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Response of soybean to *Rhizobium* biofertilizer under different levels of phosphorus

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Abstract

A field experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh during January to May 2015 to study the response of soybean to *Bradyrhizobium* biofertilizer under different levels of phosphorus on growth, yield, nutrient content and uptake by soybean. The experiment was laid out in a randomize complete block design with three replication of each treatment. The treatments were: T_o = Control, T_1=(100% Nitrogen), T_2=*(Bradyrhizobium*), T_3=(*Bradyrhizobium* inoculation+75% P), T_4=(*Bradyrhizobium* inoculation+10 P), T_5=*Bradyrhizobium*+125% P), T_6=(100% RFD+*Bradyrhizobium*). Potassium and Sulphur at recommended dose were applied as basal to all the treatments. Urea was applied in three spits. Phosphorus in the form of TSP was applied as per treatment during final land preparation. Soybean seeds were inoculated with *Bradyrhizobium* as per treatments. Intercultural operations were done as and when necessary. Data were collected on pods weight, grain and stover yields. The N and P contents in grain stover and total N and P uptake by soybean were determined. Inoculation of seeds with *Bradyrhizobium* and application of N and P fertilizer at recommended dose recorded the highest grain and straw yields of soybean. The results suggest that soybean cultivation can effectively be done in the Old Brahmaputra Floodplain soils by Inoculating with *Bradyrhizobium* biofertilizer in combination with recommended dose of N, P, K and fertilizer.

Key words: *Rhizobium* inoculants, nutrient uptake, yield, soybean

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Introduction

Soybean (*Glycine max* L. Merr) is one of the most important recognized oil seed and protein rich crops in the world. In Bangladesh, about 5 thousand hectares of land is under soybean cultivation and annual production is approximately 4 thousand metric tons with an average yield of 1.5-2.3 t ha^{-1} (BARI, 2006). Soy protein products can replace animal-based foods-which also have complete proteins but tend to contain more fat, especially saturated fat without requiring major adjustments elsewhere in the diet. Because soybeans contain no starch, they are a good source of protein for diabetics (USFDA, 2000).Soybean is called "miracle bean" or protein hope of future in Bangladesh. Soybean contains 40-45% protein, 18-20% edible oil and 24-26% carbohydrate (Gowda and Kaul, 1982).

Excessive uses of chemical fertilizers are increasing environmental pollution every day. On the contrary, bio-fertilizer technology is inexpensive and environmentally sound. As a legume crop, soybean in association with *Bradyrhizobium* has the unique ability of fixing atmospheric nitrogen for their growth and enriching nitrogen fertility as well as organic matter content in soil (Hoque *et al.,* 1982 and Podder *et al.,* 1999).

Atmosphere contains 78.08% N_2, which is practically unavailable to plants. Some plants, basically leguminous plants can assimilate atmospheric N_2, with the help of bacteria such as *Rhizobium.* The cooperation of plants and bacteria in nodules to convert atmospheric N_2 to reduced N is called symbiotic N_2-fixation. Successful *Rhizobium*-legume symbiosis will definitely increase the incorporation of biological N_2-fixation into soil ecosystem (Vance, 2001). On a global basis these symbiotic association between legume and *Rhizobium* may reduce about 70 million tons of atmospheric N_2 to ammonia per annum which amounts to about 40% of all biologically fixed N per year (Burns and Hardy, 1975). Legumes play a pivotal role in developing new strategic approaches to ensure sustainable increase in agricultural productivity, without harming the environment (Hardarson *et al.,*1987). Leguminous crops like soybean could utilize atmospheric N_2 and then the volume of N fertilizers applied to fields could be reduced, without losing the maximum biomass of crops (Kannaiyan, 1999). The symbiotically fixed N can meet up to 30-90% N requirement of the plant (Vessey, 2004). This fixed N also acts as a renewable source of N if the legume crop is rotated with a non-leguminous one.

