years ago from the extracts of Asparagus. It is responsible for the unique flavor of Asparagus. When plants are grown in dark, asparagine content in Asparagus increases, while there is decrease in woody tissues; that is why etiolated plants are both tastier and softer.*] Asparagine is synthesized from

Box 11.9: Pyridoxal 5'-Phosphate (PLP)

PLP serves as the coenzyme for variety of enzymes. Its primary role is in the metabolism of molecules with amino groups since it is also the cofactor of aminotransferases. All aminotransferases catalyze similar reactions. Pyridoxal phosphate (PLP) functions as an intermediate carrier of the amino group. It is derivative of vitamin B₆. Vitamin B₆ in diet consists of three different forms of PLP, pyridoxamine, pyridoxine, and pyridoxal. In the cell, all of these three forms of PLP can be converted to pyridoxal phosphate.



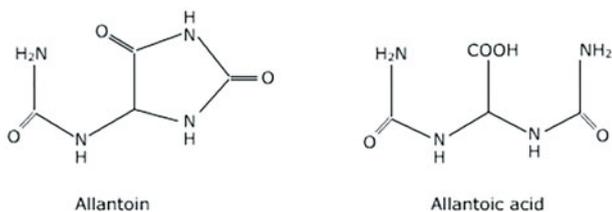
glutamine and aspartate, catalyzed by the enzyme **asparagine synthetase (AS)** (Fig. 11.24).



AS is present in the cytosol of leaf and root cells and in nodules. Similar to GS, activity of AS is also regulated by the adenylation of the enzyme. Two types of AS have been reported in plants. One type of AS utilizes glutamine, while the other type is ammonium-dependent asparagine synthetase. However, K_m value of the ammonium-dependent AS is higher, so possible direct amidation of aspartate to asparagine is of no significance. Transport of nitrogen in the form of glutamine is induced by light or availability of sucrose, while transport of asparagine is promoted by dark and in the conditions when sucrose supply is limited.

In some legumes, such as alfalfa and pea, nitrogen is transported as amides, i.e., glutamine and asparagine, while in tropical legumes, such as soybean (*Glycine max*) and cowpea (*Vigna sp.*), nitrogen is transported in the form of **ureides**, such as allantoin and allantoic acid (Fig. 11.25). Biosynthesis of these compounds involves various compartments of the cells, both infected as well uninfected. Purine biosynthesis and their degradation to uric acid occur in the infected cells of nodules. Uric acid is transported to uninfected cells and is degraded to ureides. Peroxisomes in the uninfected cells are also involved in formation of ureides. The ureides, allantoin and allantoic acid, are transported through xylem to other parts of the plants. Ureides have an advantage over amides since they have a very high N/C ratio which is 1:1 in comparison to glutamine and asparagine which have N/C ratio of 2:5 and 2:4, respectively (Fig. 11.25).

A.



B.

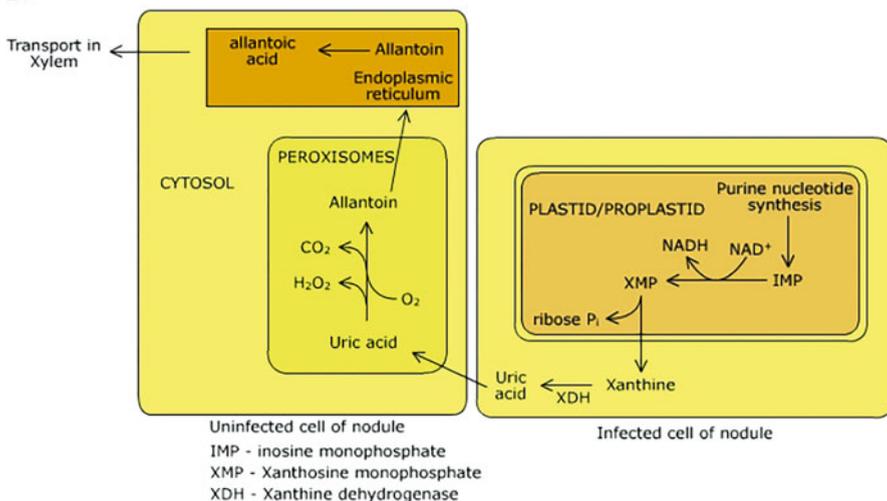
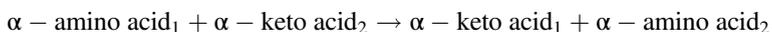


Fig. 11.25 (a) Structure of ureides (compounds which are related to urea). (b) Cellular and subcellular compartmentation in nodule cells for ureide biosynthesis

pathway and citric acid cycle, besides other sugars such as ribose 5-phosphate and erythrose 4-phosphate, which are produced either in reductive or oxidative pentose phosphate pathway.

