Review Article

BENEFICIAL EFFECT OF Bradyrhizobium sp. ON BLACKGRAM (Vigna mungo L.) CULTIVATION: A REVIEW

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Abstract

Plants are exposed to diverse species of microbes that are present in soil. Plants are the prime source of nutrients for microbes and provide nutrients indirectly from root exudates and dead plant matter. In some cases, nutrients are provided directly to microbes that form close relationship with plants. Plant microbes lead to many harmful and beneficial sequences. During recent years, studies on beneficial aspects of plant-microbe interactions, metabolites mediating microbial plant communication and plant strategies to manage nutrient fluxes and nutrient acquisition have gained great importance. The potential of variety of soil microorganisms to exert beneficial effects on various crops is now well established. Rhizosphere bacteria may promote plant growth directly by providing nutrients or growth factors or indirectly by antagonizing soil borne phytopathogens through secondary metabolites. Many of the studies with PGPR show plant growth promotion but only under gnotobiotic condition or in potting media where these bacteria do not compete with the normal array of soil microorganisms. In this present review paper, we explained the beneficial role Bradyrhizobium sp. on the growth and yield of Blackgram.

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1. Introduction

Biofertilizers are natural fertilizers which are microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. Biofertilizers (microbial nutrients) are the products containing living cells of different types of microorganisms which have an ability to mobilize nutritionally important elements from non usable to usable form through biological process. In recent years, biofertilizers have emerged as an important component of INSS and hold a promise to improve the crop yields and nutrient supplies. The

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use of biofertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers.

Biofertilizing agents control the plant pathogenic fungi directly as well as indirectly. Directly they parasitize the pathogens; application of *Rhizobium* culture on the legume seeds control seed borne fungi such as *Colletotrichum* sp., *Ascochyta* sp., *Helminthosporium* sp., etc. The *Rhizobium* produce a toxic substance when they multiply on the seed and rhizosphere. Phosphate solubilizing fungi such as *Aspergillus niger* and other *Penicillium* sp. produce antibiotic substances and thus kill the pathogenic fungi. Indirect killing of the plant pathogens is achieved by producing healthy seedlings and phytoalexins. Application of *Bradyrhizobium* sp. produce better root systems which overcome the attack of root rotting and soil borne pathogens. Numerous reports are available that applications of biofertilizers in the soil stimulate and augment the activity of saprophytic microorganisms.

Biofertilizers are apparently environment and farmers friendly renewable source of non-bulky, low cost organic agro-input. While Brady *Rhizobium* sp., Blue Green Algae (BGA) and *Azolla* sp. are crop specific, bioinoculants like *Azotobacter* sp., *Azospirillum* sp., Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Myorrhiza (VAM) etc. could be regarded as broad spectrum biofertilizers. Their use has so far not received desired attention. This is mainly because of inadequate awareness both among the extension workers and farmers regarding their utility, their short shelf life, lack of ready availability in time and in desirable quality, and inconsistency in results with their use.

Blackgram (*Vigna mungo* L.) is one of the important pulse crops in India. It has been reported that Blackgram has been cultivated in India since ancient times. It disbelieved that Blackgram is a native of India and Central Asia and grown in these regions since prehistoric times. It is widely cultivated throughout the Asia, including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, Indonesia, Malaysia, south China, and Taiwan. In Africa and U.S.A. it is probably recent. Blackgram is a protein rich staple food. It contains about 25 per cent protein, which is almost three times that of cereals. It supplies protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. The moong dal Khichdi is recommended to the ill or aged person as it is easily digestible and considered as complete diet. Roti with Moong dal and Moong dal chawal is an important ingredient in the average Indian diet. The biological value improves greatly, when wheat or rice is combined with Greengram because of the complementary relationship of the essential amino acids. It is particularly rich in Leucine, Phenylalanine, Lysine, Valine, Isoleucine, etc.

Blackgram is one of the important pulse crops gaining importance all over the world in recent years. It is rich in proteins and contains amino acids higher quantities than any other cereals and pulses. Blackgram is an annual food legume. Blackgram seeds are boiled and eaten whole or after splitting into dhal. The dried seeds contain approximately 9.7 % water, 23.4 % protein, 1 % fat, 57.3 % carbohydrate, 3.8 % fibre and 4.8 % ash. It is very nutritious and is recommended for diabetics.

