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## Root nodulation process and its soil ameliorating benefits: A review

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### Abstract

Legume plants have an extraordinary ability to combine with microorganisms which range from non-specific to very specific interaction. Legume rhizobial symbiosis results in large growth and metabolic changes for both microorganisms and hosts, while providing plants with fixed nitrogen. In a complex signal exchange rhizobia leads to selective colonization of plant cells within nodules, development of new organ known as nodule on the roots of host plants. Although the nodulation mechanism is highly specific, it contains same subset of phytohormones of plants, i.e. auxin, cytokinin, and ethylene, which are essential for root development. In addition, the formation of nodule triggered by rhizobia affect the development of host root system and indicating that microorganisms may change the developmental pathways of host. A prime example of nodulation by rhizobia is how microorganisms and plants have coevolved and illustrate how microbial colonization may affect plant developmental pathways.

**Keywords:** Root nodule, nod factor, rhizobia, nitrogenase and nitrogen assimilation

### Introduction

Besides anchoring plants in the soil, roots are essential for mineral nutrition and water uptake. When exposed to fluctuations in the soil environment such as drought or nitrate or phosphate availability, roots activate compensating mechanisms ranging from changes in nutrient uptake ability to changes in root architecture (Desnos 2008). Nitrogen is essentially the most important nutrient required by plant cells for the synthesis of enzymes, proteins, chlorophylls, DNA and RNA, which is essential for the growth of the plant and the production of food (Matiru *et al.* 2004) [41]. However, the availability of nitrogen in many soils is limited, and although 78.1% of the Earth's atmosphere contains nitrogen gas (N<sub>2</sub>), plants are unable to use this kind of nitrogen (Ferguson *et al.* 2010) [25]. However certain plants are blessed with the capacity of fixing atmospheric nitrogen due to their ability of establishing symbiotic relation with some bacteria. Root nodules are among such globular structure formed on the roots of certain plants, notably legumes and alder, by symbiotic association between the plant and a nitrogen-fixing microorganism. Rhizobium in the case of legumes and Frankia in the case of alder and a variety of other plants (Lackie 2013) [36]. Root nodules are fully differentiated plant organs that harbor prokaryotic nitrogen-fixing endosymbionts. The capacity to establish a root nodule symbiosis is confined to a single group of higher plants, the Rosid clade I. Within this clade, six tribes have evolved the capacity to nodulate with *Frankia* (a Gram-positive endosymbiont) while, within the Leguminosae (Fabaceae), symbiosis is established exclusively with members of the *Rhizobium* superfamily (which are all Gram-negative endosymbionts). The study of nodules has mainly involved crop legumes such as soybean, peas, beans, and alfalfa (Brewin 2001) [7].

N utilizes from soil and seed is required for plant productivity. About 25-30% of the total plant N (80-110 kg N per hectare per season) is supplied through the N<sub>2</sub> fixation process (Harper 1971) [29]. Through symbiotic association with soil microbes, the legumes act as soil-improvement components of the agricultural system and increase the agricultural ecosystem. They are able to meet the great demand of N through the assimilation and absorption of inorganic N from the soil for crop plants (International Atomic Energy Agency, 2008) [34].

Rhizosphere a narrow region of the soil surrounding the roots that is directly influenced by root secretions and associated soil Microorganisms. The leguminous plants with *Rhizobium* in nodules are responsible for converting NH<sub>3</sub> from atmospheric N in the N-accumulation process where nitrogenase enzymes are involved. Many bacteria having hydrogenase to oxidize hydrogen in a reaction performed nitrogen fixation.

In the absence of N, nitrogenase produces hydrogen when ATP is supplied (Hirsch 2009) <sup>[30]</sup>. Legumes play an important role in improving the productive capacity of the soil as a source of

N<sub>2</sub>-fixation from the atmosphere, as well as improving recycling of nutrients, protection from soil erosion, the buildup and maintenance of soil organic matter and the suppression of weeds (Hairiah *et al.* 1992; Skerman *et al.* 1988). The use of leguminous plants as 'cover crop' is one of the oldest agricultural practices for improving soil fertility. In this review we are try to present an overview to root nodulation and its importance in improving soil health to get the benefit for crops other than legumes. Legumes represents the third largest group of angiosperms and are the second largest group of food and feed crops grown globally. It includes major food and feed crop species, such as soybean, peas, clover, gram, alfalfa and mungbean. (Berris *et al.* 2001) <sup>[12]</sup>.

