

# Summary Note on Nitrogen Fixation, Legume Nodulation and Abiotic Factors Affecting Biological Nitrogen Fixation Inside the Soil

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## Abstract

However nitrogen is one of the most important nutrient that plants require for healthy growth only plants of the legume family are able to fix their own nitrogen. They accomplished this through a symbiotic relationship with *Rhizobium* bacteria that naturally exist in the soil. In this regard many past researches has largely find out the plant flavonoid signals that induce bacterial *nod* gene expression and the return bacterial Nod signal that induces nodule formation. This inventory is mainly focused on the ways of the nitrogen fixation process particularly with biological nitrogen fixation through nodulation and legume symbiosis. Many literatures narrate the physiological and environmental factors for the success of nitrogen utilization with the legumes and this article highlighted legume nodulation for biological nitrogen fixation and its *edaphic* environmental stresses.

**Keywords:** Nitrogen, Rhizobium, Nodules, Symbiosis, Legumes, Bacterial, Nutrients

## 1. Introduction

Nitrogen is one of the most important nutrients that plants require for healthy growth. Except plants of the legume family which are able to fix their own nitrogen almost all plants depend on the outside source of nitrogen. At the time where the geochemical reserves of fixed nitrogen in the biosphere were depleted the nitrogen fixation is likely evolved. The opposing views of pre biological paleo atmosphere composition highlighted the considerable uncertainty about when prokaryotes acquired the ability of fixation. This view may have been contained large amount of ammonia, methane, carbon monoxide and hydrogen sulfide (Hart, 1979).

With the beneficial role of legumes the atmospheric nitrogen which is unusable by all plants is converted to the form of nitrogen that plants can use. Legumes accomplished it through the relationship with rhizobium bacteria found naturally inside the soil. At the time when rhizobium bacteria associate with legumes nodules are formed on their roots. These are, in essence, the nitrogen-fixing factories. In this mutually beneficial relationship, legumes provide a means to acquire atmospheric nitrogen and an ideal environment for *Rhizobium* growth, while Rhizobium bacteria convert unusable nitrogen into a form that can be utilized by the host legume plant.

After diazotrophy appeared, fixed-N was no longer the limiting nutrient in balanced ecosystems.

However, when such ecosystems are perturbed, nutrients recycle geo biochemically and fixed-N

usually becomes limiting again. One of life's greatest paradoxes is that all living things depend on a source of utilizable nitrogen but only some of the smallest members of this community can produce it from the "sea" of otherwise inert, molecular nitrogen that surrounds and saturates us. This "fixed" nitrogen utilized in all its forms comprises less than 0.0007% of the nitrogen on Earth and in its atmosphere. Nitrogen fixation is that part of the geo biochemical nitrogen cycle that drives the conversion of atmospheric to ammonia, whereas nitrification and denitrification act to return nitrogen to the atmosphere as is fixed by abiological, natural processes, including lightning, combustion and volcanism, which account for about 10% of the annual fixation. Man-made processes, mainly industrial ammonia production, contribute about 25% of the total annual fixation (Newton, 1996)

A near-term strategy for increased fixed-N input to legumes involves a better match of *rhizobial*

Microsymbiont to its host cultivar, earlier initiation and prolongation of symbiotic fixation, that would fix in the presence of fixed-N and improving utilization of applied fertilizer. Of course, the selected strains have to out-compete any indigenous microbial strain. The best approach may be to engineer the most competitive *rhizobial* strains for maximal fixation rather to select the best fixers. These efforts will be assisted by our increasing knowledge of the signaling between plant and microbe (Dénarié *et al.*, 1993) namely Biological nitrogen fixation where about 65% of nitrogen is, fixed annually and it is the major sustainer of life on Earth.

The intimate interaction between *rhizobia* and their legume hosts is known to involve the mutual exchange of diffusible signal molecules. Although past research has largely focused on the plant flavonoid signals that induce bacterial *nod* gene expression and the return bacterial Nod signal that induces nodule formation, it is now clear that a multitude of additional signals are likely involved in the nodulation process. Consequently, their role must be viewed against the backdrop of the extensive exchange of signals between host and symbiont including Environmental factors that influence all aspects of nodulation and Biological N-fixation. Therefore, the objective of this article is to review Nitrogen fixation and provide highlighted information on legume nodulation through Biological nitrogen fixation and the abiotic stresses for biological nitrogen fixation inside the soil.