2. PGPR and Symbiotic nitrogen fixation

Plant growth promoting rhizobacteria (PGPR) may promote growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus and potassium, production of siderophore that solublize and sequester iron, or production of plant growth regulators (Kloepper *et al.*, 1997). Some bacteria support plant growth indirectly, by improving growth restricting conditions either via production of antagonistic substances or by inducing host resistance towards plant pathogens. Since associative interactions of plant and microorganisms must have come into existence as a result of co-evolution, the use of either former or latter groups of bioinoculants
form one of the vital components for a long term sustainable agriculture system (Tilak et al., 2005).

In recent history, cereals productivity was dramatically increased under high input systems whilst legume yields have neared a plateau, stagnated or even reduced. This has resulted in unbalanced cereal-legume global production, and thus in higher and unsustainable dependence on Nitrogen chemical fertilizer inputs which reached $20 billion annually (Crouch et al., 2004). Nitrogen is one of the major important nutrients essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a Blackgram performs N$_2$ fixation by establishing a symbiotic relationship with the *Rhizobium* sp. Symbiotic nitrogen fixation resulting from mutual beneficial interaction between Blackgram and soil nodule bacteria provides a significant boost to Nitrogen fertilization and additionally, does not cause any hazard to environment. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculate in Blackgram fields through the world Caldwell and Vest (1970).

The beneficial effects of *Bradyrhizobium* inoculation to various leguminous crop plants have been investigated by several workers (Thakare et al., 1999). The beneficial effects of *Rhizobium* and *Bradyrhizobium* in legume in terms of biological N$_2$ fixation has been a main focus in the recent past (Deshwal et al., 2003), as it is an important aspect of sustainable and environmental friendly food production and long term productivity.

Nitrogen is one of the major important nutrients essential for plant growth. The atmosphere contains about 10$^{15}$ tonnes of N$_2$ gas and the nitrogen cycle involves the transformation of some 3 x 10$^{9}$ tonnes of N$_2$ per year on the Global basis (Delgodo et al., 1993). However, transformations e.g., N$_2$ fixations are not exclusively biological. Lightning probably accounts for about 10 per cent of the world's supply of fixed nitrogen. The fertilizer industry also provides very important quantities of chemically fixed nitrogen. World's production of fixed nitrogen from dinitrogen for chemical fertilizer accounts for about 25 per cent of earth's newly fixed nitrogen and the biological process accounts for about 60 per cent. The international emphasis on environmentally sustainable development with the use of renewable sources is likely to focus attention on the potential role of biological nitrogen fixation in supplying N for agriculture. Annually, biological nitrogen fixation is estimated to be around 175 million tonnes of which 79 per cent is accounted by terrestrial fixation. *Rhizobium* symbiotic association observed to fix 60 - 100 kg N ha$^{-1}$ crop$^{-1}$ (Anonymous, 2000).

In recent history cereals productivity has dramatically increased under high input system whilst legume yield have neared plateau, stagnated or even reduced. This has resulted in unbalanced cereal legume global production, and this in higher and unsustainable dependence on Nitrogen chemical fertilizer inputs which reached $2 billion annually (Crouch et al., 2004). Increasing cultivation of legumes will be required to ameliorate environmental degradation, reduce depletion of non-renewable resources and provide adequate nitrogen for sustainable agriculture. Most of the new lands to be opened for legume cultivation in the developing countries are located in the dry desert areas.

Symbiotic nitrogen fixation (SNF) by legumes plays major role in sustaining crop productivity of marginal lands and in small holders systems. Farmers in the dry areas depend on legumes as an important crop in their cropping systems due to the capacity of these plants to fix nitrogen from air by the interaction with nitrogen fixing *Rhizobium* sp. It is well known that nitrogen is abundant in the atmosphere, but plants cannot directly utilize the elemental nitrogen from the air. Symbiotic nitrogen fixation occurs mainly through symbiotic association of legumes with N$_2$ fixing *Rhizobium* sp. that convert elemental nitrogen fixation (BNF) in to ammonia. This type of biological nitrogen is therefore less costly and more sustainable as compared with nitrogen fertilizers for production of plant proteins. Scientific and technological progress has opened

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tremendous opportunities for the benefit of small farmers (Abdel Aal et al., 2004). Tong and Sadowky (1994) developed a novel non-antibiotic containing medium which allows selective isolation of Bradyrhizobium japonicum and Bradyrhizobium elkanii strains from soils. The medium, BSJM, in based on the resistance of Bradyrhizobium japonicum and Bradyrhizobium elkanii to more than 40 µg of the metals ions Zn²⁺ and CO²⁻ per ml. BJSM does not allow the growth of Rhizobium sp. strains.

