International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 10 [1] March 2019 : 19-24

[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA **RESEARCH ARTICLE**

DOI: .10.15515/iaast.0976-4828.10.1.1924

Nature nitrogen factories- Nodules and legumes- A Review

Ranju Gulati
DAV College Chandigarh
Email: ranjugulatidav@gmail.com

ABSTRACT

Strange but true the most abundant mineral in the earth atmosphere, Nitrogen is unavailable to plants due to stable triple bond between two nitrogen atoms. This inert nitrogen is converted to useable ammonia form via few groups of prokaryotes in mini factories called nodules on legume roots. Chemical fertilizers though increase crop yields but in return cause heavy damage to environment. The present article is an attempt to explain Biological Nitrogen Fixation – A Key to Sustainable development in a lucid manner.

Keywords: *Nitrogen fixation, legumes, nodules*

Received 10.12.2018 Revised 08.01.2019 Accepted 09.02.2019

CITATION OF THIS ARTICLE

Ranju Gulati. Nature nitrogen factories- Nodules and legumes- A Review. Int. Arch. App. Sci. Technol; Vol 10 [1] March 2019 : 19-24

INTRODUCTION

Nitrogen is the most essential element for plant growth and development. Nitrogen is an integral component of chlorophyll (the important pigment needed for photosynthesis), amino acids (the key building blocks of proteins), nucleic acids and most other biomolecules like ATP [1]. Despite its abundance in the atmosphere, it cannot be directly utilized by the plants due to its inert nature. Plants can acquire nitrogen by three processes (i) Atmospheric nitrogen fixation via lightening (approximately 15%) (ii) Chemical nitrogen fixation via Haber- Bosch process (approximately 25%) and (iii) Biological nitrogen fixation discovered by Beijerinck in 1901 in specialized group of prokaryotes (approximately 60%) [2]. Prokaryotes include organisms like cyanobacteria, free living soil bacteria, bacteria with associative relationship with plants and bacteria in symbiotic relation with legumes and non legumes.

Biological nitrogen fixation is a phenomenon occurring in all known ecosystems and is, undoubtedly, of greatest agricultural importance as it represents the most significant contributor to the global nitrogen cycle which is major source of nitrogen in agriculture where symbiotic N_2 fixing organisms are used. From the agricultural point of view, leguminous plants, in symbiotic association with appropriate bacteria (*Rhizobium* species), are very important and can be suitably exploited to catalyze the fixation of atmospheric nitrogen [3].

Leguminous plants, rather root nodules are by far the largest sole source of organic nitrogen in the global nitrogen cycle. In symbiotic nitrogen fixation, the plant supplies the carbon source for the energy dependent reduction of dinitrogen and protects the most effective Nitrogen fixing organisms and in turn obtains a part or the total Nitrogen required for plant growth from its symbiotic partner i.e. bacteria [1].

The term 'legumes' broadly applied to all plants of the pea and bean family leguminosae, now named as Fabaceae (third largest and economically important family of flowering plants), consists of three subfamilies namely Caesalpinoidae ,Mimosoidae and

Papilionoidae which are valued for their ability to fix atmospheric molecular nitrogen thus making largest contribution to the global biological nitrogen fixation , through a symbiosis with specific soil bacteria. Bacteria belonging to the family Rhizobiaceae (*Rhizobium, Bradyrhizobium, Azorhizobium, Mesorhizobium, Sinorhizobium* etc.) collectively referred to as Rhizobia), elicit on their host, a successful infection that leads to the development of specialized organs, the nodules, where biological nitrogen fixation takes place, by the creation of ecological niche required for atmospheric nitrogen thus rendering the plant independent of soil nitrogen [4] . Nodules induced by rhizobia are of two general kinds, determinate and indeterminate. Determinate nodules lack a persistent meristem, are usually round and are found in legumes of tropical origin (*Glycine max*, *Phaselous vulgaris*) whereas indeterminate nodules seen in legumes of temperate origin (*Pisum sativum*, *Medicago sativa*) are elongated with persistent meristem that continually gives rise to new nodule cells that are subsequently infected by rhizobia residing in the nodule [5].