11.5.1 Aminotransferase Reaction (Transamination)

Key reaction during amino acid biosynthesis is transamination, catalyzed by transaminases (also known as aminotransferases). The reaction involves transfer of an amino group from one α -amino acid to α -carbon of an α -keto acid resulting in the formation of a new α -amino acid and a new α -keto acid. The reaction is catalyzed by the enzymes which are known as aminotransferases.



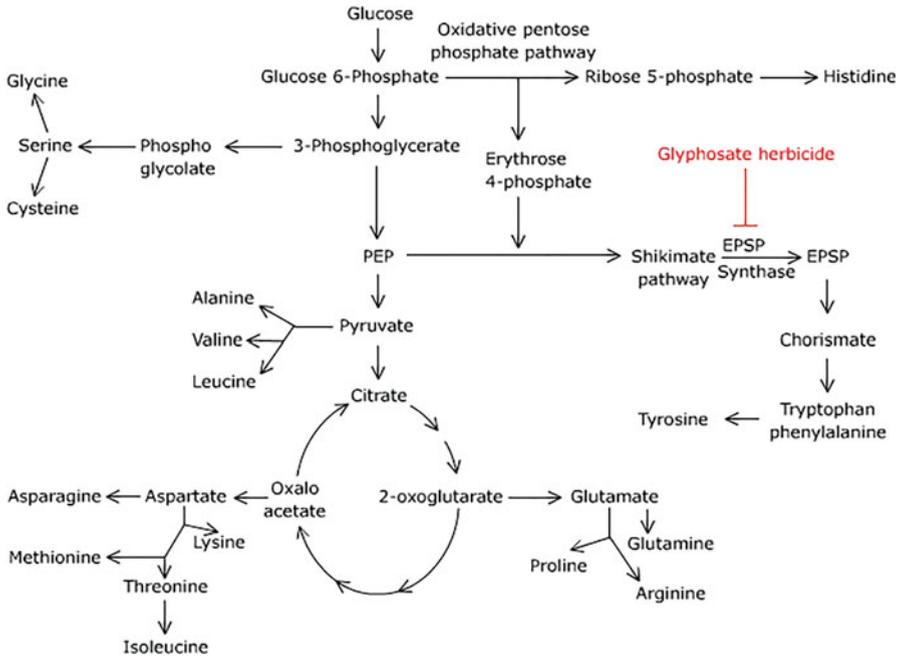


Fig. 11.26 An overview of biosynthesis of amino acids. EPSP, 5'-enolpyruvylshikimate-3-phosphate

Table 11.4 Essential and nonessential amino acids

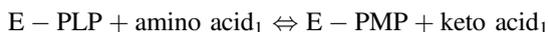
Essential amino acids	Nonessential amino acids	Conditional essential amino acids ^a
Histidine	Asparagine	Arginine
Leucine	Aspartate	Cysteine
Isoleucine	Alanine	Tyrosine
Lysine	Glutamate	
Methionine	Glycine	
Phenylalanine	Proline	
Valine	Serine	
Tryptophan	Glutamine	
Threonine		

^aConditional essential amino acids are those which may be required in specific condition, e.g., arginine is required at the growing stage; cysteine can be synthesized provided methionine is provided in diet. Tyrosine can be produced only from phenylalanine

In most of the cases, either glutamine (an amine) or glutamate are the amino group donors, while an α -keto acid is the amino group acceptor. Some of the aminotransferases prefer aspartate or asparagine as the group donor, which

themselves are produced at the expense of glutamate/glutamine. There are various aminotransferases. Some of them may be specific for 2-oxoglutarate as the amino group acceptor but may differ for the amino group donor α -amino acids. Aminotransferases are named on the basis of the amino acids which serve as the amino group donors, e.g., alanine aminotransferase and aspartate aminotransferase, the enzymes which catalyze amino group transfer from alanine and aspartate, respectively.