## Ways of Nitrogen Fixations

Although nitrogen gas ( $N_2$ ) accounts for about eighty percent of the Earth's atmosphere it is the most limited nutrient to plant growth. This is because the  $N_2$  molecule is very stable chemically and it has to be "fixed" into ammonium ( $NH_4^+$ ) and nitrate ( $NO_3^-$ ) so as to be assimilated by plants (Fisher and Newton, 2002).

Nitrogen can be chemically fixed by lightning, combustion and volcanism. The enormous energy of lightning ionizes the molecules in the atmosphere and enables them to combine and form nitrogen oxides and contributes to 10% of the total annual yield of fixed nitrogen (Fisher and Newton, 2002). Nitrogen can be synthetically fixed by Haber and Bosch process by combining atmospheric  $N_2$  gas (freed from contaminating  $O_2$ ) and hydrogen ( $H_2$ ) gas (usually derived either from natural gas or petroleum) under a high pressure and high temperature to form ammonia ( $NH_3$ ) in large scale production of nitrogen fertilizer (urea) (Fisher and Newton, 2002). The process produces an equilibrium mixture that contains approximately 20% ammonia (Bockman, 1997). The production of fertilizer ammonia together with high yielding crop varieties contributed to the green revolution of the 1960's (William, 2000) . .

The second most important source of fixed nitrogen derives from the activity of certain soil bacteria (biological nitrogen fixation) that absorb atmospheric  $N_2$  gas and convert it into ammonium. The biological process contributes nearly 79 % of the total annual yield of fixed nitrogen globally (Burns and Hardy, 1975).

### 1.1. Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is a process by which  $N_2^{14}$  in the atmosphere is reduced into a biologically useful, combined form of N-ammonia by nitrogen fixing bacteria (Giller, 2001). The most important N fixing agents in agricultural systems are the symbiotic associations between legumes and the microsymbiont rhizobia via the formation of nodules (Giller, 2001). Nodules are formed on roots or, in some cases, stem (Tamimi and Timko, 2003).

Each year, about 175 million ton of N is contributed by BNF globally (Burns and Hardy, 1975), of which nearly 79% is accounted for by terrestrial fixation. Therefore, symbiotic nitrogen fixation is of great importance not only in the production of leguminous crops but also in the global nitrogen cycle (Ben Romdhane *et al.*, 2008). Both nodulation and phenological traits of chickpea were improved by inoculation, consequently resulting in improved yield and yield related traits indicating the positive impact of biological nitrogen fixation on the crop productivity (Ibsa Aliyi, 2013). The rhizobial population plays a major role in meeting the nitrogen requirements of the plants.

### 2.0. Rhizobia–Legume Symbiosis

The interaction between rhizobia and their corresponding specific legume host plants leading to nodule formation is a complex process that requires a continuous and adequate signal exchange between the plant and the bacteria (Perret *et al.*, 2000). During this interaction, rhizobia are attracted by root exudates and colonize plant root surfaces. Flavonoids, the plant signal compounds present in the exudates, trigger the transcription of bacterial nodulation (*Nod*) genes leading thereby to the synthesis of *lipochito-oligosaccharide* signals called *Nod* factors.

These signal compounds (*lipochito-oligosaccharide*) in turn cause the legume root hairs to curl. *Nod* factors together with additional microbial signals, such as polysaccharides and secreted proteins, allow bacteria attached to root hairs to penetrate the root through a tubular structure called the infection thread, through which the rhizobia enter, move into root hair, and subsequently reach to the dividing cortical cells. When the thread reaches the primordium, the bacteria are released into the plant cytoplasm, where they differentiate into endosymbiotic form, the  $N_2$  -fixing bacteroids. Inside the central nodule cells, rhizobia are housed as symbiosome that are horizontally acquired organelles and are involved in the enzymatic reduction of atmospheric nitrogen to ammonia and make this N accessible to their hosts. In return, the bacteria are supplied with carbohydrates in a protected environment. The host plant, however, regulates the number of nodules formed, the maturation of nodules, and the  $N_2$  fixation of the nodules dependent upon available nitrogen (Fabio *et al.*, 2000).