Bradyrhizobium japonicum has the beneficial effect on the growth and yield of soybean through producing root nodules. Fortunately, the soybean nodulating bacteria *Bradyrhizobium japonicum* can fix sufficient atmospheric nitrogen (about 300 kg ha^{-1} yr^{-1}) in symbiosis with soybean (Keyser and Li, 1992). So, the use of bradyrhizobial inoculant in soybean production can play a vital role in improving soil environment and agricultural sustainability. Increased nodulation, higher dry mater and grain yield production due to *Bradyrhizobium* inoculation have been documented by several workers (Hoque *et al.,* 1982; Solaiman and Hoque, 1983; Moward *et al.,* 1988; Hoque and Jahiruddin, 1988 and Singh, 2005). Again, effective nodulation and high N_2-fixation depend largely on the efficiency of the nodule bacteria. The efficiency of the symbionts depends on many factors, important of which are genetic variability of the symbiont host plant, soil and environmental factors (Danso *et al.,* 1987; Douka *et al.*, 1986 and Somasegaran *et al.,* 1990).

After nitrogen, phosphorus (P) is another plant growth-limiting nutrient despite being abundant in soils in both inorganic and organic forms. However, many soils throughout the world are phosphorus -deficient because the free phosphorus concentration (the form available to plants) even in fertile soils is generally not sufficient (Gyaneshwar *et al*., 2002). Root improvement, stalk and stem vigor, flower and seed formation, crop production, crop maturity and resistance to plant pests and diseases are the attributes associated with phosphorus availability. Phosphorus is needed in relatively large amounts by legumes for growth and has been reported to promote leaf area, biomass, yield, nodule number and nodule mass in different legumes (Berg and Lynd, 1985: Pacovsky *et al*., 1986: Kasturikrishna and Ahlawat, 1999). Furthermore, phosphorus has important effects on photosynthesis, root development, fruiting and improvement of crop quality (Sara *et al*., 2013). Large amount of phosphorus applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Aluminium (Al+) and Iron (Fe3+) in acidic, and Calcium (Ca2+) in calcareous or normal soils (Gyaneshwar *et al*., 2002: Hao *et al*., 2002). Efficiency of P fertilizer throughout the world is around 10 - 25 % (Isherword, 1998), and concentration of bioavailable phosphorus in soil is very low reaching the level of 1.0 mg kg-I soil (Goldstein, 1994). In soybean production, phosphorus and inoculation with the appropriate *Rhizobium* strains have quite prominent effects on nodulation, growth and yield parameters (Shahid *et al*., 2009: Kumaga and Ofori, 2004).The factors which control the amount of nitrogen fixed include available soil nitrogen, genetic determinants of compatibility in both symbiotic partners and lack of other yield-limiting factors like edaphic factors associated with phosphorus deficiency, soil acidity, mineral elements nitrogen and other various microelements like Cu, Mo, Co, B which are necessary for N2 fixation (Harold *et al*., 1992).The absence of the required *Rhizobia* species and optimal phosphorus levels limit legume production in different parts of the world. Inoculation with compatible and suitable *Rhizobia* with optimum phosphorus levels may be essential where a low population of native rhizobial strains prevail and is one of the key components of which grain legume farmers can use to optimize yields.

The use of fertilizers and bio-fertilizer in soybean production can play a significant role in terms of biomass production, yield, improving soil environment and agricultural sustainability. Research on the contribution of *Bradyrhizobium* and fertilizers especially phosphorus on the growth, yield and N_2-fixation of soybean is very limited in our country. Therefore, the present investigation was carried out to evaluate the performance of *Rhizobium* biofertilizer on yield of soybean under different levels of phosphorus and to know the effect of *Rhizobium* inoculants on N, P content and uptake by soybean under different levels of phosphorus.

Materials and Methods

Experimental field and site**:** The experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University, Mymensingh during the winter (Rabi) season (January, 2015 to May, 2015). The experimental site is located about 24°75′ North latitude and 90°50′ East longitude. The land selected for the experiment was medium high land.

Soil**:** The initial soil samples were collected from 0-15 cm depth of the Old Brahmaputra Floodplain (AEZ-9) before opening the field.

Crop**:** The crop under the study was soybean (*Glycine max.* L. Merr.). The variety of the crop was PB-I (Shohag) was collected from Department of Genetics and Plant Breeding of Bangladesh Agricultural University, Mymensingh.

Characteristics of the initial soil sample

Morphological characteristics		**Physiological characteristics**		**Chemical characteristics**	
Locality	BAU, Mymensingh	Sand (%)	7.64	pH (soil : water 1:2.5)	6.94
AEZ	Old Brahmaputra Floodplain (AEZ-9)	Silt (%)	82.00	Organic matter (%)	1.62
Soil Series	Sonatola	Clay (%)	10.36	Total nitrogen (%)	0.067
Physiographic unit	Old Brahmaputra Floodplain	Textural class	Silt loam	Available phosphorus (ppm)	10.45
Drainage	Moderate			Exchangeable K (me/ 100g soil)	0.08
Flood level	Above flood level			Available sulphur (ppm)	12.00
Topography	Medium high land, fairly leveled			Cation exchange capacity (me/ 100g soil)	15.00
General soil type	Non-calcareous Dark Gray Floodplain				

Climate: Details of the climatic data during the study period have been recorded.