3. Nitrogen fixing efficiency of Bradyrhizobium isolates

Effectiveness of the symbiosis can be measured by two ways, either directly by determining the amount of nitrogen fixed or indirectly by measuring the plant dry weight. Bradyrhizobium japonicum bacteria’s were isolated anaerobically and were supplied with ¹⁴C-labelled trehalose, sucrose UDP-glucose, glucose or fructose under low O₂ (2 %) in the gas phase. The overall results support the view that, although bacterioids metabolize sugars the rates are very low and are inadequate to support nitrogenase in comba et al. (1998).

The nitrogenase activity under water logged condition in soyabean inoculated with Bradyrhizobium japonicum was studied by Sung (1993). After four days of inoculation, the ARA was 104 µ mol (C₂H₂ g⁻¹ dw h⁻¹) and 80 µ mol C₂H₂ g⁻¹ dw h⁻¹ under waterlogged conditions. The normally grown plants of Vicia fabae (Vidal et al., 1992) and Phaseolus sp. (Jamro et al., 1994) showed maximum nitrogenase activity of flowering stage and declined after pod filing. Nitrogenase activity was positively correlated with nodule number during this stage of both Vicia and Phaseolus plants.

In Blackgram, a strong correlation existed between leghaemoglobin content, nitrogenase activity and ureide concentration in Xylem sap (Dakora and Felix, 1995). The effectiveness and competitive ability to Bradyrhizobium strains were studied by Narendrakumar et al. (1996). The nodulation, plant biomass, N uptake and grain yields of inoculated plants were significantly higher in loamy soils than alluvial sandy soils. Thakare et al. (1999) tested out of 39 isolates of Blackgram Bradyrhizobium screened, 24 isolates increased nodulation significantly over control.

The symbiosis of hup⁺ and hup⁻ and hup² strains of Bradyrhizobium elkanii in cowpea cultivars were studied by Souza (1999). Nitrogenase activity, hemoglobin content and nitrogen fixation is more in hup⁺ and hup⁻ inoculated cultivars than hup² strains inoculated plants. The effects of lectins from Greengram on the symbiotic activity of Bradyrhizobium japonicum strain was investigated by Kirichenko and Malichenko (2000) preincubation 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 but the lectin isolated from the non specific host plant had no such effect.

There was an inverse relationship between nitrate and ureide contents in the different plant parts in both nodulating and non-nodulating Blackgram cultivars. The nitrogenase activity was negatively correlated with NO₃ content of plant. The nitrogenase activity of Blackgram var. Bragg peaked at 60 DAS, which was positively correlated with the concentration of ureides. The ureide concentration was maximum at R₂ - R₃ stages in all cultivars and gave a positive correlation with a grain yield of Blackgram cultivars.

Castro and Acuna (1992) in green house trails determined the ureide content at three growth stages in leaves and stems of Blackgram and bean. In both cases, the ureide content was higher in stem and decreased with an increasing age. Avline et al. (1995) proposed that ureides assay method has been found to be suitable for Blackgram to assess nitrogen fixation in wide range of genotypes. Their results confirmed that the relative abundance of ureides was closely related to the Nitrogen derived from symbiotic N₂ fixation at vegetative and reproductive stages. Fue et al. (1995) screened eight wild cultivars of Greengram for their nitrogenase activity and ureide content at different stages of growth. The ureide
content in the stem was positively correlated with the nitrogenase activity.

4. Exopolysaccharides production by *Bradyrhizobium* sp.

Several *Rhizobium* polysaccharides were found to be effective stabilizing agents of most soils than either the synthetic soil conditioner or the other reference compounds. Streeter and Salmen (1993) reported that the polysaccharides of *Bradyrhizobium japonicum* is a bacterial product and not a plant product. Two types of polysaccharides are formed corresponding to the two DNA-homologus groups of *Bradyrhizobium japonicum*. The polysaccharide produced in nodules (NPS) is different in composition from that produced in culture (EPS).

Bacterial polysaccharides are necessary for a functional Brady *Rhizobium* legume symbiosis. Exopolysaccharide (EPS), Lipopolysaccharide (LPS), Capsular polysaccharides and cyclic β-(1-2) glucan play essential role in the formation of the infection thread and in nodule development (Kannenberg and Brewin, 1994). The exopolysaccharide production by *Rhizobium meliloti* is influenced by salt. The halotolerant strain *Rhizobium meliloti* EFB 1 modifies the production of EPS in response to salt. This bacterium grown in the presence of 0.3M NaCl showed decrease in mucoidy and when grown in salt supplemented liquid medium this organism produced 40 percent less exopolysaccharides (Javierlloret et al., 1998).