### Nitrogen Fixation

Atmospheric nitrogen cannot be directly utilized by plants and animals for their requirement (Dobereiner 1997) <sup>[21]</sup>. Nitrogen fixation is the process by which nitrogen in the atmosphere is converted into a more active form that can be used by plants. Nitrogen fixation is carried out by free living soil bacteria and also symbiotic biological nitrogen fixation occurs in leguminous and non-leguminous plants. (Borris 2001) <sup>[12]</sup>. Some bacteria denominated diazotroph, which possesses an enzymatic apparatus capable to break the triple bond between two nitrogen atoms from the atmospheric nitrogen, forming ammonia that is similar to the industrial process (Dobereiner and Baldani, 1998; Okon and Vanderleyden, 1998 <sup>[45]</sup>; Victoria *et al.* 1992) <sup>[57]</sup>. Free-living nitrogen-fixing microorganisms contribute significantly to the N budgets of a number of ecosystems (Kahindi *et al.* 1997 <sup>[35]</sup>; Deslippe *et al.* 2005 <sup>[19]</sup>; Unkovich and Baldock 2008 <sup>[53]</sup>; Hsu and Buckley 2009) <sup>[33]</sup>. Some nitrogen-fixers may also promote plant growth by synthesizing and releasing antibiotics and growth-promoting substances (Aquilanti *et al.* 2004 <sup>[2]</sup>; Beneduzi *et al.* 2008) <sup>[3]</sup>. Free-living nitrogenfixers of soil or on soil surface include Cyanobacteria, Proteobacteria, Archaea and Firmicutes (Kahindi *et al.* 1997 <sup>[35]</sup>; Widmer *et al.* 1999; Diallo *et al.* 2004 <sup>[20]</sup>; Duc *et al.* 2009) <sup>[24]</sup>. *Azotobacter* and *Azospirillum* genera are free-living bacteria and fix 20-40 kg ha<sup>-1</sup> nitrogen per year cereal crops fields (Essam A. Abd El-Lattief, 2016). Although majority of nitrogen fixation in terrestrial ecosystems is carried out by symbiotic bacteria in association with plants (especially leguminous plants).

### Legume and Nitrogen Fixation

Nitrogen is an important limiting factor for the growth and development of plants, the ability of legumes to perform symbiosis with nitrogen-fixing rhizobia gives them a distinct advantage over other plant species. Fixation of nitrogen through biological mean is an option for nitrogen fertilizer which may help in reducing the overburden of inorganic harmful nitrogen fertilizer. The symbiotic association between legume-rhizobia is the most important symbiosis in terms of biological nitrogen fixation, producing about 200 million tons of nitrogen per year (Graham and Vance 2003 <sup>[28]</sup>; Peoples *et al.* 2009) <sup>[47]</sup>. Leguminous plants developed a symbiotic relationship with specific soil bacteria called *Rhizobia* including the genera *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* (Vance 1990) <sup>[56]</sup>. These prokaryotes by using

an enzyme complex grouped as nitrogenase carried out reduction of atmospheric N<sub>2</sub> in the forms such as ammonia, nitrate and nitrites (Frans J. de Brjun 2016) <sup>[9]</sup> which plant can easily utilize for their nitrogen requirement. Selecting a compatible legume plant, rhizobia invades into the root leading to the development of special root structures called nodules. In this process of "symbiotic nitrogen fixation" the bacteria differentiate into bacteroids in root nodule and begin the reduction of N<sub>2</sub> into ammonia (Graham and Vance 2003; Peoples *et al.* 2009) <sup>[28, 47]</sup>.

### Nodule organogenesis

Encountering the low nitrogen condition legume plants provoke a signaling cascade (nodulation factor or nod factor) by releasing flavinoids to establish a symbiotic relationship with rhizobia that stimulates nodule formation in root of the plants (Gresshoff *et al.* 2010; Redmond *et al.* 1986) <sup>[50]</sup>. Through the process of infection by compatible and specific bacteria suitable to infect particular host legume (Pueppke and Broughton 1999) (Figure: step1). Nod factors (NF) are strain-specific lipo-chito-oligosaccharides resultant of *nod* (nodulation) gene expression activated by root secreted flavinoids (Caetano-Anoll'es and Gresshoff 1991 <sup>[13]</sup>; D'enari'e *et al.* 1996; Spaink 2000) <sup>[51]</sup>. NFs have oligosaccharide backbone of *N*-acetyl-D-glucosamine units with a fatty acyl group attached to the non-reducing sugar and a major determinant of host-symbiont specificity is attributed to the different NF substituents attached to the oligosaccharide backbone (Lerouge *et al.* 1990 <sup>[39]</sup>; D'enari'e *et al.* 1996) (figure 1: step 2).