Treatments: The experiment consists of 7 treatments with 3 replications. The treatments under study were as follows: T_o: Control, T_1: Recommended dose of N, T_2: *Rhizobiun* biofertilizer inoculation, T_3: *Rhizobium* biofertilizer inoculation+75% P, T_4: *Rhizobium* biofertilizer inoculation+100% P, T_5: *Rhizobium* biofertilizer inoculation+125% P, and T_6: *Rhizobium* biofertilizer inoculation +N+P (100%)

Experimental design: The total land was divided into three blocks and each block was sub-divided into 7 unit plots. The total number of plots were 7 × 3 = 21 and the size of each unit plot was 4 m × 2.5m = 10 m^2.

Crop cultivation

Land preparation: Land preparation was started on November, 2014.

Fertilizer application: At the final land preparation a basal recommended dose of K and S were applied. Potassium @ 40 kg K ha^{-1} from muriate of potash (MoP) and sulphur @ 10 kg S ha^{-1} from gypsum were applied as basal dose. Triple Super Phosphate (TSP) was applied as per treatments. Urea was applied in 3 splits. The fertilizers were mixed well with the soil by spading.

Seed germination test: Germination test was conducted in petri-dish using water soaked blotting paper. It was found that more than 95% seeds were viable and germinated successfully after 4-5 days.

Preparation of rhizobial inoculants (broth): The broth culture of *rhizobial* strain was prepared following the method of Vincent (1970). Yeast mannitol broth medium was prepared in 250 ml Erlenmeyer flask.

Composition of medium:

Composition			
K_2HPO_4	0.5 g	NaCI	0.1 g
$MgSO_4.7H_2O$	0.2 g	Yeast extract	0.5 g
Mannitol	10 g	Distilled water	Up to 1L

Inoculation of seeds: At first uninoculated seeds were sown according to the experimental design for T_0. Then inoculated seeds were taken in small polythene bag equal in weight for each plot and mixed with 40% gum acacia. Then the selected inoculum was mixed with the seeds @ 50 g inoculum/ kg seeds for T_2, T_3, T_4, T_5 and T_6 treatments and mixed well with the seeds by shaking the bag thoroughly.

Sowing of seeds: Seeds were sown on the furrow on 01 January, 2015 and the furrows were covered by soils soon after seeding.

Germination of seeds: On the 8^{th} to 9^{th} day, the percentage of germination was more than 80% and on the 11^{th} day nearly all plants came out of the soil.

Collection of plant samples: Plant samples were collected from each plot at 40 and 60 days after sowing (DAS) and at harvest. The shoot, root and nodule materials were first air dried and then oven dried at 65°C for 72 hours.

Harvesting: The crop was harvested on 20 may (140 days after sowing), 2015 at maturity of whole plants

Grain yield and Stover yield: From each plot randomly selected 10 square meter plants were harvested. After drying, threshing and processing were done plot wise carefully. The processed seed and stover were again dried in the sun for 3 days. Seed and stover yields were recorded plot wise, which were then converted into yield in kg ha^{-1}.

Determination of N, P, K, and S from chemical analysis of grain and straw samples

Total nitrogen: Total N content in soil was determined by micro-Kjeldahl method

Available phosphorus: Phosphorus was extracted from plant sample with 0.5 M $NaHCO_3$ solution at pH8.5 following the method of Olsen *et al*. (1954).

Exchangeable potassium: The K was determined from the extract by using flame photometer. This method was proposed by Brown and Lilleland (1946).

***Available sulfur*:** The intensity of turbidity was measured by spectrophotometer at 420 nm wave length. The extraction method was described by Page *et al*. (1989).

***Statistical analysis*:** Data were analyzed statistically using analysis of variance (ANOVA) to examine the treatment effects, and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) and ranking was indicated by letters.