The cell surface carbohydrates of bacteria within the Rhizobiaceae family provide important functions during legume nodulation. *Rhizobium* lipopolysaccharides which have been shown to elicit root hair deformation cortical cell division and nodule organogenesis (Frenssen et al., 1992). Genetic studies have also provided evidence that a second class of *Rhizobium* cell surface carbohydrate, the exopolysaccharide is required for nodule development in plant (Leigh and Coplin, 1997). Gore and Miller, (1993) reported a third class of *Rhizobium* cell surface carbohydrate, the cyclic β-glucans with in the Blackgram root nodules rhizobial cell surface carbohydrate, the exopolysaccharides, is required for nodule development in plants.

5. Indole acetic acid production by *Bradyrhizobium* sp.

Naturally occurring substances with indole nucleus possessing growth-promoting activity are referred to as auxins, chemically it is Indole acetic acid (IAA). Not only plants but also microorganisms can synthesize auxins and cytokinins. The ability to synthesize phytohormone is widely distributed among plant-associated bacteria. Nearly, 80% of the bacteria isolated from plant rhizosphere produce Indole acetic acid (Sahasrabudhe, 2011).

Sridevi and Mallaiah (2007) *Bradyrhizobium* isolates from root (*Sesbania procumbens*) and stem nodules (*Sesbania rostrata* and *Sesbania procumbens*) of *Sesbania* species were shown to produce Indole-3-acetic acid (IAA) in culture supplemented with L-tryptophan. Among the three isolates, maximum amount of IAA was produced by the *Rhizobium* isolate from *Sesbania procumbens*. The IAA from this isolate was extracted, purified and identified by thin layer chromatography.

Kumari et al. (2009) isolated *Bradyrhizobium* 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 examined for production of acid, exopolysaccharide (EPS) and indole acetic acid (IAA) by utilizing 10 different carbon sources.

7. Effect of inoculation of *Bradyrhizobium* sp. on Blackgram

The influence of five Thai Blackgram cultivars on nodulation competitiveness of four *Bradyrhizobium japonicum* strains was investigated by Payakapong et al. (2004). At harvest, nodule occupancy by each strain was determined by fluorescent antibody technique. Nodulation competitiveness of the...
Bradyrhizobium japonicum strains was affected by the cultivars of Blackgram used. Keyser and Li (1992) reviewed the following characters of Bradyrhizobium to be used as inoculant for pulses: 1. Ability to form nodules and fix nitrogen 2. Ability to compete 3. Ability to fix N at different environmental range 4. Ability to grow in the artificial media 5. Ability to persist in soil for long time 6. Ability to migrate 7. Ability to colonize in the soil 8. Genetic stability 9. Compatibility 10. Ability to colonize in rhizosphere soil. Appunu et al. (2008) studied the symbiotic interactive effect of different Bradyrhizobium japonicum strains with 6 Blackgram cultivars. Plants inoculated with strain ASRO11 produced higher plant dry matter accumulation and seed yield over all other cultivars.

Morote et al. (1990) reported that inoculation with Bradyrhizobium japonicum increased nodule mass significantly in Blackgram. Daramola et al. (1994) reported that soil inoculation with Bradyrhizobium japonicum resulted in more nodules, more uniform distribution on the root and greater nitrogen fixation. Dakora and Felix (1995) used light, scanning, and transmission electron microscopy to show that roots of sorghum and millet landraces from Africa were easily infected by Rhizobium isolates from five unrelated legume genera. With sorghum, in particular, plant growth and phosphorus (P) uptake were significantly increased by Rhizobium inoculation, suggesting that filed selection of suitable Rhizobium cereal combination could increase yields and produce fodder for livestock production. Zhao (1997) reported the effect of Bradyrhizobium inoculation on biomass and nodulation cowpea. Cowpea seeds inoculated with Rhizobium strains increased nodulation, fodder yield and seed yield.

Egamberdiyeva et al. (2004) reported the effect of Bradyrhizobium spp. strains on dry matter yield, nodulation and seed yield of Blackgram varieties grown in Nitrogen deficient soil in pot and field experiments. They noticed the significant effects on growth, nodule number and yield of Blackgram were obtained after inoculation with Bradyrhizobium spp. strains.

