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Plant growth promoting activity of *Bradyrhizobium* communities with special reference to Soybean (*Glycine max* L.) cultivation: A review

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Abstract

Soybeans (*Glycine max* L.) is one of the most valuable crop cultivated in the world, it serves as a good dietary source of protein for human diet and oil seed and as a feed for livestock and aquaculture and also as a feed stock for biofuel. Soybean oil is used directly in food and preventing high blood pressure caused by arteriosclerosis. It also contains lot of the essential vitamins for the body. Soybean cultivation in India started in 1976. Soybean production in India has increased to about 966 ha. Yield levels have stabilized at about 2895 metric ton per hectare. This present review is focusing on the plant growth promotion activity of *Bradyrhizobium* communities with special reference to cultivation of Soybean.

Keywords: Soybean, Bradyrhizobium communities, Plant growth promoting

INTRODUCTION

Nitrogen is one of the significant nutrients for plant growth. Legume crops' economic and environmental importance is mainly due to their ability to fix atmospheric dinitrogen in symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). Like most legumes, soybeans perform nitrogen fixation by establishing a symbiotic relationship with the *Rhizobia*. *Bradyrhizobium japonicum* is a slow-growing root nodule symbiont, widely used as an inoculant in soybean fields worldwide.

Soybean inoculated with *Bradyrhizobium japonicum* forms effective bolder nodules and increased yields, especially in fields where soybeans are cultivated for the first time [1,46,47,48,49,50,51,52].

2. BIOLOGICAL NITROGEN FIXATION BY *Bradyrhizobium* ISOLATES

Biological nitrogen fixation (BNF) is one of the most important natural phenomena, only exceeded by photosynthesis [2,46]. One of the most common limiting factors in plant growth is the availability of nitrogen [3,46]. Although 4/5th of earth's atmosphere comprises nitrogen, utilizing atmospheric nitrogen is restricted to a few groups of prokaryotes that can convert atmospheric nitrogen to ammonia and, in the case of the legume symbiosis, make some of this available to plants.

Soybean has a tremendous capacity for nitrogen fixation and has thus been heavily promoted as a crop in low nitrogen settings. It was estimated that the soybean acquires up to 250 kg nitrogen ha-1, approximately 80 % of the total tissue nitrogen through BNF [4]. For legume crops to effectively add nitrogen to a cropping system, they must have appropriate *Rhizobium* partners. While native *Rhizobium* populations exist in most soils, it is difficult to predict whether significant numbers will be capable of nodulating a particular host legume.

The capacity for nitrogen fixation in differing *Rhizobium* sub-species or strains varies. The issue is further complicated because multiple types of Rhizobium can nodulate many host legumes, and many *Rhizobium* types are capable of nodulating multiple host plant species. In commercial agriculture, farmers will apply purchased inoculants, which tend to contain strains of *Rhizobium* that have been shown to effectively nodulate a legume crop and fix adequate amounts of nitrogen in controlled settings. The amount of nitrogen accumulated in soybeans has been shown to correlate with the inoculation rate of appropriate *Rhizobium* [5].

However, access to inoculants is limited in many regions, and farmers rely solely on native *Rhizobium* populations for legume nodulation and BNF. If the population of soil *Rhizobium* is low, or if the existing population is unable to form nodules on a particular crop, little to no nitrogen fixation will occur. On the other hand, a large, diverse *Rhizobium* population is likely to include *Rhizobium* capable of forming nodules on a wide range of legumes [6], and inoculant performance can vary according to the competitiveness of indigenous populations and the encountered soil environment, with different strains of *Rhizobium* demonstrating differential competitiveness in their ability to form nodules as well in their ability to survive in various soil environments. If the native population is less efficient at nitrogen fixation than the inoculant, this can reduce overall nitrogen fixation and cause reduced growth and yield of the crop. Understanding the background diversity of *Rhizobium* populations can provide for better use of inoculant strains and improved nitrogen fixation of legumes [7].

Predominantly, members of the plant family Leguminosae have evolved with nitrogen-fixing bacteria from the family Rhizobiumceae. Plants excrete specific root exudates, which has chemical signals to attract the nitrogen-fixing bacteria towards their roots, which permit the bacteria to access their roots, allowing them to colonize and reside in the root nodules, where the modified bacteria (bacteroids) can perform the fixation of nitrogen [8]. This process is of great interest to scientists in general, and agriculture specifically, since this highly complex recognition and elicitation is co-ordinated through gene expression and cellular differentiation, followed by plant growth and development; it has the potential to minimize the use of artificial nitrogen fertilizers and pesticides in crop management.