Compatible rhizobia species and their corresponding NF is generally sufficient to trigger nodule development. Emerging root hairs tips are primary target for infection by rhizobia because their thinner and less cross-linked cell walls allow the re-arrangement of underlying microtubules. Such changing vesicle trafficking stimulates root hair deformation within 6-8 h (Yao and Vincent 1969 <sup>[58]</sup>; Bhuvanewari *et al.* 1981 <sup>[4]</sup>; Bhuvanewari and Solheim 1985) <sup>[5]</sup> and also promotes cortical cell divisions (Calvert *et al.* 1984; Mathews *et al.* 1989) <sup>[40]</sup> (Figure 1; Step 3).

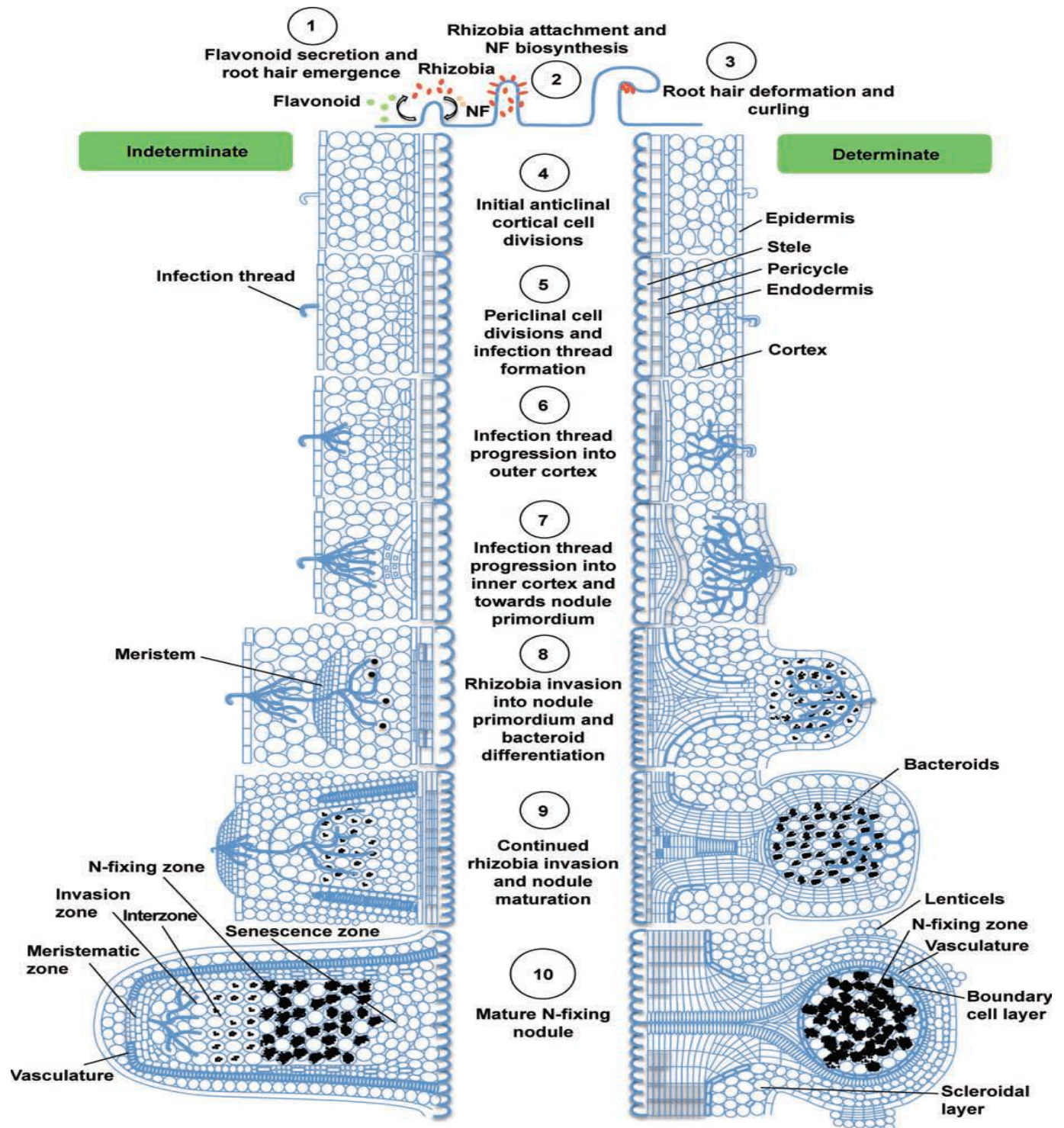
**Mode of bacterial infection:** Basically rhizobia gain entry into their hosts through three different paths. Root hair infection is most common and best understood method, here bacteria gain entry into (usually) curled root hairs and host cell wall material grows around the developing infection forming infection thread. It grows through the cortex of the root, branching repeatedly to invade newly form daughter cortex cells due to simultaneous division of cortex cell. After that bacteria are released from the tips of these infection threads, into host-membrane-bound units called symbiosomes, where the bacteria differentiate into their N<sub>2</sub>-fixing (bacteroids) form and synthesize the nitrogenase enzyme. Number and nature of different variants on this pattern leads to the formation of either determinate or indeterminate nodule (Doyle, 2011). For determinate nodule products of N fixation (Sprent 2001 <sup>[52]</sup>) may be either ureides (phaseoloid legumes) or amides (many Loteae).