Result

Effect of *Bradyrhizobium* under different P levels on N content of soybean

***Nitrogen content in stover at 60 DAS*:** The values ranged from 2.167 to 3.623 % at 60 DAS due to different treatments. The highest N content in stover (3.623%) was observed in the treatment T_6 (100%RFD+ *Bradyrhizobium*). The second highest N content in stover (3.013%) was observed in the treatment T_5 which was also statistically similar to the treatment T_4 with the value of 2.960%. The lowest N content in stover (2.167%) was obtained in the treatment T_0 (control). The results indicated that the treatment T_6 (100%RFD + *Bradyrhizobium*) accumulated the highest % N in stover compared to other treatments. The combined application *Bradyrhizobium* with chemical fertilizers was found more effective to increase % N content in stover of soybean.

***Nitrogen content in stover at harvest*:** The nitrogen content in stover of soybean cv. Shohag was influenced significantly due to inoculation of soybean with *Bradyrhizobium* under different levels of P (Table 1). The results indicated that the treatment T_6 (100% RFD +*Bradyrhizobium*) accumulated the highest % N content in stover compared to other treatments. The combined application *Bradyrhizobium* with chemical fertilizers recorded the highest % N content in stover of soybean.

***Nitrogen content in nodules at 60 DAS*:** The values ranged from 3.573 to 4.917% at 60 DAS due to different treatments. The highest N content in nodule (4.917%) was observed in the treatment T_6 (100% RFD+*Bradyrhizobium*) which was also statistically similar to that of the treatments T_5 and T_4 (Table 1). The lowest N content in nodule (3.573%) was obtained in the treatment T_0 (control). The results indicated that the treatment T_6 (100% RFD+*Bradyrhizobium*) accumulated the highest %N content in nodule compared to other treatments. The combined application *Bradyrhizobium* with chemical fertilizers was noted the highest % N content in nodules of soybean.

***Nitrogen content in grain*:** Nitrogen content of soybean grain differed significantly due to inoculation of soybean with *Bradyrhizobium* under different levels of P (Table 1). The N content in grain ranged from 5.273 to 6.110% due to different treatments and the highest value was found in the treatment T_6 (100%RFD+*Bradyrhizobium*). The combined application *Bradyrhizobium* and chemical fertilizers was found more effective in increasing % N content in grain of soybean.

Effect of *Bradyrhizobium* under different P levels on P content of soybean

***Phosphorus content in Stover*:** Phosphorus content in soybean stover varied significantly due to inoculation of soybean with *Bradyrhizobium* under different levels of P (Table 2). The highest p content in stover was found in the treatment T_6 (100% RFD+ *Bradyrhizobium*).The treatments $T_{4,}$ T_5 and T_6 exerted statistically similar effect on the P content of soybean stover. Application of phosphorus fertilizers at variable rates and in different combinations showed a significant effect on the P content in soybean stover. All the treatments resulted comparatively higher P content in soybean stover over the control (T_0).

The P content in stover at harvest ranged from 0.22 to 0.30%. The highest P content in stover was found in the treatment T_6 (100%RFD+ *Bradyrhizobium*).The treatments T_4, T_5 and T_6 exerted statistically similar effect on the P content in soybean stover. Application of phosphorus fertilizers at variable rates and in different combinations showed a significant variation on the P content in soybean stover. All the treatments resulted comparatively higher P content in soybean stover over the control (T_0).

Table 1. Effect of *Bradyrhizobium* under different P levels on N content of soybean

Treatments	N content(%) in Stover at 60 days	N content(%) in Stover at harvest	N content(%) in nodule at 60 days	N content(%) in grain at harvest
T_0	2.167e	1.347c	3.573d	5.273c
T_1	2.847bc	1.940b	3.970c	5.930ab
T_2	2.660d	1.857b	4.230bc	5.787b
T_3	2.70cd	1.867b	4.143bc	5.927ab
T_4	2.960b	1.887b	4.673a	5.930ab
T_5	3.013b	1.893b	4.540ab	5.917ab
T_6	3.623a	2.173a	4.917a	6.110a
SE(±)	0.058	0.062	0.127	0.087
Level of significance	**	**	**	**
CV (%)	3.58	5.81	5.16	2.60

** = Significant at 1% level of probability, T_0=Control, T_1=100% N+P, T_2= *Bradyrhizobium,* T_3= *Bradyrhizobium* +75% P, T_4= *Bradyrhizobium* +100% P, T_5= *Bradyrhizobium* +125% P, and T_6= *Bradyrhizobium* +100% RFD.