Up to 25% of a legume's net photosynthate may be required for nitrogen fixation by rhizobia (Minchin *et al.*, 1981). Faster fixation rates (mol nitrogen per s) can be beneficial for hosts, but carbon costs can also be important. Rhizobia that fix more nitrogen per carbon respired could free more carbon for other functions, including the option of supporting more nodules with the same amount of photosynthate.

### 2.1. Nodulation and Nitrogen Fixation in the Leguminosae

Most legumes genera are able to form rhizobial symbioses. This is perhaps not surprising considering the vastness and diversity of the family, currently estimated to contain 16,000–19,000 species in about 750 different genera (Allen and Allen, 1981). The family *Leguminosae* is divided into three subfamilies: the *Papilionoideae*, the *Mimosoideae* and the *Caesalpinioideae*. From these the *Caesalpinioideae* is accepted as the most primitive

group and the *Papilionoideae* and *Mimosoideae* are likely to have evolved from a common, nodulated caesalpinoid ancestor (Sprent, 2001). *Papilionoideae* is the biggest subfamily that contains most of the legume tribes such as *Vicieae*, *Cicereae*, and *Phaseoleae* e.t.c.

The *Mimosoideae* and the *Caesalpinioideae* are almost completely restricted to the tropics. The *Papilionoideae* contains the majority of the most important grain legumes. Nodulation capacity has been surveyed in several thousand species, representing about 20% of leguminous species and including members of about 60% of the legume genera (Sprent, 2001). Of these, 97% of the examined *papilionoid* species form nodules, as do more than 90% of *mimosoid* species. In contrast, only 23% of the examined *caesalpinoid* species nodulate. These species all fall within eight genera – seven in the tribe *Caesalpinieae* and one sole genus, *Chamaecrista*, within the tribe *Cassieae* (De Faria *et al.*, 1989).

Rhizobia, in general, produce both indeterminate and determinate types of nodules. Indeterminate nodules are characterized by different zones: (1) the distal meristem, where bacteria are internalized, (2) an inter zone with amyloplast accumulation and differentiation of bacteroids, and (3) a fixation zone that includes plant cells and a senescent zone in comparison, determinate nodules are typically round shaped and are derived from the cessation of meristem activity after nodule initiation and growth of the nodule mainly by cell expansion (Jeroen *et al.*, 2006).

The survey by Corby (1988) recognized three types of indeterminate nodule: *caesalpinoid*, *crotalarioid* and *lupin* type; and two types of determinate nodule: *aeschynomoid* and *desmodoid*.

## 2.2. The Nod genes and Legume Promiscuity in Rhizobia

In the 1970s, it was discovered that symbiosis and pathogenicity genes are harbored in plasmids. These plasmids are conjugative and found in both genera *Agrobacterium* (Ledeboer *et al.*, 1976) and *Rhizobium* (Zurkowski and Lorkiewicz, 1979). The symbiotic genes included those involved in legume nodulation (*nod*) and in nitrogen fixation (*nif*). The *nod* genes are responsible for the synthesis of nod factors (*lipochitin-oligosaccharides*) that are receptors for the plant flavonoid signal (Broughton *et al.*, 2000). The *nodD* is a regulatory gene of the operon *nodABC* whose genes are determinants of the host range (Perret *et al.*, 2000). The *nif* genes are involved in nitrogen fixation and are carried by rhizobia but also by free-living nitrogen fixing bacteria (Zehr *et al.*, 2003). Symbiotic genes are harbored in plasmids in fast and in some intermediate-growing species of rhizobia, whereas these genes are integrated in the chromosome in the intermediate and slow-growing rhizobia, harbored in symbiotic islands (Young *et al.*, 2006). Symbiotic genes also named “auxiliary” or “accessory” genes, are commonly included in species description of rhizobia. From these genes most commonly studied are *nodD*, *nodA*, *nodC*, and *nifH* (Laguerre *et al.*, 2001). Nevertheless, these genes are not useful in taxonomy because of their ability to be transferred in nature from plasmids to chromosomal islands (Nakatsukasa *et al.*, 2008), from bacteria to plants (Broothaerts *et al.*, 2005), and among bacteria (Rogel *et al.*, 2001). Therefore, the analysis of symbiotic genes is overall useful to identify new-rhizobial species forming nodules and to carry out biogeographical studies of legume endosymbionts. Particularly, the nodulation genes are useful to define biovars within rhizobial species (Leon Barrios *et al.*, 2009).