Nitrogen fixation in legumes with regards to physiology and biochemistry can be studied under the following subheadings:-

I) Nodule Organogenesis
II) Symbiotic Nitrogen fixation
III) Nitrogen assimilation

I) Nodule Organogenesis

The first step in the nodulation process is the attachment of the rhizobia to the plant root hairs for a long term association [1]. After rhizobia attach to the tips of emerging root hairs, next step is the deformation and curling of the root hairs, from which bacteria penetrate the cell, using a special tunnel structure called infection thread. Parallel to the infection thread development, cortical cell divisions occur and a nodule primoridium is formed. Bacteria in the infection thread reach the nodule primoridium and are engulfed into the nodule cell cytoplasm, and are differentiated into specific nitrogen fixing forms called bacteroids .

The bacterium are surrounded by the membrane of plant origin termed as symbiosomes (peribacteroid membrane - PBM). Parallel to bacteroid differentiation, the nodule primoridium develops into a nodule with a typical histological zoning. An externally triggered organogenesis leads to the formation of nitrogen fixing nodules [5]. This extraordinary phenomenon unites and develops bondage between the plant host and symbiotic bacteria in a microenvironment suitable for the occurrence of the bacterial nitrogen fixation [6].

(a) *Rhizobia* bacteria

Using modern methods of bacterial systematic Rhizobia were classified in to three genera namely *Rhizobium* (fast growing), *Bradyrhizobium* (slow growing), and *Azorhizobium* (nitrogen dependent growth in free living conditions). Rhizobia are gram negative belonging to the large and important division *Proteobacteria* division. Mostly, rhizobia have a narrow host range and nodulates only a very narrow range of symbionts and this host specificity to the symbiotic interaction depends on recognition signals exchanged between Rhizobia and their hosts [8].

(b) Nodule Formation

Process of nodule induction depends upon the specific expression of plant and bacterial genes. The identification of appropriate genes is a prerequisite for studying root nodule development and the underlying communication between the bacterium and the plant [9]. The plant controls the expression of bacterial nodulation genes via phenolic compounds whereas the rhizobia signal back to the plant by secreting specific lipooligosaccharides. Flavonoids, isoflavonoids, betaines and phenolic compounds secreted by roots of legumes act as signals for the induction of nodulation (nod) genes in the rhizobial partner which return signal as N acylated chitin oligosaccharide. Nodulation (nod) factors essential for root hair deformation, formation of nodule primoridia, are synthesized by the products of rhizobial nodulation genes which are induced by plant secreted molecules such as flavonoids and are recognized by membrane receptor like kinases [10]. Nod factors are considered the main rhizobial inducer molecules for nodulation as they result in calcium spiking signaling pathway and ion balance, actin induced alternation in cytoskeletal

organization and morphology of root hairs, the initiation of cortical cell divisions and the triggering the development of nodule development in the host plant [11].