The symbiosis's effectiveness can be measured in two ways, either directly by determining the amount of nitrogen fixed or indirectly by measuring the plant dry weight. The measurement of ARA for estimating N_2 fixation rate by nitrogenase preparations and bacterial cultures in the laboratory and by legumes and free-living bacteria insitu were reported by Hardy *et al.* [9]. This assay was done based on the nitrogenase catalyzed reduction of C_2H_2 to C_2H_4 and quantitative measurement of C_2H_4 using a gas chromatograph with flame ionization detector.

The effectiveness and competitive ability of *Bradyrhizobium* strains were studied by Narendrakumar *et al.* [10]. The nodulation, plant biomass, N uptake and grain yields of inoculated plants were significantly higher in loamy soils than sandy alluvial soils. Thakare *et al.* [11] tested out of 39 isolates of soybean *Bradyrhizobium* screened, 24 isolates increased nodulation significantly over control. The symbiosis of hup+ and huphr and hup- strains of *Bradyrhizobium elkanii* in cowpea cultivars were studied by Souza [12]. The effects of lectins from soybean on the symbiotic activity of *Bradyrhizobium japonicum* strain was investigated by Kirichenko and Malichenko [13] pre-incubation with lectins isolated from the specific host plant resulted in both enhancement of nodulation and stimulation of the nitrogen-fixing activity of fully developed nodules. However, the lectin isolated from the non-specific host plant had no such effect.

3. EXOPOLYSACCHARIDES PRODUCTION BY *Bradyrhizobium* ISOLATES

Exopolysaccharides (EPS) can either be homopolysaccharides or heteropolysaccharides. For example, cellulose is one of the crucial homopolysaccharides produced by plant-associated bacteria such as *Rhizobium* and *Agrobacterium* whereas leaves are produced by *Erwinia* and *Pseudomonas* [14]. On the other hand, Sutherland [15] defined EPS are polysaccharides found external to the structural surface of the microbial cell and the term can be applied to carbohydrate polymers of diverse compositions and different physical types.

Several *Rhizobium* polysaccharides were more effective stabilizing agents of most soils than the synthetic soil conditioner or the other reference compounds. The exopolysaccharides, cellular polysaccharides and extracellular protein of *Rhizobium* have been identified as the root curling factor [16]. The strains of *Bradyrhizobium* are diverse in their composition, often varying from strain to strain. The chemical composition of the polysaccharides of Rhizobium and Bradyrhizobium by chemical, Physico-chemical and immunological methods have shown that the Rhizobium strain produces homopolysaccharides. For example, *Rhizobium meliloti* SU47 can produce two EPS, a succinoglycon (EPS I) and a galacto glucan (EPS II). Most physiological and genetic studies have been performed with derivatives of this strain, which are under normal growth conditions [17].

Bacterial polysaccharides are necessary for a functional *Rhizobium* legume symbiosis. Exopolysaccharides, Lipopolysaccharides, capsular polysaccharides and cyclic b (1-2) glucon plays a vital role in the formation of infection thread formation and thereby initiates the process of nodule development [18]. The exopolysaccharide production by *Rhizobium meliloti* is influenced by salt. The halotolerent strain *Rhizobium meliloti* modifies the production of EPS in response to salt. These bacteria grow in the presence of 0.3 M NaCl showed a decrease in mucoid and when grown in salt supplemented liquid medium, this organism produced 40 per cent fewer exopolysaccharides [19].

The cell surface carbohydrates of bacteria within the Rhizobiumceae family provide essential functions during legume nodulation. *Rhizobium* lipopolyosaccharides have been shown to elicit root hair deformation, cortical cell division, and nodule organogenesis. Genetic studies have also provided evidence that the second class of *Rhizobium* cell surface carbohydrate, the exopolysaccharide is required for nodule development in plants [20]. Gerretsen [21] reported the third Rhizobium cell surface carbohydrate class, the cyclic b-glucans in the Glycine max root nodules Rhizobium cell surface carbohydrate, the exopolysaccharides is required for nodule development in plants.

4. INDOLE ACETIC ACID PRODUCTION BY *Bradyrhizobium* ISOLATES

5.