The second mode of bacterial infection (aeschynomenoid nodule) is associated with infection occurs at the sites of lateral root emergence, rather than by root hair curling, and infection threads are very rarely seen and then not in the infected region (James *et al.* 2001). It is found in dalbergioid legumes (Lavin *et al.* 2001) <sup>[38]</sup> as a specific characteristic of this clade of legumes and was first described by Corby

(1988). Here internal structure of the nodules is quite different from those formed following hair infection, although they are determinate in nature.

Third mode is associated with infected directly through the root epidermis directly and infection threads are never formed in these nodules. Rhizobia get attached to the root hair tips, the tips curl tightly and bacteria become entrapped in the

curls. Later local hydrolysis take place in plant cell at the curled region (Callaham and Torrey, 1981; Van Spronsen *et al.* 1994) and this invaginates plasma membrane and new plant cell wall material is deposited (Bauer, 1981; Newcomb, 1981; Brewin, 1991; Kijne, 1992) resulting in formation of a tubular structure regarded as infection thread, by which the bacteria enter the plant. (Figure: step 4 & 5).



**Determinate and Indeterminate Nodule Structures**

Based on the site of first internal cell divisions, maintenance of a meristematic region and the form of the mature nodules (Newcomb *et al.* 1979; Gresshoff and Delves 1986; Rolf Gresshoff 1988 and (Hirsch 1992) <sup>[30]</sup> nodules are classified as indeterminate and determinate.

**Indeterminate nodule**

Indeterminate nodules are typically cylindrical or elongated in shape with a persistent apical meristem (Patriarca *et al.* 2002 <sup>[46]</sup>; Vasse *et al.* 1990) <sup>[56]</sup>. And accordingly bacteroids within these nodules undergo large changes in morphology, typically becoming elongated and/or branched (some bacteroids form a characteristic Y-shape) through endoreduplication of the



genome and membrane permeabilization (Mergaert *et al.* 2006) [44].

Here the apical meristem generates new nodule cells (Zone I) that are infected by rhizobia released from branched infection threads in the sub meristematic cells (Zone II), where they differentiate into nitrogen-fixing bacteroids (Zone III) and ultimately undergo senescence (Zone IV) (Fig. 2).

Legume species of the inverted repeat lacking-clade (IRLC), which includes the genera *Pisum*, *Medicago* and *Trifolium*, form indeterminate nodules. Recent evidence suggests that antimicrobial peptides produced by legumes of the IRLC induce these changes in bacteroids (Mergaert *et al.* 2008) [43]