All aminotransferases require **pyridoxal 5'-phosphate (PLP)** as the cofactor. PLP is covalently bound to the protein (E-PLP) at the active site of the enzyme through an **aldimine (Schiff base)** linkage with the ϵ -amino group of a lysine residue. PLP can undergo reversible transformations. The incoming amino acid donates its amino group to the aldehyde form of the cofactor (PLP) (Box 11.9). The amino group donor, amino acid, is converted to the respective keto acid and leaves the active site of the enzyme, while PLP, on receiving the amino group, is converted to its amino form, i.e., **pyridoxamine phosphate (PMP)**. The second substrate (another α -keto acid) binds to the active site of the enzyme and receives the amino group from PMP in a typical "Ping-Pong" reaction and gets converted to respective amino acid, and PLP is regenerated.



Reactions catalyzed by the aminotransferases are reversible reactions with a $\Delta G^{0'} \approx 0$.

Understanding of the amino acid biosynthesis pathways in plants has mainly been inferred from the pathways worked out in bacteria and fungi. However, the pathways occurring in plants are much more complex than those in bacteria, since bacterial genome is small and organelles are absent in bacteria. There are multiple genes present in plants, which regulate each step of the pathway. Most of the pathways involved in amino acid biosynthesis are quite complex and involve a number of enzymes. Various amino acid transporters have been identified for inter- and intracellular transport of the amino acids in plants; however the mechanism of amino acid transport is still poorly understood. Enzymatic pathways for the biosynthesis of many of amino acids have been worked out, which are commercially exploited for regulating synthesis of especially the essential amino acids, as these are not synthesized in human body and need to be provided in their diet. Additionally, since enzymes for the synthesis of essential amino acids are absent in humans and present in plants and bacteria, using inhibitors of these enzymes can be exploited for producing potential antibiotics against animal pathogens and also for developing **herbicides** (Box 11.10). Amino acids are classified under the following categories on the basis of their precursors and the amino group donors required for their synthesis.

Box 11.10: Blockers of Synthesis of Essential Amino Acid in Bacteria Can Be Potential Antibiotics

Unlike plants, fungi, and bacteria, animals are not able to synthesize essential amino acids. Some of the enzymes required for biosynthesis of these amino acids are missing in animals, so they have to depend on the supply of these amino acids in their diet. Learning about the biosynthetic pathways for these amino acids can be exploited in developing new antibiotics and antifungal medicines. This becomes significant with the growing antibiotic resistance in bacteria. One of the potential targets is the enzyme aspartate- β -semialdehyde dehydrogenase (ASADH) which catalyzes the second reaction of aspartate biosynthetic pathway. Almost a quarter of the amino acids are synthesized through this pathway. Besides essential amino acids, this pathway provides many other compounds required for various critical functions of the organisms, and any disruption of the pathway will be fatal. Blocking lysine biosynthesis in *Mycobacterium tuberculosis* is fatal for the organism which requires de novo synthesis of the amino acid for its survival. Another significance is in developing the herbicides, which can work by blocking the biosynthesis of essential amino acids in weeds. One of the examples is the use of glyphosate as the potential herbicide. Glyphosate is the inhibitor of enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, which is the sixth enzyme of shikimic acid pathway. The herbicide-tolerant crop plant can be produced by genetic engineering.