Seeds of Greengram plants were inoculated with antibiotic mutants of the Bradyrhizobium strains. Increased shoot dry weight, % N, total N and seed yield were a result of increased nodulation by the effective and competitive inoculant Bradyrhizobium strains. The results showed that there was high potential for increasing growth and seed yield of promiscuous Greengram cultivar by inoculation with foreign Bradyrhizobium strains (Okereke et al., 2000). Meghvansi et al. (2005) isolated three pH tolerant strains of Bradyrhizobium japonicum and their efficiency were determined under the mist conditions in the sterilized sandy soil at pH 8.4. Maximum and minimum nodulation and vegetative growth were observed in Bj-3 and Bj-2 inoculated Greengram plants respectively. Three pH tolerant strains could also pose better results in the efficiency determination experiments.

Rhizobium form root nodules that fix nitrogen (N2) in symbiotic legumes, extending the ability of these bacteria to fix N2 in non-legumes such as cereals would be useful technology for increased crop yields. Agraw (2012) conducted an experiment at Assossa Agricultural Research Centre (AARC) station during 2008 cropping season in order to study the effects of co-inoculation of Bradyrhizobium japonicum (TAL-378 and TAL-379) and phosphate-solubilizing bacteria (PSB) (Pseudomonas spp.), and conventional farmers’ fertilizer level (combined and individual application of 46 N kg ha−1 and 46 P2O5 kg ha−1) on nodulation, seed yield and yield components of Greengram. Analyses of variance indicated that most of the parameters measured were significantly (P>0.05) affected by the treatments. Accordingly, dual inoculation with TAL-378 and PSB significantly increased plant height at harvest, number of nodules per plant, nodule volume per plant, nodule fresh weight per plant, and shoot height at late flowering and early pod setting compared to the other treatments.

Ibrahim et al. (2011) carried out an experiment at Shambat, Sudan (Latitude 15° 40’N and Longitude 32° 32’E) in three consecutive
seasons (2000/03) to investigate the effect of \textit{Bradyrhizobium} inoculation and chicken manure or sulphur fertilization on growth, nodulation and yield of green gram. The results showed that inoculation, chicken manure, sulphur and their interaction significantly ($P = 0.05$) improved the dry weight of shoots and roots, nodulation, yield and yield components.

According to Mendes \textit{et al.} (2003), \textit{Bradyrhizobium} inoculation has successfully replaced the use of N fertilizer on Greengram crops. N rates promoted reductions of up to 50\% in the nodule number at 15 days after the emergence. Regardless of the management system, these reductions disappeared at the flowering stage and there was no effect of Nitrogen rates on either the number or dry weight of nodules or on Blackgram yields.

Seed inoculation of \textit{Bradyrhizobium} sp. increased number of pods plant$^{-1}$, number of grain pod$^{-1}$ and 1000 seed weight in Blackgram (Sable and Khuspe, 1997). Inoculation of Blackgram with \textit{Bradyrhizobium japonicum} increased seed yields by 25 to 41\% with no significant differences between seed and soil inoculation (Pandzoa \textit{et al.}, 1990). Inoculation with Hup$^{-}$ \textit{Bradyrhizobium japonicum} strains yielded 3.08 tonnes ha$^{-1}$ while the commercial strain inoculated and uninoculated Blackgram control yielded 2.87 + ha$^{-1}$ and 1.96 tonnes ha$^{-1}$ respectively (Hume and Shelp, 1992).

The cell load of \textit{Bradyrhizobium japonicum} in commercial inoculants on Blackgram seed yield possessed positive correlation with nodule number, mass and seed yield and recorded 19\% yield increase when inoculum load from $10^5$ to $10^6$ cells/ seed (Hume and Blair, 1992). Gurung and Sherchan (1997) studied on the native green gram \textit{Bradyrhizobium} isolates to increase biological nitrogen fixation. Among the native isolates tested 3 of them increased green gram yield up to 24\% over uninoculated treatment and showed tolerance to low pH and high levels of A1 (5.72 m mol) in soil. The seed yield of \textit{Rhizobium fredii} inoculated green gram were two folds greater than uninoculated controls and on par with nitrogen fertilizer (200 kg ha$^{-1}$) applied plots (Temprano, 1998).

Baskaran \textit{et al.} (2009) carried out the research work has been carried out to understand the effect of different concentrations of sugar mill effluent on growth, yield, biochemical contents and enzymatic activities of Blackgram (\textit{Vigna radiata} L. Wilczek). The effluents severely affect crop plants and soil properties when used for irrigation. Pot culture experiments were conducted with Blackgram at different concentrations of sugar mill effluent.

8. References


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