***Phosphorus content in nodule*:** The P content of nodule ranged from 0.14 to 0.18% at 60 DAS due to different treatments (Table 2). The highest P content in nodule was found in the treatment T_6 (100%RFD+ *Bradyrhizobium*).The treatments T_4, T_5 and T_6 exerted statistically similar effect on the P content of soybean nodules. Application of phosphorus fertilizers at variable rates and in different combinations showed a significant variation on the P content in nodule. All the treatments resulted comparatively higher P content in soybean nodule over the control (T_0).

***Phosphorus content in grain*:** The highest p content in grain was found in the treatment T_5 (125% P+ *Bradyrhizobium*) (Table 2). The treatments T_4, T_5 and T_6 exerted statistically similar effect on the p content in soybean grain. Application of phosphorus fertilizers at variable rates and in different combinations showed a significant effect on the p content in grain. All the treatments resulted comparatively higher p content in soybean nodule over the control (T_0).

Effect of *Bradyrhizobium* under different P levels on N uptake of soybean

***Nitrogen uptake by grain*:** The N uptake by grains of soybean cv. Shohag varied significantly due to inoculation of soybean with *Bradyrhizobium* under different levels of P (Table 3). Nitrogen uptake by grain due to different treatments ranged from 42.01 to 88.57 kg ha^{-1}.The highest N uptake in grain (88.57 kg ha^{-1}) was observed in the treatment T_6 (100%RFD+*Bradyrhizobium*) which was statistically different and superior to all other treatments. The second highest N uptake in grain (82.00 kg ha^{-1}) was obtained in the treatment T_4 and it was statistically similar to that of the treatment T_5 with value of 77.49 kg ha^{-1}. The lowest N uptake in grain (42.01 kg ha^{-1}) was obtained in the treatment T0 (control). The combined application of *Bradyrhizobium* and chemical fertilizers was found more effective in increasing the N uptake of soybean grains. The results revealed that the treatment T_6 (100% RFD+*Bradyrhizobium*) recorded the highest N uptake by soybean grains.

Nitrogen uptake by stover: Nitrogen uptake by stover due to different treatments ranged from 105.75 to 229.68 kg ha^{-1}.The highest N uptake by soybean grains (229.68 kg ha^{-1}) was observed in the treatment T_6 (100% RFD+*Bradyrhizobium*) which was statistically different and superior to all other treatments(Table 3). The second highest N uptake in stover (198.31 kg ha^{-1}) was obtained in the treatment T_5 and it was statistically similar to that of the treatments T_4 and T_3 with values of 196.21 and 189.58 kg ha^{-1}, respectively. The lowest N uptake by stover (105.75 kg ha^{-1}) was obtained in the treatment T_0 (control). The combined application of *Bradyrhizobium* and chemical fertilizers increased the N uptake in stover of soybean. The results revealed that the treatment T_6 (100% RFD +*Bradyrhizobium*) increased the N uptake of stover compared to other treatments.

Table 2. Effect of *Bradyrhizobium* under different P levels on P content of soybean

Treatments	P content(%) in Stover at 60 days	P content(%) in Stover at harvest	P content(%) in nodule at 60 days	P content(%) in grain at harvest
T_0	0.23c	0.22c	0.14c	0.8467b
T_1	0.23c	0.23c	0.14c	0.8567b
T_2	0.23c	0.23c	0.14c	0.8633b
T_3	0.28b	0.26b	0.16b	0.9067b
T_4	0.31a	0.29a	0.18a	0.9833a
T_5	0.31a	0.30a	0.18a	1.003a
T_6	0.33a	0.30a	0.18a	0.9967a
SE(±)	0.007	0.0035	0.005	0.018
Level of significance	**	**	**	**
CV (%)	4.24	3.57	5.31	4.08

**=Significant at 1% level of probability, *=Significant at 5% level of probability, Values in a column having same letter (s) do not differ significantly at 1% level by DMRT.

Total N uptake: The total N uptake by soybean ranged from 147.76 to 318.26kg ha^{-1} and the treatment T_6 (100% RFD +*Bradyrhizobium*) recorded the highest N value which was significantly superior to all other treatments (Table 3). The treatments T_5 and T_4 exerted similar effect on the total N uptake by soybean. Again, the treatments T_1 and T_2 also recorded statistically similar results. The effect of the *Bradyrhizobium* and fertilizers in various combinations on the total N uptake by soybean was significant.