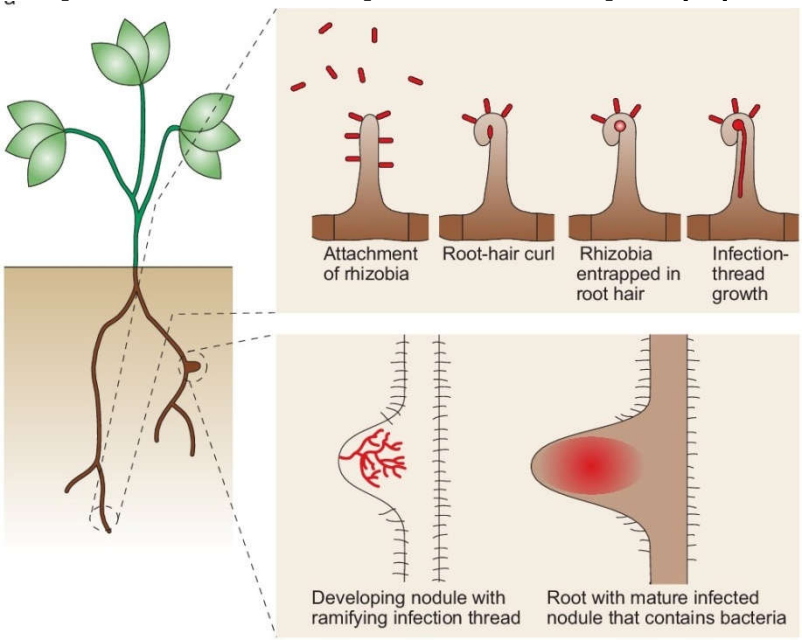

Fig 1: Legume infection by *Rhizobium*

(Images courtesy of Simon Walker, John Innes Centre, UK., with permission)

II.SYMBIOTIC NITROGEN FIXATION

Symbiotic nitrogen fixation takes place in the modified bacterial cells called bacteroids, whose niche are the legume nodules. Enzymatic conversion nitrogen fixation which involves conversion of nitrogen to ammonia is catalyzed by the bacterial enzyme nitrogenase. Nitrogenase enzyme complex, which has been purified from different sources is composed of two components and is metalloenzyme that catalyses the MgATP dependent reduction of nitrogen to yield two molecules of ammonia [12]. This catalytic reduction of nitrogen is called Biological nitrogen fixation, and the stiochiometry of the reduction is usually indicated as:-

$N2+8e-+8H++16Mg\ ATP \longrightarrow 2NH3+H2+ 16MgADP+ 16Pi$

(a) Nitrogenase**: -** Nitrogenase is made up of two distinct parts, dinitrogenase reductase (Fe Protein) which is an electron carrier and dinitrogenase (MoFe protein) which is responsible for nitrogen reduction [2]. It comprises about 30% of the total protein in the infected cells. The MoFe protein (Component 1) is an $\alpha 2\beta 2$ heterotetramer of 220,000 Da and contains 30 Fe atoms and 2 Mo atoms organised into 2 pairs of metalloclusters referred to as P clusters and FeMoCo cofactors whereas Fe protein (Component 2) is a homodimer of 68,000Da formed by identical subunits and contains 4 Fe atoms organised into Fe4S4 cluster [13].

(b) Leghemoglobin**:-**The nitrogenase enzyme is an oxygen sensitive complex but oxygen is required in the nodules to support the highly active respiratory process that takes place aerobically in the plant and bacteroid compartments. To protect nitrogenase from inactivation by oxygen, a very low level of oxygen is maintained in the infected cells of functional legume nodules via combination of high rate of bacteroid and mitochondrial respiration as well as a barrier to oxygen diffusion, located in the nodule inner cortex called as nodule parenchyma in the form of haemoprotein namely leghaemoglobin [3]. Leghemoglobin (Lb), a myoglobin is a red coloured pigment discovered in soyabean nodules, is synthesized jointly by leguminous plants and rhizobia. Leghemoglobin acts as an oxygen buffer in the nodule and facilitates the transport of oxygen at a strictly controlled concentration to rapidly respiring bacteroids thus fulfilling the dual role for oxygen scavenging and oxygen transporter [14].

The microsymbiont, i.e. the bacteroid, depends upon oxygen and photoassimilates supplied by the host plant. The photosynthate in the form of sucrose is translocated to the root nodules, which is metabolized to produce dicarboxylic acids, mainly malate, which further

provides the bacteroids with carbon and energy through the symbiosome membrane [9].
Carbon metabolism has two main roles in the nodule, first to provide energy and reducing power to both bacteroids and host cell cytosol and the second role is to provide carbon skeletons for the transport of fixed nitrogen.