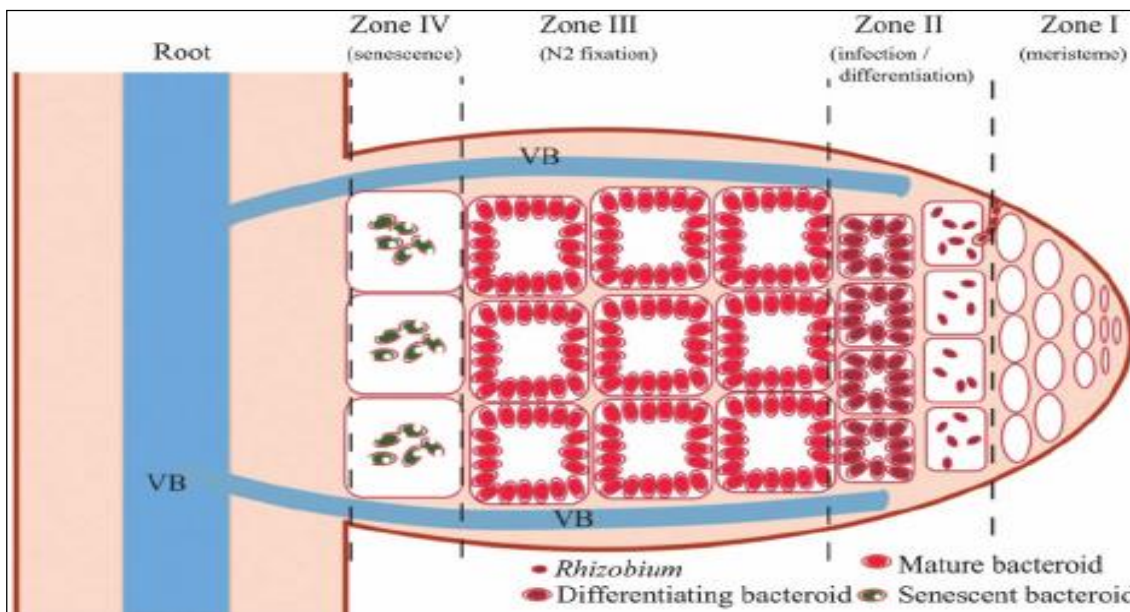


Fig 2: Scheme of the indeterminate elongated nodule. VB indicates Vascular Bundle (Rodriguez-Haas *et al.* 2013)

**Determinate nodule**

Determinate nodules are typically globular in shape and meristematic activity does not persist once formed ((Fig 3). Here tissues lack different developmental zones as compared to indeterminate and thought to contain a relatively homogenous population of bacteroids. However, gene expression in *P. vulgaris* nodules indicated that bacteroids are not all at the same stage of differentiation (Patriarca *et al.*

2002 [46]; Cermola *et al.* 2000; Patriarca 1996) and the lysis of infected cells initiates predominantly within the central nodule tissue. *P. vulgaris*, *G. max* and *L. japonicas* are examples of determinate nodule legumes. Bacteroids within determinate nodules resemble their free-living form in terms of cell size and genome content and remain viable following release from the nodule (Mergaert *et al.* 2006).

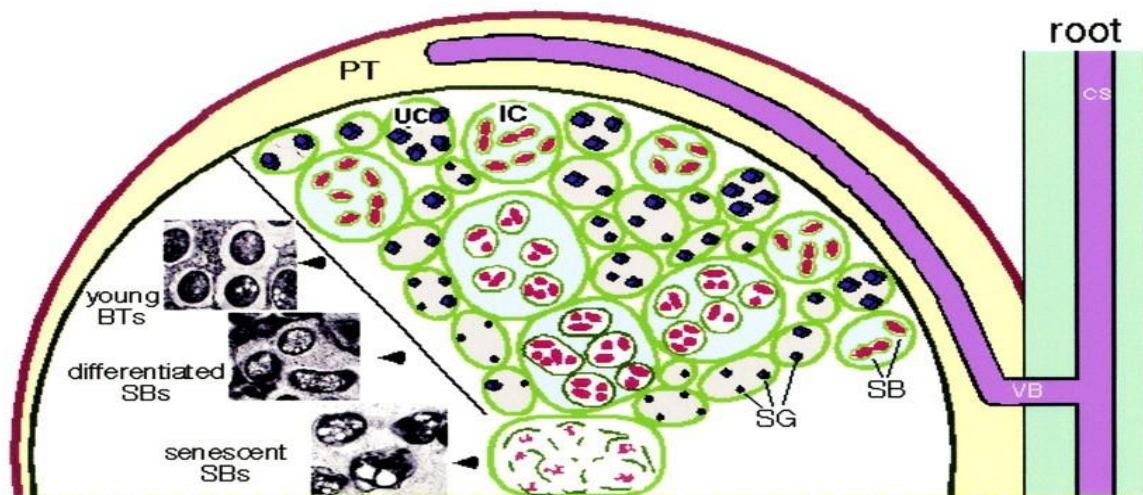


Fig 3: Scheme of the determinate globose nodule. CS, central stele; VB, vascular bundle; PT, peripheral tissue; BTs, bacteroids; SBs, symbiosomes; SG, starch grain; IC, invaded cell; UC, un-invaded cell

**Nitrogen Assimilation**

Nitrogen assimilation is essential course of action controlling plant growth and development. Inorganic nitrogen is assimilated into the amino acids. There are some amino acids which function as important nitrogen carriers in plants glutamine, glutamate, asparagine, and aspartate. The enzymes

responsible for the biosynthesis of these nitrogen- carrying amino acids are glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (Asp AT), and asparagine synthetase (AS) (Lam *et al.* 1996) [37].