Glutamate-Derived Amino Acids Amino acids included in this category are proline, arginine, and a non-protein amino acid ornithine. Initial reactions require ATP and NADPH leading to formation of glutamate semialdehyde. This is followed by the formation of a ring because of condensation of the carbonyl group of glutamate semialdehyde with α -amino group of the molecule, resulting in the formation of proline. The reaction is coupled with oxidation of yet another molecule of NADPH. Proline accumulates in plant tissues in response to drought and salt stress. It serves as a compatible solute, so it can accumulate at higher concentration without disturbing normal cellular activities and can protect the cell against osmotic stress. Proline also serves in scavenging ROS. Thus, synthesis of proline by the plants is significant in developing transgenics, which are more suitable to grow under drought. Arginine, another amino acid, has a high N/C ratio (4:6). It serves as the nitrogenous compound for storage in the seeds, both in protein bound form and in free form. Arginine also serves as a precursor of many alkaloids and signaling molecule—NO (nitric oxide). Synthesis of arginine requires acetyl CoA along with glutamate. Acetylation of α -amino group of glutamate protects it from cyclization. Three more amino groups are added from glutamate, **carbamyl phosphate**, and aspartate, resulting in synthesis of arginine. Prior to arginine, ornithine is produced, which is a non-protein amino acid. In animals, arginine is the immediate precursor of urea. So, it is important in urea cycle.

Aspartate-Derived Amino Acids Amino acids included in this category are lysine, threonine, isoleucine, and methionine. Since these amino acids are not synthesized in animals, these are required in their diet. Some of the foods, such as corn, are poor in lysine, methionine, and tryptophan, while soybean is rich in lysine. Corn can be supplemented with soybean even for the grain-fed animals for their better growth. Initial steps in the biosynthesis require ATP and NADPH. There is a bifurcation of the pathway after intermediate aspartate semialdehyde is synthesized. For biosynthesis of lysine, pyruvate is required. For synthesis of threonine and methionine, NADH and ATP are consumed. Synthesis of methionine is sulfur requiring and is a complicated multistep process. All steps of aspartate-derived amino acids, including methionine, are known to occur in plastids. Methionine occupies central position in sulfur metabolism. It is entirely obtained by the mammals from their diet. Carbon skeleton of the methionine is provided by aspartate, methyl group comes from β carbon of serine, and sulfur comes from cysteine. Methionine has two functions. It is the structural component of proteins and is required for generation of S-adenosylmethionine (SAM). It is of considerable interest for the plant scientists to produce plants which are able to synthesize more lysine. Generally, lysine accumulation leads to inhibition of its biosynthesis because of feedback regulation of the enzyme dihydrodipicolinate synthase. Plants with mutant dihydrodipicolinate synthase have been produced in which enzyme is insensitive for lysine accumulation. Another strategy adopted to produce transgenic plants is by incorporating bacterial dihydrodipicolinate synthase in plants, since the bacterial enzyme is less sensitive to lysine accumulation. However, only a small amount of lysine is increased, which is incorporated in the proteins because free lysine gets degraded. So, protecting lysine from degradation can be a better strategy, which has been tested in *Arabidopsis*.

Aromatic Amino Acids Synthesis of aromatic amino acids occurs in plastids. This category of amino acids includes tryptophan, phenylalanine, and tyrosine. Precursors for biosynthesis of aromatic amino acids are phosphoenolpyruvate and erythrose 4-phosphate. Shikimate is formed as an intermediate, so the pathway is known as shikimate pathway. Pathway is common till the formation of an intermediate chorismate. Subsequent to chorismate formation, the pathway branches off. Enzymes of this pathway are located in plastids and have not been found in animals, so inhibitors for these enzymes would be potential antibiotics against animal pathogens. Besides synthesis of aromatic amino acids, numerous other aromatic compounds (plant hormones, e.g., auxins, salicylic acid; pigments, e.g., anthocyanins; signal molecules, e.g., isoflavonoids and many others) are also synthesized through this pathway. Products of the shikimate pathway can be as high as up to 50% of the total dry matter of the plant; therefore it is regarded as one of the major pathways.

Branched-Chain Amino Acids Isoleucine, leucine, and valine are included in this category. These amino acids represent three of the ten essential amino acids and are nutritionally important. Two molecules of pyruvate (precursor) are converted to

α -acetolactate. The reaction is catalyzed by the enzyme acetolactate synthase, which requires thiamine pyrophosphate (TPP) as the cofactor for this reaction. α -Acetolactate is reduced to valine using NADPH, a dehydration reaction and transamination by glutamate. Branching of pathway results in the synthesis of leucine. Isoleucine is synthesized from threonine. Biosynthetic pathway of these amino acids exists in chloroplasts.

