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Biological nitrogen fixation: Its mechanism and role of boron in nitrogen fixation

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Abstract

Environmental issues over the increased level of reactive nitrogen in the atmosphere as a result of the production and usage of chemical fertilizers have prompted a re-evaluation of the value of biological nitrogen fixation (BNF). The fixation of nitrogen and photosynthesis form a foundation of any earthly existence. Because symbiotic nitrogen fixation is a major source of nitrogen, and different legume crops and pasture species can fix up to 300 kg nitrogen per hectare, the Rhizobium-legume symbiosis is superior to other nitrogen-fixing systems. Legumes' nitrogen-fixing properties benefit the environment by increasing soil fertility and reducing the need for inorganic fertilizers. Boron deficiency (B) adversely influences symbiotic relationships between legume and rhizobia and develops N2-fixing nodules. There has been great progress in boron treatment in the number of nodules, nodule size and nodule weight.

Keywords: biological nitrogen fixation, rhizobia, boron, nodule

Introduction

In 1886, the German scientists Hellriegel and Wilfarth discovered nitrogen fixation when they observed that gaseous (molecular) nitrogen can be used in legumes containing root nodules. A Dutch microbiologist named Beijerinck successfully isolated a bacterial strain from root nodules in 1888. This isolate turned out to be a strain of Rhizobium leguminosarum. Biological nitrogen fixation (BNF) in plants is an effective mechanism for maintaining sustainable agricultural production and the proper functioning of the ecosystem. Crop production is reliant on nitrogen (N), which is a finite resource with a widening gap between supply and demand. BNF is produced by diazotrophic microorganisms from the bacteria and archaea domains, and only a few prokaryotes can use atmospheric nitrogen via BNF by encoding nitrogenase, an enzyme that catalyzes the conversion of dinitrogen (N2) gas to ammonia (NH3). This process is extremely important because it eliminates the reliance on nitrogen fertilizers for plants and, as a result, for agriculture as a whole. Nitrogenases are involved in all biological nitrogen fixation processes. Iron is present in these enzymes, along with a second metal, usually molybdenum, and sometimes vanadium. Urea, amino acids (proteins), nucleic acids (DNA and RNA), adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NAD) are all key elements of all living cells. Increased biological nitrogen fixation, lower energy costs, improved soil physical conditions, and biodiversity can all help legumes play a bigger role in cropping systems, which is especially important in the current state of agriculture. The majority of the reactive nitrogen needed for protein formation and plant growth is provided by biological nitrogen fixation (BNF) in nature. Legumes are the host of BNF, so understanding BNF is the basis for numerous decisions in legumes cultivation. Free-living bacteria from genera such as Azotobacter, Azospirillum, Bacillus, or Clostridium are involved, as are symbiotic bacteria such as Rhizobium associated with legumes, Frankia associated with actinorhizal plants, and cyanobacteria associated with cycads.

More than 80 years have passed since boron has proved to be necessary for normal higher plant development (Luis Bolaños *et al*., 1994) [23]. The importance of boron for plant growth was discovered in the early 1920s (K. Warington *et al*., 1923) and thus Boron has been recognized as an important micronutrient for all vascular plants since then. Bolanos *et al*. investigated the impact of boron on Rhizobium-legume cell-surface interaction and nodule formation in pea. In this study, it was reported that the frequency of Rhizobia infecting host cells as well as infection threads were decreased in boron-deficient plants, and thus the infection threads formed morphological abnormalities.

Mechanism of Biological Nitrogen Fixation

Beijerinck discovered the BNF in1901, which is carried out by a particular type of prokaryote. In this process, microorganisms convert atmospheric nitrogen to ammonia, which plants can use. For the reduction of each mole of nitrogen, these microorganisms require 16 moles of adenosine triphosphate (ATP), which they acquire through the oxidation of organic molecules. Photosynthetic microorganisms, such as cyanobacteria, use sugars synthesized throughout photosynthesis, whereas non-photosynthetic free-living microorganisms acquire these molecules from other organisms. The reaction for BNF is:

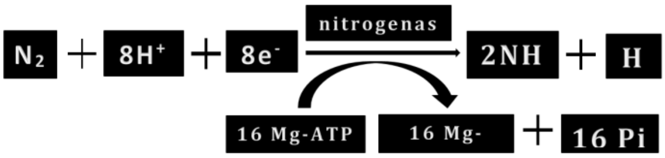

Fig 1: Reaction for Biological Nitrogen Fixation (Source: Adopted from SR Reddy & CNagamani, 2017).

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The following are the different kinds of biological nitrogen fixation:

A. Non- Symbiotic/ Asymbiotic Biological Nitrogen Fixation

Soil comprises a variety of free-living nitrogen-fixing species. A variety of aerobic and anaerobic bacteria, as well as blue green algae, are among them. Non-symbiotic nitrogen fixation refers to biological nitrogen fixation by microorganisms that live freely outside of plant cells. Many heterotrophic bacteria live in ground soil and are capable of fixating considerable amounts of nitrogen without interacting with other organisms. These organisms seek their own source of energy, which they obtain through the oxidation of organic molecules released by other organisms or through decomposition. The free-living asymbiotic nitrogen fixers are very primitive. Fixation is a reduction procedure that does not require respiration. Under poor aeration, these organisms fix nitrogen more aggressively, as long as no hydrogen gas is emitted.