In root nodules of legume plants atmospheric dinitrogen atoms directly fixed into ammonia (Burries *et al.* 1993; Vance *et al.* 1992; Vance 1990). In non-legume plants, ammonia is produced by the combined reactions of nitrate reductase and nitrite reductase (Crawford *et al.* 1993<sup>[16]</sup>; Hoff *et al.* 1994)<sup>[31]</sup>. In most tropical and subtropical species, nitrate taken up by the roots is largely transported to leaves where it is reduced to ammonia in plastids (Andrews, 1988)<sup>[1]</sup>.

### Nitrogenase

Nitrogenase plays a key role in global nitrogen cycle. Harbored in a group of microorganisms called diazotrophs, nitrogenase is capable of catalyzing the reduction of atmospheric dinitrogen (N<sub>2</sub>) to bioavailable ammonia (NH<sub>3</sub>) in a nucleotide-dependent process (Burgess *et al.*, 1996<sup>[10]</sup>; Howard *et al.* 1996)<sup>[32]</sup>. The overall reaction catalyzed by nitrogenase is usually depicted as  $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$ . Under the ambient conditions, the ability of nitrogenase to break the inert N-N triple bond not only enables the production of sufficient supply of "fixed" nitrogen through biological process, but also makes nitrogenase interesting subjects from the perspective of chemical energy. As such, nitrogenase has remained a topic of intense research for decades.

### Effect of Legumes on Soil Quality and other crops

Legumes have long been recognized and valued as "soil building" crops. Legumes when grown under organic/ bio fertilizer application (Ghosh *et al.* 2004) improves soil quality through their beneficial effects on soil biological, chemical and physical conditions further farming system residual effect of these leguminous crops may prove an option to inorganically provided nitrogen (Berry *et al.* 2002).

When properly managed, legumes will:

- Enhance the N-supplying power of soils
- Increase the soil reserves of organic matter
- Stimulate soil biological activity
- Improved soil structure
- Reduce soil erosion by wind and water
- Increase soil aeration
- Improve soil water-holding capacity
- Make the soil easier to till

The extent of these soil improvements depends mainly on the type of legume used, the quantity of plant material returned to the soil, and the soil and climate conditions. Legumes benefited by plants in many ways it include: increasing soil organic matter, improving soil porosity, recycling nutrients, improving soil structure, decreasing soil pH, diversifying the microscopic life in the soil, and breaking disease build-up and weed problems of grass-type crops.

**Soil Organic Matter:** As mentioned previously, legumes are high in protein, and therefore, nitrogen rich. Because most crop residues contain much more carbon than nitrogen, and bacteria in the soil need both, the nitrogen supplied by legumes facilitates the decomposition of crop residues in the soil and their conversion to soil building organic matter.

**Soil Porosity:** Several legumes have aggressive taproots reaching 6 to 8 feet deep and a half inch in diameter that open pathways deep into the soil. Nitrogen-rich legume residues encourage earthworms and the burrows they create. The root channels and earthworm burrows increase soil porosity,

promoting air movement and water percolation deep into the soil.

**Recycle Nutrients:** Because perennial and biennial legumes root deeply in the soil, they have the ability to recycle crop nutrients that are deep in the soil profile. This results in a more efficient use of applied fertilizer and prevents nutrients (particularly nitrate nitrogen) from being lost due to leaching below the root zone of the rotation.

**Improve Soil Structure:** Research in both the United States and Canada indicate improved soil physical properties following legumes. The improvements are attributed to increases in more stable soil aggregates. The protein, glomalin, symbiotically along the roots of legumes and other plants, serves as "glue" that binds soil together into stable aggregates. This aggregate stability increases pore space and tilth, reducing both soil erodibility and crusting. (Bowren, 1995)<sup>[6]</sup>.

Cereal and legumes which has become a popular combination among farmers was probably due to legumes ability to combat erosion and raise soil fertility levels (Matusso *et al.* 2012)<sup>[42]</sup>.

