

# Rhizobial Symbiosis in Legumes and Non-Legumes Cereals

Bezabih Addisu\*

Gambella University, College of Agriculture and Natural Resources, Department of Plant Science

## Abstract

Studying any living organism, it is important to know how each species grows and responds to certain conditions that affect its growth and development. In the case of legumes, the symbiotic relationship with rhizobia is a key factor in their ability to fix atmospheric nitrogen. This process is essential for the growth and development of legumes, as it provides them with a source of nitrogen that is readily available to them. In non-legume cereals, the symbiotic relationship with rhizobia is less well understood, but it is believed to play a role in their growth and development. This study aims to investigate the symbiotic relationship between rhizobia and legumes and non-legume cereals, and to determine the conditions that affect this relationship. The results of this study will be used to develop strategies for improving the growth and development of legumes and non-legume cereals.

**Keywords:** Nitrogen fixation; Symbiosis; Nodulation; Non-legume; Legumes

## Introduction

Nitrogen is one of the most abundant elements in the earth's atmosphere; however, more than 78.08% of the total nitrogen is in the form of nitrogen gas which is unavailable to living organisms, and plays a critical role for plant growth and production. Plants can use nitrogen in the form of ammonium or nitrate ions [1]. Biological nitrogen fixation (BNF) is one of the pathways of nitrogen (N) inputs performed by free living or symbiotic N fixing organisms represents an important source of N in terrestrial ecosystems. The process of converting atmospheric nitrogen gas into these ions is known as nitrogen fixation and it being carried out by nitrogen-fixing microbes such as bacteria and algae [1].

Rhizobia are diazotrophic bacteria that fix nitrogen after becoming established inside the root nodules of legumes (Fabaceae). To express

\*Corresponding author: Addisu Bezabih, Gambella University, College of Agriculture and Natural Resources, Department of Plant Science, Gambella, Ethiopia. Email: bezabih.addisu@gambella.edu.et

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organic substances from the rhizosphere of host plants [5].

Nitrogen fixing prokaryotes (diazotrophs) which are capable of producing the enzyme nitrogenase can fix nitrogen. These diazotrophs can be classified into three types: symbiotic (fixes nitrogen only in specialized organelle, nodules, formed on specific hosts of the Leguminosae family, which forms symbiotic nodules on a variety of non-leguminous tree and shrub families), endophytic (exists inside the root of the plant of both legumes and non-legumes) and free-living (enhances BNF in the rhizosphere of both legumes and non-legumes).

Symbiotic nitrogen fixation (SNF) has the highest efficiency of BNF, but it is limited to a few genera of the Leguminosae. While, most of the crop plants are not capable of forming SNF, they are able to benefit from the nitrogen fixed by free-living and associative diazotrophs which exist in the rhizosphere [6]. Several mega-grand challenge projects have been commissioned around the world focusing on transfer of the BNF traits from legumes into cereals [7]. Most of the attempts to engineer these BNF traits involve transfer of a minimal set of genes from legumes into cereals [8].

Different authors recognized that competent rhizobial strains could be applied to legumes and non-legumes to increase their production. According to Abdul (2012), *Rhizobium* culture significantly affected the growth and yield components of mung-bean. Similarly Mahmoodi reported that the use of appropriate strains of inoculants in nitrogen deficient soils may offer an excellent opportunity for improving legume growth and development.

Other studies have also indicated the contribution of biological nitrogen fixation in the growth promotion of non-leguminous plants through associative interaction with diazotrophs. Rhizobial inoculants have been recognized as endophytes (those microorganisms that live within host plants for at least part of their life and do not cause apparent symptoms of diseases) in the roots of non-legumes. Moreover, Sessitsch reported that rhizobia are now considered rhizobacteria of non-leguminous plants.