B. Associative Biological Nitrogen Fixation

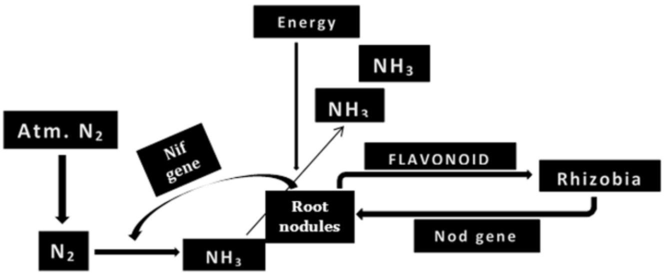
Nitrogen is fixated by bacteria that live in close proximity to cereal and grass roots. Associative Symbiosis is the term given for this loose mutualism. Within the rhizosphere of the host plants, these bacteria fix significant quantities of nitrogen. The bacteria live in the rhizosphere (the zone between soil and root) and sometimes enter the roots. Some of the fixed nitrogen is taken up by the roots, and in return, the bacteria get nourished through the carbohydrates released by the roots. Azospirillum species are closely associated with a number of Poaceae (grass) members including agriculturally economic and important grain crops such as rice, barley, oats, wheat and maize.

C. Symbiotic Biological Nitrogen Fixation

By collaborating with a host plant, many microorganisms fix nitrogen in a symbiotic manner. Sugars are formed by plants during photosynthesis and used by the nitrogen-fixing microorganism to provide the energy it needs to fix nitrogen. The microbe supplies the host plant with fixed nitrogen in exchange for these carbon sources for its growth. Water fern Azolla symbiosis is a kind of example for this type of nitrogen fixation, the symbiosis with a cyanobacterium anabasa azollae. Plants provide a niche and fixed carbon to bacteria in exchange for fixed nitrogen in a mutualistic relationship known as symbiotic nitrogen fixation. Despite the fact that the symbiotic partners discussed above play an essential role in the global ecology of nitrogen fixation, the most significant nitrogen-fixing symbiotic associations to date are those between legumes and Rhizobium and Bradyrhizobium bacteria. Leguminous plants form endosymbiotic relationships with rhizobia (nitrogen-fixing soil bacteria), and form root nodules where resident rhizobia fix atmospheric nitrogen. The association is considered symbiotic since the host plant supplies the required organic carbon with the nodule bacteria (carbohydrates). *Bradyrhizobium japonicum* is a symbiont of soybeans that grows slowly. In Sesbania species, *Azorhizobium caulinodans* is a stem nodule forming symbiont.

Biological Nitrogen Fixation in Rhizobia

Rhizobial biological nitrogen fixation takes place mainly in root or stem nodules and is caused by bacteria found in legume plants. At normal temperature and pressure, symbiotic nitrogen fixation uses solar energy to convert inert N2 gas to ammonia. The relationship between rhizobia and their legume hosts has thus been dissected at the agronomic, plant physiological, microbiological, and molecular levels, yielding a wealth of knowledge about the processes involved, but finding significant bottlenecks in nitrogen fixation performance has proved difficult. Rhizobia are the most well-known symbiotic nitrogen-fixing bacteria. The symbiotic nitrogen fixing relationship between rhizobia and legumes is a mutually beneficial symbiosis that benefits both plants and bacteria. The nod, nif, and repair genes regulate symbiotic nitrogen fixation in rhizobia. Nod factors are rhizobial lipochito-oligosaccharide signal molecules encoded by a particular group of rhizobial genes known as nod genes. The association between legume roots and rhizobia is triggered by legumes exuding flavonoids, which are recognised by rhizobial NodD, which controls the transcription of other nodulation genes (nod, nol, and noe) in rhizobia. In most rhizobia, the three nod genes, nodA, nodB, and nodC, occur as single copy genes.



(Source: Adopted from Kristina Lindstrom and Seyed Abdollah Mousavi, 2019).

Fig 2: Symbiotic nitrogen fixation by legumes in rhizobia

Development of Nodules in Root

The formationof a nodule necessitates complex hormone signalling, which includes the activation of cytokinin and the inhibition of polar auxin transport in the root cortex. Mature nodules are made up of infected core tissue with a combination of infected and uninfected cells, surrounded by uninfected tissues that bind to the root vascular system. Plants emit signals such as flavonoids, which are identified by compatible bacteria in the rhizosphere, resulting in the formation of Nod factors, which initiate the nodulation phase early. The rhizobial bacteria bind to the ends of the root hairs, twisting them into an infection thread that enables the bacteria to enter the host plant's root cells. The bacteria are released into the cells of the newly developed root nodule, where nitrogen fixation occurs, as the infection thread progresses into the root's center. The bacteria induce the development of leghemoglobin in the host plant cells. Since the nitrogen that is fixed is accessible to the entire host plant, high-yielding legume crops do not need nitrogen fertilizer.