III.NITROGEN METABOLISM

Atmospheric inorganic nitrogen is converted to a biologically useful form either by the molecular nitrogen fixation or the assimilation of nitrate. The nitrate, after its uptake by roots, is converted to ammonia in an eight electron reduction process that occurs in two steps with the help of enzymes [15].
The first step in the nitrate assimilation is a two electron reduction of nitrate to nitrite catalyzed by the enzyme nitrate reductase (NR) located in the cytosol. The second step is a six electron reduction of nitrite to ammonia, catalysed by the enzyme nitrite reductase (NiR) [16] .The conversion of nitrate to ammonia consumes eight electrons and occurs in chloroplast. Nitrate reductase is the substrate inducible enzyme and it is homodimer where each monomer consists of three prosthetic groups : flavin adenine dinucleotide (FAD), heme (cyt. b 557) and Molybdenum cofactor (MoCo) which serve as redox centers for the transfer from NADH or NADPH to nitrate. This enzyme is considered to be rate limiting and regulatory step in nitrate utilization. The enzyme nitrite reductase (NiR) mediates the reduction of nitrite to ammonia, is induced by nitrate. Unlike nitrate reductase which is located in leaf tissue, nitrite reductase is associated with plastids of roots and leaf cells and instead of NADH or NADPH, the ferredoxin is the reducing agent in the both root and leaf cells [17].
Ammonia, either directly absorbed by plant roots or as a result of reduction of nitrate, is further assimilated and incorporated into the amide amino group in the plastid/ chloroplast by the action of glutamine synthetase (GS) and subsequently in to glutamic acid by glutamate synthase or glutamine 2- oxoglutarate amino transferase (GOGAT) frequently designated as GS/ GOGAT cycle [4]. Earlier, glutamate dehydrogenase (GDH) was considered to be primary route for the assimilation of ammonia but GDH enzyme has a high Km value (Michaelis-Menton constant) for ammonia which argues strongly against its role in ammonia assimilation [15].
The ammonia produced by Nitrate reductase is condensed with glutamate to form glutamine by glutamine synthetase (GS) in the plastids and cytosol which the help ATP . Two isoforms of glutamine synthetase have been identified in plants: one located in the cytosol (GSI) and other in the chloroplast (GS2) [19]. GSI which is predominantly located in roots is responsible for root nitrogen assimilation whereas GS2 which is predominantly located in leaves serves mainly purpose of primary assimilation of ammonia reduced from nitrate in chloroplasts .
Glutamate synthase or glutamate 2- oxoglutarate aminotransferase (GOGAT) mediates the conversion of □-keto-glutarate and glutamine to glutamate. Two different types of GOGAT enzymes exist in plants in two isoforms called respectively NADH-GOGAT and Fd GOGAT, depending on the use of either NADH or ferredoxin as electron donors. Fd GOGAT is localised in chloroplasts and is involved in the assimilation of ammonia derived from the light dependent reduction of nitrate and from photorespiration. It is monomeric enzyme with molecular mass of 140-160 KD and is considered to be a Fe- S protein and Fd GOGAT is a flavoprotein containing an iron- sulphur cluster with a molecular mass of 200 to 240 KD and is well characterized in legume roots nodules [20]. The two amino acids glutamine and glutamate which are products of ammonia assimilation act as key donors in various cellular reactions.

NH_3 + glutamate + ATP $\xrightarrow{GS}$ Glutamine+ADP+ Pi $\longrightarrow$

Glutamine + 2- oxoglutarate + NADPH + H+ $\xrightarrow{GOGAT}$ 2 Glutamate + NADP+

The Nitrogen which is assimilated in form of the glutamine or glutamate is exported from the nodules as other amides like asparagine, glutamine along with asparate, glutamate, alanine, γ□ aminobutyric acid and serine or ureides like allantoin and allantonic acid in proportions which depend on legume species. For Biological Nitrogen Fixation, the carbon requirement for initial assimilation of nitrogen is met by Krebs cycle intermediates α-

ketoglutarate and oxaloacetate. Combined activity of Phosphoenolpyruvate carboxylase (PEPC) and malic dehydrogenase (MDH) provide a substantal amount of malate in nodules which serves as the principal energy substrate for bacteriods and after conversion to oxaloacetate serves as carbon source for amino acid synthesis [12]. Thus, the functioning of this symbiotic nitrogen fixation and its subsequent assimilation to ammonia is dependent upon carbon metabolism.