### Rhizobial symbiosis in legumes

Some microorganisms can fix nitrogen symbiotically by partnering with a host plant. There are several examples of symbiotic nitrogen fixation such as the water fern *Azolla*'s symbiosis with a cyanobacterium, the symbiosis between actinorhizal trees and shrubs, such as Alder (*Alnus* sp.), with the actinomycete *Frankia*. One of the well-known symbioses occurs between two partners, legume plants and rhizobia. The rhizobium-legume symbiotic interaction induces specialized organs known as nodules on the roots of their host, and obtains their nutrients from the host plant. Inside nodules rhizobia reduce atmospheric  $N_2$

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## Endophytes

Endophyte has been known for long that some nitrogen-fixing endophytic bacteria form nodule-independent association with cereal crops. One of the potent approaches for increasing biological N<sub>2</sub> fixation in cereals is to enhance associative or endophytic root colonization by desirable bacteria. The inoculation of non-legumes, especially cereals, with various non-rhizobial diazotrophic bacteria has been undertaken with the expectation that they would establish themselves intercellularly within the root system, fixing nitrogen endophytically and providing combined nitrogen for enhanced crop production. Azorhizobium caulinodans is known to enter the root system of cereals, other non-legume crops and Arabidopsis, by intercellular invasion between epidermal cells and to internally colonize the plant intercellularly, including the xylem. A particularly interesting, naturally occurring, non-nodular xylem colonizing endophytic diazotrophic interaction with evidence for endophytic intracellular symbiotic nitrogen fixation, without the need for nodulation is that of Gluconacetobacter diazotrophicus in sugarcane.

A rhizosphere-associated nitrogen fixation can occur in different ways. They may be endophytic, which reside in the internal tissue of the plant when a lack of Nod genes, which results in a Nod factor-independent infection process, and rhizospheric, which reside within the rhizosphere.

## Effect of inoculation of rhizobia on non-legumes crops

Following section highlights the improvement of growth and yield parameters of different non-legumes upon inoculation with rhizobia. Several studies indicate that rhizobia may act as natural elicitor for improving the growth and yield of rice. Growth stimulation of rice followed by inoculation with rhizobium has been reported by many workers.

According to Gutierrez and Martinez, (2001) reported that increased in maize yield upon *R. etli* inoculation. Similarly, while testing nine different rhizobial strains for their growth promotion effects with six different non-leguminous plants in laboratory and greenhouse experiments reported that the rhizobial strain PAR-401 was the best for *Zea mays* and increased shoot and root dry weight of plants.

Furthermore, Hoich (2000) conducted a series of experiments under greenhouse and field conditions to see the growth stimulating effects of rhizobia. He reported that *R. leguminosarum* bv. *trifolii* strain R39 promoted the shoot growth of maize grown in greenhouse experiments whereas in field experiments. A recent report demonstrated that an indigenous maize landrace, characterized by an extensive development of aerial roots that secrete large amounts of mucilage, can acquire 28–82% of its nitrogen from atmospheric dinitrogen. Although the Sierra Mixe maize landrace is unique in the large quantity of mucilage produced, other cereal crops secrete mucilage from underground and aerial roots and we hypothesize that this may represent a general mechanism for cereals to support associations with microbial diazotrophs. A key feature of the Sierra Mixe maize landrace mucilage is the abundance of sugars that potentially serve as a source of energy for the diazotrophs.

The aerial root mucilage was found to maintain oxygen levels below 5% at a depth of 8 mm, suggesting that the mucilage could sustain a micro aerobic environment compatible with nitrogenase activity. *Azoarcus* sp. Strain BH72, a mutualistic endophyte of rice, is of agrobiotechnological interest because it supplies biologically fixed nitrogen to its host and colonizes plants in remarkable numbers without

eliciting disease symptoms. Used four (BHUE-2, 3, 4 and 5) isolates of rhizobia for rice plant growth response studies under laboratory and greenhouse conditions. All four isolates gave a positive response in enhancing the plant growth measured in terms of plant height and shoot dry weight.

Similarly, tested six rhizobial strains isolated from a wide range of legume hosts to determine their growth promoting activities in lowland rice in a potted soil supplemented with varied amounts of mineral N and reported that inoculation with *R.leguminosarum* bv.*trifolii* E11Rhizobium sp. IRBG74, and Bradyrhizobium sp.

### **Factors affecting symbiotic nitrogen fixation**

Several environmental conditions are limiting factors to the growth and activity of the N<sub>2</sub>-fixing plants. When studying any living organism, it is important to know how each species grows and responds to certain