### Reference

1. Andrews M. The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant Cell Environ.* 1986; 9:511-19.
2. Aquilanti L, Mannazzu I, Papa R, Cavalca L, Clementi F. Amplified ribosomal DNA restriction analysis for the characterization of Azotobacteraceae: a contribution to the study of these free-living nitrogen-fixing bacteria. *Journal Microbiol Methods.* 2004; 57:197-206.
3. Beneduzi A, Peres D, da Costa PB, Zanettini MHB, Passaglia LMP. Genetic and phenotypic diversity of plant-growth-promoting bacilli isolated from wheat fields in southern Brazil. *Res Microbiol.* 2008; 159:244-50.
4. Bhuvanewari TV, Bhagwat AA, Bauer WD. Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. *Plant Physiol.* 1981; 68:1144-1149.
5. Bhuvanewari TV, Solheim B. Root hair deformation in the white clover/Rhizobium trifolii symbiosis. *Physiol. Plant.* 1985; 63:25-34.
6. Bowren KE, Biederbeck VO, Bjorge HA, Brandt SA, Goplen BP, Henry JL *et al.* Soil Improvement with Legumes. Saskatchewan Agriculture and Food. (Internet site; www.gov.sk.ca), 1995, 24.
7. Brewin NJ. Nodulins Encyclopedia of Genetics, 2001, 1334-1335
8. Brewin NJ. Nodulins Encyclopedia of Genetics, 2001, 1334-1335.
9. Bruijn. Biological Nitrogen Fixation Book Summary Advances in Microbiology. 2016; 6:407-411.
10. Burgess BK, D Lowe DJ. Mechanism of molybdenum nitrogenase, *Chem.* 1996; 96:2983-3012.
11. Burris RH, Roberts GP. Biological nitrogen fixation. *Annu. Rev. Nutr.* 1993; 13:317-35.
12. Burris RH. Nitrogen fixation. *Journal of encyclopedia of life sciences*, 2001, 1-5.
13. Caetano-Anoll'es G, Gresshoff PM. Plant genetic control of nodulation. *Annu. Rev. Microbiol.* 1991; 45:345-382.
14. Calvert HE, Pence MK, Pierce M, Malik NSA, Bauer WD. Anatomical analysis of the development and distribution of *Rhizobium* infection in soybean roots. *Can. J Bot.* 1984; 62:2375-2384.