Role of Boron during Fixation in Nitrogen

Yamagishi and Yamamoto (1994) [25] observed that low B availability induced major changes in N2 fixation in soybean plants. The disruption of root tip development and the emergence of lateral roots were apparently observed in soybean plants grown in the boron-free culture medium held in plastic containers, suggesting boron deficiency symptoms. The most common symptom of boron deficiency in tomatoes is the presence of lateral roots. Root nodules began to show signs of boron deficit. The development and nitrogenase activity of the nodules had been reduced. However, though long-term culture in the boron-free medium adversely decreased the growth of the whole plant, it is uncertain if boron deficiency hindered the nodules. To evaluate the primary impact of boron, short-term boron removal was performed. Boron removal hindered the growth of nodules and declining nitrogenase activity. Thus, these findings suggest that boron is essential for nodule formation and nitrogen fixation.

Boron is an important plant micronutrient taken up mainly as boric acid from its roots. Boron serves a key role in the formation of nodules (Luis Bolafios *et. al*., 1996) [22]. In the study done on the impact of boron (B) deficiency in bean (Phaseolus vulgaris) nodule formation and nitrogen fixation by lldefonso Bonilla *et al*., (1997) [21], it was observed that plants grown without boron in the nutrient solution developed smaller nodules with lower weights than controls, furthermore, after two weeks of treatment, boron deficiency induced a substantial decline in acetylene reduction activity, thus indicating that these results signify that boron is a required component for the normal nodule formation and function.

Milka Brdar-Jokanovic (2020) [27] reviewed that the inhibition of DNA synthesis caused by pyrimidine base deficits results in root growth suppression in conditions of inadequate boron availability, thus indicating that boron involvement is indeed required in nitrogen fixation in legumes and mycorrhiza. Milka Brdar-Jokanovic (2020) [27] also mentioned that boron deficiency affects root growth rather than shoot growth. Root elongation is hindered as cell division in the apical meristem ceases due to boron deficiency leading to the vanishing of the root cap and ultimately growth declines, and root tips die out as a result of severe deficiency i.e., boron deficiency.

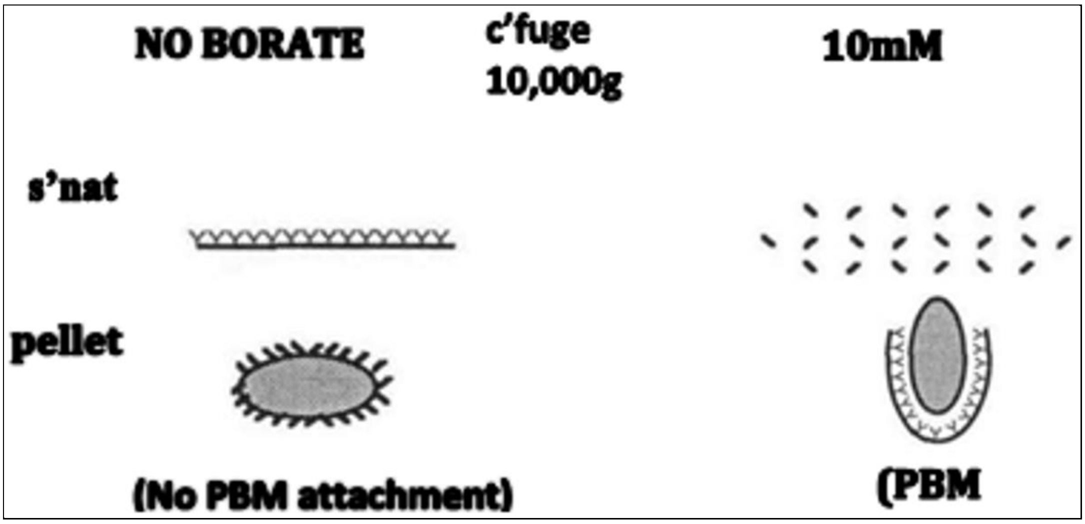


Fig 3: Effect of Boron on Rhizobium-plant cell-surface interactions (Source: Adopted from Luis Bolafios *et al*., 1996) [22].

Conclusion

Biological nitrogen fixation in plants has the potential to be a long-term source of nitrogen, potentially reducing our reliance on industrial nitrogen production. Organic matter degradation, synthetic fertilizer applications, and biological nitrogen fixation (BNF) through nitrogenase enzyme activity all play a role in soil nitrogen input for plant nutrition and crop productivity. BNF by diazotrophic bacteria contributes nearly half of the total nitrogen in crop fields, accounting for approximately half of the total nitrogen in the biosphere. It's a low-cost, non-polluting way to increase soil fertility and crop production. Boron has an impact on the Rhizobium-legume cell-surface interaction and nodule formation in peas. The element boron is required for normal nodule formation and operation. It can be inferred that, up to a certain threshold value, boron has a substantial positive impact on nodule formation.

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