Within rhizobia, the concept of biovar is directly linked to the concept of legume promiscuity. It is known for many years that legume has different promiscuity degree and whereas some of them can be nodulated by several species of *rhizobia* such as *Macroptilium* and others are restrictive hosts for nodulation such as *Cicer* (Perret *et al.*, 2000). In the same way, rhizobial strains can have broad or narrow host range. For instance, *R. leguminosarum* bv. *Trifolii* can only nodulate plants of genus *Trifolium* whereas *Rhizobium* sp. NGR234 nodulates over 100 legumes as well as the non legume *Parasponia* (Pueppke and Broughton, 1999). Within these genes, *nodC* has been widely analyzed in rhizobial strains and found related with the host range of rhizobia and the promiscuity degree of the hosts (Iglesias *et al.*, 2008). Moreover it was described the biovar *Ciceri* based on the *nodC* gene analysis (Rivas *et al.*, 2007) concluded that *Cicer arietinum* is a very restrictive host, because although it can be nodulated by several species of *Mesorhizobium*, all of them carry nearly identical *nodC* genes. By contrast *P. vulgaris* is a very promiscuous legume since it is nodulated by the highest number of taxonomic species, which carry very divergent symbiotic genes (Zurdo Pineiro *et al.*, 2009).

## 2.3. Taxonomy of Rhizobia

Rhizobia are bacteria capable of forming nodules on leguminous plants. The description of the first rhizobial species was mainly based on the legume, which acted as host. Bacterial genome is consisting of two parts. The basic genome is composed of housekeeping genes that are needed under almost all growth conditions, they are carried on the chromosome, their organization is reasonably stable, and they are predominantly inherited vertically from mother to daughter cells. By contrast, the accessory genome consists of genes that are entirely selfish or offer adaptations to special circumstances, these are carried on plasmids, islands, transposons and phages, they undergo frequent rearrangement and are often transferred horizontally between cells (Fabio *et al.*, 2000).

In rhizobia three sizes of replicon are generally distinguished: plasmids (<1MB), mega plasmids (1-2MB) and chromosome (>4MB, so far as known). The organization differs: *Rhizobium leguminosarum* has a chromosome and 2-8 plasmids, *Sinorhizobium meliloti* has a chromosome, two mega plasmids, and sometimes 1-2 plasmids, *Mesorhizobium* has a chromosome, but may lack other independent replicons. In each case the nodulation genes are carried in the accessory genome: on a plasmid, a mega plasmid, and a symbiotic island inserted in the chromosome, respectively (Fabio *et al.*, 2000). Early *Rhizobium* taxonomy has been mainly based on the nodulating host range, although overlapping host ranges have already been reported more than fifty years ago. The development of molecular techniques accelerated the taxonomic evaluation and led to the identification of many new rhizobial genera. Based on the sequence of the 16S rRNA gene; rhizobia could be grouped in the alpha subdivision of the Proteobacteria and several genera have been defined including *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium* (Young and Haukka, 1996). The identification of *M. amorpha* (Wang *et al.*, 1999) showed the clear existence of symbiotic plasmids containing *nif* and *nod* genes in *Mesorhizobium*. *M. loti* and *M. plurifarum* have these genes in the chromosome and in *M. loti*; symbiotic islands have been described (Sullivan and Ronson, 1998). This finding adds support to *Mesorhizobium* being intermediate between *Rhizobium* (having mainly symbiotic plasmids) and *Bradyrhizobium* (with symbiotic determinants in chromosome).