15. Corby HDL. Types of rhizobial nodule and their distribution among the Leguminosae. *Kirkia*. 1988; 13:53-123.
16. Crawford NM, Arst HN Jr. The molecular genetics of nitrate assimilation in fungi and plants. *Annu. Rev. Genet.* 1993; 27:115-46
17. Crutzen P, Mosier AR, Smith KA, Winiwarter W. N<sub>2</sub>O release from agro-fuel production negates global warming reduction by replacing fossil fuels. *Atmos. Chem. Phys. Discussions.* 2007; 7:11191-11205.
18. Denarie J, Debelle F, Prome JC. *Rhizobium* lipochitooligosaccharide nodulation factors: Signalling molecules mediating recognition and morphogenesis. *Annu. Rev. Biochem.* 1996; 65:503-535.
19. Deslippe JR, Egger KN, Henry GHR. Impacts of warming and fertilization on nitrogen fixing microbial communities in the Canadian High Arctic. *FEMS Microbiol Ecol.* 2005; 53:41-50.
20. Diallo MD, Willems A, Vloemans N, Cousin S, Vandekerckhove TT, de Lajudie P. Polymerase chain reaction denaturing gradient gel electrophoresis of the N<sub>2</sub>-fixing bacterial diversity in soil under *Acacia tortilis* ssp. *raddiana* and *Balanites aegyptiaca* in the dryland part of Senegal. *Environ Microbiol.* 2004; 6:400-15.
21. Dobreiner J. *Soil. Biol. Biochem.* 1997; 29:771-774.
22. Dobreiner J, Baldani VLD. *Biotechnol. Sci. Dev. Bras.* 1998; 1:16-17.
23. Doyle JJ. Phylogenetic perspectives on the origin of nodulation. *Molecular*, 2011.
24. Duc L, Noll M, Meier E, Burgmann H, Zeyer J. High diversity of diazotrophs in the forefield of a receding alpine glacier. *Microb Ecol.* 2009; 57:179-90.
25. Ferguson BJ, Indrasumar A, Hayashi S, Lin YR, Lin YH, Reid DE *et al.* Molecular Analysis of Legume Nodule Development and Autoregulation. *Journal of Integrative Plant Biology.* 2010; 52:61-76.
26. Flooding tolerant legumes from the Brazilian Pantanal. *New Phytologist.* 2001; 150; 723-738.
27. Giller KE. Nitrogen fixation in tropical cropping systems, CABI publishing, Oxon, 2001, 167-168.
28. Graham PH, Vance CP. Legumes: Importance and constraints to greater use. *Plant Physiol.* 2003; 131:872-877.
29. Harper JE, Hageman RH. Canopy and seasonal profiles of nitrate reductase in soybeans (*Glycine max* L. *Merr.*). *Plant Physiology.* 1971; 49:146-154.
30. Hirsch AM. Brief History of the Discovery of Nitrogen-fixing Organisms. Retrieved from History Discovery N<sub>2</sub> fixing Organisms, 2009.
31. Hoff T, Truong HN, Caboche M. The use of mutants and transgenic plants to study nitrate assimilation. *Plant Cell Environ.* 1994; 17:489-506
32. Howard JB, Rees DC. Structural basis of biological nitrogen fixation, 1996.
33. Hsu SF, Buckley DH. Evidence for the functional significance of diazotroph community structure in soil. *Int Soc Microb Ecol.* 2009; 3:124-36.
34. International Atomic Energy Agency. Management of agroforestry systems for enhancing resources use efficiency and crop productivity, 2008.
35. Kahindi JHP, Woome P, George T, de Souza Moreira FM, Karanja NK, Giller KE. Agricultural intensification, soil biodiversity and ecosystem function in the tropics: the role of nitrogen-fixing bacteria. *Appl Soil Ecol.* 1997; 6:55-76.
36. Lackie JM. The Dictionary of Cell & Molecular Biology (Fifth Edition), 2013, 553-580.
37. Lam HM, Coschigano KT, Oliveira IC, Oliveira R, Melo Coruzzi GM. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Journal of Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1996; 47:569-93.
38. Lavin M, Pennington RT, Klitgaard BB, Sprent JI, de Lima HC, Gasson PE. The Dalbergioid legumes (Fabaceae): delimitation of a pantropical monophyletic clade. *American Journal of Botany.* 2001; 88:503-533.
39. Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, Prome JC *et al.* Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature.* 1990; 344:781-784.
40. Mathews A, Carroll BJ, Gresshoff PM. Development of *Bradyrhizobium* infection in supernodulating and non-nodulating mutants of soybean (*Glycine max* [L.] Merrill). *Protoplasma.* 1998; 150:40-47.
41. Matiru VN, Dakora FD. Potential Use of Rhizobial Bacteria as Promoters of Plant Growth for Increased yield in Landraces of African Cereal Crops. *African Journal of Biotechnology.* 2004; 3:1-7.
42. Matusso JM, Mugwe JN, Mucheru-Muna M. Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of sub-Saharan Africa Research Application Summary. Third RUFORUM Biennial Meeting September, Entebbe, Uganda, 2012, 24-28.
43. Mergaert P, Velde WVD, Zehirov G, Ishihara H, Szatmari A, Nagy A *et al.* Terminal differentiation of nitrogen fixing bacteroids induced by antimicrobial plant peptides in *Medicago truncatula* nodules. In 8th European Nitrogen Fixation Conference, 2008.
44. Mergaert P. Eukaryotic control on bacterial cell cycle and differentiation in the *Rhizobium*-legume Symbiosis. *Proc Natl Acad Sci USA.* 2006; 103(13):5230-5.
45. Okon Y, Vanderlayden J. Features. 1998; 63:366-370.
46. Patriarca EJ, Tate R, Iaccarino M. Key role of bacterial NH<sub>4</sub> + metabolism in *Rhizobium*-plant symbiosis. *Microbiol Mol Biol.* 2002; 66(2):203-22.
47. Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S *et al.* The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis.* 2009; 48:1-17.
48. *Plant-Microbe Interactions*, 24, 1289-1295.
49. Pueppke SG, Broughton WJ. *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. *Mol. Plant-Microbe Interact.* 1999; 12:293-318.
50. Redmond JW, Batley M, Djordjevic MA, Innes RW, Kuempel PL, Rolfe BG. Flavones induce expression of nodulation genes in *Rhizobium*. *Nature.* 1986; 323:632-635.
51. Spaink HP. Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol.* 2000; 54:257-288.
52. Sprent JI. Nodulation in Legumes. Royal Botanic Gardens, Kew, Richmond, 2001.
53. Unkovich M, Baldock J. Measurement of asymbiotic N<sub>2</sub> fixation in Australian agriculture. *Soil Biol Biochem.* 2008; 40:2915-21.

54. Vance CP, Gantt JS. Control of nitrogen and carbon metabolism in root nodules. *Physiol. Plant.* 1992; 85:266-74.
55. Vance CP. Symbiotic nitrogen fixation: recent genetic advances. 1990; 70:43-88.
56. Vasse J. Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. *Journal Bacteriol.* 1990; 172(8):4295-306.
57. Victoria RL, Picolo MC, Vargas AAT. Microbiology of the ground campinas, Brazilian science of the ground. The cycle of Nitrogen. 1992, 105-117.
58. Yao PJ, Vincent JM. Host specificity in the root "curling factor" of *Rhizobium* spp. *Aust. J Biol. Sci.* 1969; 22:413-423.