Histidine The pathway for histidine biosynthesis has been completely worked out in plants. Eight enzymes are involved in the biosynthesis of histidine. Complete pathway for histidine biosynthesis occurs in the plastids. Precursors for biosynthesis of histidine are ribose-5-phosphate and ATP.

Alanine Alanine is derived from pyruvate, an intermediate of glycolytic pathway, by a single transamination step.

Glycine and Serine The major route for the biosynthesis of these amino acids is during photorespiration. Serine may also be synthesized by an alternate route as well, i.e., 3-phosphoglycerate-dependent synthesis in chloroplasts.

Cysteine Mammals are not able to reduce sulfates, so they are dependent on the plants for the supply of sulfur-containing amino acids: methionine and cysteine. Cysteine is required for maintaining protein structure. Sulfur-containing R groups of cysteine can be easily converted from thiol (-SH) to the disulfide bond formed in between two cysteine amino acids (-S-S-) on being oxidized and vice versa. So, it plays an important role in maintaining protein structures. In plants, enzymes for cysteine biosynthesis are present in the cytosol, plastids, and mitochondria. This might be due to the possibility of cysteine being unable to cross the cell membranes. Cysteine is synthesized from serine, which first gets acetylated followed by addition of the thiol (-SH) group, which is coupled with the removal of acetate group. Methionine is synthesized from cysteine.

Non-protein Amino Acids Various amino acids are present in free form and are not bound to form the proteins. In legume seeds, many of these types are present, which function in chemical defense against predation and disease. Toxic action of many of the non-protein amino acids may be through a range of their actions, e.g., through inhibiting catalytic activity of the key enzymes. One of the examples of non-protein amino acids is γ -aminobutyric acid (GABA) which is produced in response to abiotic and biotic stress.

Nucleotide biosynthesis steps are similar in all the organisms. This includes purine and pyrimidine biosynthesis. Purine biosynthesis requires 5-phosphoribosyl 1-pyrophosphate (PRPP). Nitrogen in the purines is furnished by the glutamine, glycine, and aspartate. Pyrimidine biosynthesis requires carbamoyl phosphate and aspartate. Ribose-5-phosphate is attached afterward yielding pyrimidine

ribonucleotides. These are phosphorylated to form triphosphates. Ribonucleotides are converted to deoxyribonucleotide by the action of an enzyme known as ribonucleotide reductase.

Summary

- Nitrogen is an essential element for the plants. Many biomolecules in plants such as proteins, nucleic acids, plant growth regulators, and plant pigments contain nitrogen as their constituent.
- In nature, nitrogen is present either in free form as molecular nitrogen in the air or is present in the soil in bound form, such as nitrates and ammonium or as organic forms derived from dead plants or animals and animal excretion. Nitrogen is cycled in nature since all these forms of nitrogen are interconvertible due to microbial action.
- Plants are able to absorb nitrogen from the soil as nitrates or ammonium. Absorption of nitrate and ammonium ions is facilitated by the receptors present on plasma membrane of the root cells. Excess nitrates can be stored in the vacuoles, while excess ammonium, if not metabolized, becomes toxic for the plants. Nitrate can also be translocated to leaves and is metabolized there.
- Nitrate needs to be reduced in the plant cells before it is further metabolized. There are two enzymes, i.e., nitrate reductase and nitrite reductase, which catalyze reduction of nitrate to ammonium in cytosol and plastids of the plant cell, respectively. Nitrate reductase is an inducible enzyme, while nitrite reductase is a constitutive enzyme. The latter is present in much more amount so that there is no accumulation of nitrite in the cell which is otherwise toxic.
- Molecular nitrogen present in the air can be fixed by some of the prokaryotes, which are called *diazotrophs*. *Diazotrophs* can either be free living or grow in symbiotic associations with plants. Two most important nitrogen-fixing symbiotic associations are *Rhizobium*, legume, and *Frankia*, actinorhizal.
- A great degree of specificity exists in between the specific strain of rhizobia and the leguminous plant, which is due to the interaction of biochemical signals produced by the plants in the form of flavonoids, and the Nod factors of rhizobia. Specific Nod factors produced by the rhizobia species are identified by the Nod receptors present on the plasma membrane of the root hair. This is followed by the formation of an infection thread and subsequently nodules. Effective symbiosis is established in the form of symbiosomes due to the involvement of both rhizobial and host genes.
- Bacteroids are the nitrogen-fixing forms of rhizobia because of nitrogenase being synthesized by them, which is responsible for the reduction of molecular nitrogen to ammonium ions. Nitrogen-fixing genes of the bacteria, i.e., *nif* genes, are involved in the synthesis of nitrogenase.
- Ammonium ions, either absorbed by the plants or produced as a result of nitrate reduction or nitrogen fixation, need to be assimilated which is carried out by glutamine synthetase and 2-oxoglutarate aminotransferase, which are collectively abbreviated as GS/GOGAT.