The first described *Rhizobium* species, namely *R. leguminosarum* with current taxonomy of rhizobia can be grouped in to three biovars: *R. leguminosarum* bv. *trifolii* that nodulated clover, *R. leguminosarum* bv. *Viciae* that nodulate pea and fababean, and *R. leguminosarum* bv. *Phaseoli* nodulating common bean (Jordan, 1984).

#### 2.4. Environmental Factors affecting BNF

The interaction between the microsymbiont and the legume host plant are known to be complicated by unfavorable edaphic, climatic and management factors (Broughton *et al.*, 2003). It is believed that any environmental or physical stress that reduces host plant growth and development may also negatively affect the associated bacterial strain and, thereby, the amount of nitrogen fixation. It is a 'rule of thumb' that symbiotically fixed nitrogen alone may not increase production if some other nutrients are limiting unless the latter are ameliorated (Bohlool *et al.*, 1992).

The soil environment is under a constant state of change and can be relatively stressful for both macro- and microorganisms. Fluctuations in pH, nutrient availability, temperature, and water status, among other factors, greatly influence the growth, survival, and metabolic activity of soil microorganisms and plants, and their ability to enter into symbiotic interactions. Many diverse biological associations contribute to N<sub>2</sub> fixation (BNF) in both soil and aquatic systems (Sprent, 1984). However, in most agricultural systems, the primary source of biologically-fixed N via the symbiotic interactions of legumes and soil bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, and *Azorhizobium* (Sadowsky and Graham, 1988). The other 20% is contributed mainly by the actinorrhizal (e.g., by Frankia) and Anabena-Azolla types of symbiotic interactions.

Rhizobia can exist in two fundamentally different modes. Either as free-living saprophytic heterotrophs or as legume-host-specific nitrogen-fixing symbionts. The later mode of existence gives rhizobia advantages with respect to survival and persistence over most other soil bacteria. The growth of rhizobia in the rhizosphere may be stimulated by plant root exudates rhizobia in soils are associated with aggregates gives them some degree of protection from perturbations by environmental and biotic factors. Nodules can contain more than 10<sup>10</sup> rhizobia g<sup>-1</sup> (Dermott *et al.*, 1987). Nodule senescence at the end of the growing season leads to the release of a large number of rhizobia into soils.

Studies have shown that a legume host is not needed for persistence (saprophytic competence) of rhizobia in soils (Bottomley, 1992) although; nodule bacteria and bacteroids, after release into the environment are often susceptible to osmotic and other soil stress factors. Any environmental factor that negatively influences either the growth of rhizobia or the host plant itself has a dramatic impact on symbiotic N<sub>2</sub> fixation. It can negatively influence the nodulation process itself indirectly affect nitrogen fixation, or directly influence plant growth and vigor during post-nodulation events and so affect the efficient functioning of the nitrogenase enzyme complex. Some factors such as soil temperature, soil pH stress, nutrient stress, desiccation tolerance as well as soil water content and stress are mentioned as the major environmental stress factors affecting BNF (Sadowsky, 2005). Fixing atmospheric nitrogen depends on the environmental conditions, the availability of nutrients in the soil, cultural practices, and mainly the number and effectivity of native rhizobia (Rachna and Dudeja, 2009).

#### Conclusion

The unusable atmospheric nitrogen to all plants can be converted by legumes and incorporated to the soil for the nutritional use for the crops growing in the particular area. This phenomenon occurred through different ways, of which biological nitrogen fixation plays great importance in the production of leguminous crops and also in global nitrogen cycle. This process is improved by inoculation where rhizobial-legume interaction is crucial in

making nitrogen accessible to their host and in return bacteria supplied with carbohydrates in protected environment. with this interaction nodulation, promiscuity of particular legume showed different behaviors and the biological products classified into eight different genera's. Abiotic factors particularly pH, nutrient availability, temperatures and water contents greatly influence the growth, survival and metabolic activity of soil.

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