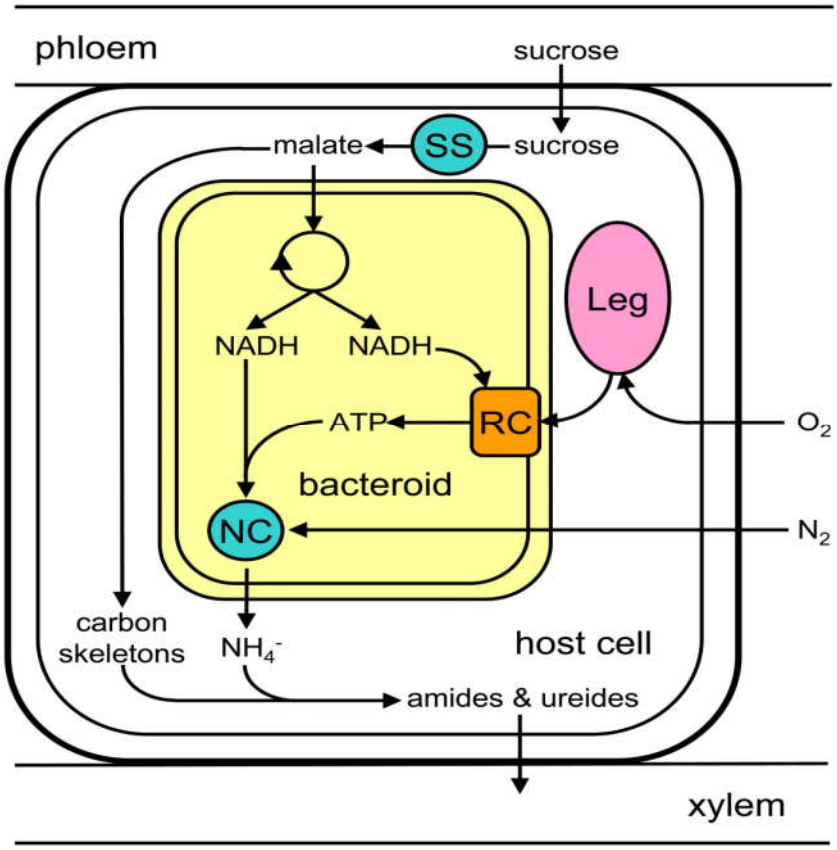

Fig 2: A root nodule showing the exchange of C and N between the host and bacteriod. Leg, Leghemoglobin; RC, bacterial respiratory chain; NC, the nitrogenase complex; SS, Suc synthase. (Reprinted from http://www.plantphysiol.org/content/151/3/1009 with permission from author Alistair Rogers (arogers@bnl.gov).

CONCLUSION

In agricultural systems symbiotically fixed nitrogen not only enhances the dry matter and seed production but also supplements the nitrogen needs of fixing species and in addition it is a boon for farmers in the form of green manure providing nitrogen to the crops grown in rotations. So this plant microbe interaction between legumes and rhizobia represents a microcosm where communication and signaling below the soil is fixing the nitrogen an important constituent for healthy plant growth. This interaction is highly useful for our agriculture industry free from harmful nitrogen fertilizers [21]. The understanding of this symbiotic relation can be used in future for designing rhizobia suitable not only for legume crops but also for cereal crops which is a staple food worldwide and this can act as an effective agent enhance crop productivity to feed the growing population and save the depleting soil nutrients particularly the nitrogen .