Naturally occurring Indole acetic acid (IAA) is present in plants and certain microorganisms can synthesize auxins and cytokinins. The capacity to synthesize hormones by plants is widely distributed in plant-associated bacterial interactions. 80 % of the bacteria isolated from the plant rhizosphere produce IAA [22]. *Rhizobium* isolates from root and stem nodules of *Sesbania* species were shown to produce indole-3-acetic acid (IAA) in culture when supplemented with L-tryptophan. Among the three different isolates, maximum IAA was produced by the *Rhizobium* isolate from *Sesbania procumbens*. The IAA from this isolate was extracted, purified and identified by Thin layer chromatography [23]. Lindsay [24] isolated *Rhizobium* strains from root nodules of five species of *Indigofera viz., Indigofera trita, Indigofera linnaei, Indigofera astragalina, Indigofera parviflora and Indigofera viscosa* on Yeast Extract Mannitol Agar (YEMA) medium. The strains were analyzed to produce acid, exopolysaccharide and indole acetic acid by utilizing ten different carbon sources.

6. SIDEROPHORE PRODUCTION BY *Bradyrhizobium* ISOLATES

Iron-containing protein figures prominently in the nitrogen-fixing symbiotic bacteria (i.e., *Azorhizobium*, *Rhizobium,* and *Bradyrhizobium* and their respective plant hosts to synthesize iron-containing compounds nitrogenase, leghaemoglobin, ferridoxin, hydrogenase and cytochromes, symbiotic bacteria must require an adequate supply of iron. Iron availability is reduced to precipitation, forming oxyhydroxide polymers of $Fe(OH)_3$. Therefore to compete successfully for iron, an organism has evolved a specific, high-affinity mechanism to acquire iron. In symbiotic bacteria, these systems are composed of ferric-specific ligands (siderophores) and cognate membrane receptors [25].

Over-production of siderophore occurs in bacteria and fungi under acute iron starvation. Siderophores dissolve the complex ferric ion chelated in the highly insoluble oxyhydroxides. Gulati [26] reported that in *E.coil, iron transport was regulated by a repressor protein* bound to the ferrous ion. Cowpea *Rhizobium* RA-1 produced catechol like siderophore, which decreased with an increase in the concentration of molybdenum (above 1 mM) and the presence of iron increased the molybdenum uptake but 2,3-dihydroxy benzoic acid did not show any increase in the uptake, thereby confirming that the entire siderophore molecule was required for the transport of molybdenum [27].

Studies on differential siderophore utilization and iron uptake by soil and rhizosphere bacteria used ferrioxamine B as the sole Fe source in Fe deficient medium, while about 12,10,2 and more than 1 per cent respectively were able to use ferric chrome and pseudobactions [28]. Introduction of transposon Tn5 (Km-1) into a siderophore production Chinese *Rhizobium fredii* resulted in mutants of overproducing siderophore. *Bradyrhizobium japonicum* utilized hydroxamate type siderophore i.e. ferric citrate and rhodotortalate under iron starving conditions. In addition to this, they also used pyoverdin type siderophore. Nodule occupancy in greenhouse experiment by these two mutants were 3 and 4 per cent compared to 19 per cent by wild strain, proving that over-production of siderophore resulted in less competitive strains [29].

Generally, applying macronutrients increases crop yield, growth, and quality [30–48]. However, dryland farmers face many challenges, such as low productivity and increased farm cost also several studies concluded that low productivity is primarily related to management practices [49–55]. Research has demonstrated substantial linkages between temperature, fertilizer applications, plant populations and planting dates [56–71]. Biological nitrogen fixation (BNF) is one of the most important phenomena occurring in nature and can be considered organic fertilizer. Numerous studies indicate that the availability of nutrients is vital for plant growth, particularly in the case of weeds that influence and inhibit crop growth for nutrients in the soil

[72–92]. Biological nitrogen fixation processes can provide good soil nitrogen, and active organic matter provides habitat and nutrition for beneficial soil organisms that help create soil structure and porosity, provide plant nutrients, and improve soil ability to retain water [93–107].

7. CONCLUSION

Bradyrhizobium synthesizes a wide array of Carbohydrates such as Lipopolysaccharides, Capsular polysaccharides, Exopolysaccharides, Nodule polysaccharides, Lipo-chitin oligosaccharides and Cyclic glucans, all of which play a role in the Biological Nitrogen Fixation symbiosis. The *Bradyrhizobium* species produce Polysaccharide degrading enzymes such as Polygalacturonase and Carboxymethyl cellulase that cleave glycosidic bonds of the host cell wall at areas where bacteria are concentrated, creating erosion pits in the epidermal layer of the roots, allowing the bacteria to gain entry to the roots. The energy source for *Bradyrhizobium japonicum* is the sugar trehalose, which is taken up readily and converted to Carbon-di-oxide. On the other hand, UDP-glucose is taken up in large quantities but metabolized slowly like sucrose and glucose. Promotion of plant growth causes more Oxygen to be released and more Carbon-di-oxide to be taken up.

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