- Nitrogen is transported either in the form of glutamine or asparagine. There are however some legumes in which nitrogen is translocated in the form of ureides. It is the ratio of nitrogen to carbon in the molecules which is significant for the nitrogen to be translocated. Ureides have the highest N/C ratio.
- Plants and bacteria can synthesize all the 20 amino acids, while animals are able to synthesize only about half of them since some of the enzymes required for the biosynthesis of these amino acids do not exist in animal systems. Since, plants, fungi, and bacteria are able to synthesize all the 20 amino acids, there is potential for developing new herbicides, antibiotics, and antifungal medicines by selectively blocking the pathway for the synthesis of these essential amino acids in weeds, bacteria, and fungi, respectively.

Multiple Choice Questions

1. The process in which NO_3^- serves as electron acceptor in place of O_2 , is known as:
 - (a) Ammonification
 - (b) Nitrate reduction
 - (c) Nitrate respiration
 - (d) Denitrification
2. Nitrate uptake into roots of the plants from soil is facilitated by:
 - (a) Uniporters
 - (b) $2\text{H}^+/\text{NO}_3^-$ symporters
 - (c) $2\text{H}^+/\text{NO}_3^-$ antiporters
 - (d) Nitrate transporters belonging to chloride channel family
3. During reduction by nitrate reductase, electrons are passed to NO_3^- :
 - (a) From heme cofactor of the enzyme
 - (b) From FAD cofactor at C-terminal of the enzyme
 - (c) From reduced molybdenum of MoCo at the C-terminal of the enzyme
 - (d) From reduced molybdenum of MoCo at the N-terminal of the enzyme
4. Electrons for NO_2^- reduction are obtained from:
 - (a) NADH
 - (b) Reduced ferredoxin
 - (c) NADPH
 - (d) Reduced glutathione
5. An example of symbiotic association of non-leguminous plants with rhizobium is:
 - (a) *Gunnera*
 - (b) *Anthoceros*
 - (c) *Casuarina*
 - (d) *Parasponia*

6. Chemical nature of Nod factors is:
 - (a) Lipoproteins
 - (b) Oligosaccharides
 - (c) Lipochitooligosaccharides
 - (d) Oligopeptides
7. The term symbiosome is used for depicting:
 - (a) Vesicular structure formed in the infected cells of nodules
 - (b) The symbiotic relationship in between the endosymbiont and host plant
 - (c) The infection thread formed at the time of nodulation
 - (d) Infected roots cells of nodules in legumes
8. Site for N_2 reduction in nitrogen-fixing enzyme is present in:
 - (a) Dinitrogenase reductase
 - (b) P cluster of dinitrogenase
 - (c) M cluster of dinitrogenase
 - (d) O-side chain of serine from the peptide backbone of P cluster
9. N_2 fixed by bacteroids is released in the cytosol of the infected cell as:
 - (a) NH_3
 - (b) NH_4^+
 - (c) Glutamine
 - (d) Ureides
10. Which of the following statements is true?
 - (a) *nifD* and *nifK* encode for nodulins.
 - (b) Fe protein is encoded by *nifF*.
 - (c) The *nif* genes are required for nitrogen fixation only by the symbiotic bacteria and not by free-living nitrogen fixers.
 - (d) *fix* genes are essential for nitrogen fixation in symbiotic nitrogen fixers but do not have counterparts in free-living forms.

Answers

1. c 2. b 3. d 4. b 5. d 6. c
7. a 8. c 9. b 10. d

Suggested Further Readings

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