REFERENCES

1. Abdel-Lateif K, Bogusz D, Hocher V. (2012). The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and *Frankia* bacteria. Plant Signaling and Behavior. 7:636–641.
2. Adams DG, Duggan PS. (2011). Signalling in cyanobacteria–plant symbioses. In: Perotto S, Baluska F, editors. Signalling and communication in plant symbiosis. Berlin: Springer; pp. 93–122.

3. Dawson JO.(2008). Ecology of actinorhizal plants. In: Pawlowski K, Newton WE, editors. Nitrogen-fixing actinorhizal symbioses. Nitrogen fixation: origins, applications, and research progress. Vol. 6. Dordrecht: Springer; pp. 199–234.
4. Godfroy O, Debellé F, Timmers T, Rosenberg C.(2006).A rice calcium- and calmodulin-dependent protein kinase restores nodulation to a legume mutant. Molecular Plant–Microbe Interactions. 9:495–501.
5. S Sulieman , L S P Tran, Symbiotic nitrogen fixation in legume nodules: metabolism and regulatory mechanisms, *International Journal of Molecular Sciences* , Vol.15, No. 11,pp.19389-93,2014.
6. B M Hoffman,D Lukoyanov, L C Seefeldt, (2014). Mechanism of nitrogen fixation by nitrogenase: the next stage, *Chemical Reviews*, Vol.114, No. 8,pp.4014-62.
7. C A Appleby, (1984). Leghemoglobin and Rhizobium respiration, *Annual review Plant Physiology*,Vol. 35,pp. 443-78.
8. S J Temple, C P Vance, J S Gantt, (1998). Glutamate synthase and N- assimilation, *Trends in Plant Science* ,Vol.3, No. 2,pp. 51-56.
9. Heckmann AB, Hebelstrup KH, Larsen K, Micaelo NM, Jensen EO.(2006). A single hemoglobin gene from *Myrica gale* retains both symbiotic and non-symbiotic specificity. Plant Molecular Biology.61:769–779.
10. Meeks JC. Symbiosis between nitrogen-fixing cyanobacteria and plants. BioScience. 1998;48:266–276.
11. Oldroyd GE, Murray JD, Poole PS, Downie J.A. The rules of engagement in the legume-rhizobial symbiosis. Annual Review of Genetics. 2010;45:119–144.
12. Silvester WB, Harris SL, Tjepkema JD. Oxygen regulation and hemoglobin. In: Schwintzer RC, Tjepkema JD, editors. The biology of Frankia and actinorhizal plants. San Diego, CA: Academic Press; 1990. pp. 157–176.
13. Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil. 2009;321:111–117.
14. Saikia SP, Jain V. (2007). Biological nitrogen fixation with non-legumes: an achievable target or a dogma? Current Science. 92:317–322.
15. Remy W, Taylor TN, Hass H, Kerp H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proceedings of the National Academy of Sciences of the USA.;91:11841–11843.
16. Pandey A, Sharma E, Palni LMS. (1998). Influence of bacterial inoculation on maize in upland farming systems of the Sikkim Himalaya. Soil Biology and Biochemistry. 30:379–384.
17. Laplaze L, Svistoonoff S, Santi C, Auguy F, Franche C, Bogusz D. (2008). Molecular biology of actinorhizal symbioses. In: Pawlowski K, Newton WE, editors. Nitrogen-fixing actinorhizal symbioses. Dordrecht: Springer; pp. 235–259.
18. James EK, Gyaneshwar P, Mathan N, et al. (2002). Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Molecular Plant–Microbe Interactions.15:894–906.
19. Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK. (2001). Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. Journal of Bacteriology. 183:2634–2645.
20. Hess WR. (2011). Cyanobacterial genomics for ecology and biotechnology. Current Opinion in Microbiology. 14:608–614.
21. Hause B, Schaarschmidt S. (2009). The role of jasmonates in mutualistic symbioses between plants and soil-born microorganisms. Phytochemistry. 70:1589–1599.