Effect of *Bradyrhizobium* under different P levels on P uptake of soybean

Grain: The P uptake by grains of soybean cv. Shohag was influenced significantly due to inoculation of soybean with *Bradyrhizobium* under different levels of P application ((Table 4). Phosphorous uptake by grain due to different treatments ranged from 6.75 to 14.43 kg ha^{-1}. The highest P uptake in grain (14.43 kg ha^{-1}) was observed in the treatment T_6 (100% RFD + *Bradyrhizobium*). The lowest P uptake by grains (6.75kgha^{-1}) was obtained in the treatment T_0 (control). The combined application of *Bradyrhizobium* and chemical fertilizers increased the P uptake by grains of soybean. The results revealed that the treatment T_6

(100% RFD +*Bradyrhizobium*) increased the P uptake in grain compared to other treatments.

Stover: Phosphorous uptake by stover due to different treatments ranged from 17.62 to 31.34 kg ha^{-1} (Table 4). The highest P uptake in stover (31.34 kg ha^{-1}) was observed in the treatment T_6 (100% RFD+ *Bradyrhizobium*) which is statistically similar to that of the treatments T_5 and T_4. The lowest P uptake by stover (17.62 kg ha^{-1}) was obtained in the treatment T_0 (control). The combined application of *Bradyrhizobium* and chemical fertilizers increased the P uptake in stover of soybean. The results revealed that the treatment T_6 (100% RFD +*Bradyrhizobium*) increased the P uptake of stover compared to other treatments.

Table 3. Effect of *Bradyrhizobium* under different P levels on N uptake of soybean

Treatments	N uptake (kg ha^{-1}) by stover	N uptake (kg ha^{-1}) by grain	Total N uptake (kg ha^{-1})
T_0	105.75d	42.01d	147.76e
T_1	177.01c	60.85c	237.85d
T_2	175.43c	62.87c	238.30d
T_3	189.58b	65.21c	254.79c
T_4	196.21b	82.00b	278.21b
T_5	198.31b	77.49b	275.80b
T_6	229.68a	88.57a	318.26a
SE(±)	3.67	1.58	4.86
Level of significance	**	**	**
CV (%)	3.50	4.01	3.37

** = Significant at 1% level of probability, Values in a column having same letter (s) do not differ significantly at 1% level by DMRT.

Total uptake of phosphorus: All the treatments caused significantly higher P uptake by soybean plants over control (Table 4). The total P uptake by soybean ranged from 24.37 to 45.76 kg ha^{-1} and the treatment T_6 (100% RFD+*Bradyrhizobium*) recorded the highest P value which was statistically similar to that of the treatments T_5 and T_4. Again, the treatments T_1 and T_2 also recorded statistically similar results. The effect of th *Bradyrhizobium* inoculation on the total P uptake of soybean under different P levels was significant.

Table 4. Effect of *Bradyrhizobium* under different P levels on P uptake of soybean

Treatments	P uptake (kg ha^{-1}) by straw	P uptake (kg ha^{-1}) by grain	Total p uptak (kg ha^{-1})
T_0	17.62d	6.75e	24.37d
T_1	20.58c	8.77d	29.36c
T_2	21.58c	9.38cd	30.96c
T_3	26.09b	9.96c	36.06b
T_4	30.42a	13.62ab	44.05a
T_5	31.21a	13.15b	44.35a
T_6	31.34a	14.43a	45.76a
SE(±)	0.626	0.357	0.709
Level of significance	**	**	**
CV (%)	4.25	5.69	3.38

** = Significant at 1% level of probability; Values in a column having same letter (s) do not differ significantly at 1% level by DMRT.

Effect of *Bradyrhizobium* under different P levels on the yield and yield contributing characters of soybean

Weight of pods plant^{-1}: A significant variation in pod weight of soybean was observed due to inoculation of soybean with *Bradyrhizobium* under different levels of P ((Table 5). The fresh and dry weight of soybean pods due to different treatments ranged from 5.190 to 6.597g and 4.327 to 5.320g, respectively**.** The treatment T_6 (100%RFD+*Bradyrhizobium*) produced the highest pods plant^{-1}. All the treatments produced higher number of pods plant^{-1} over control. All the treatments except control were statistically effective in producing the soybean pods. The combined application of *Bradyrhizobium* and chemical fertilizers was found more effective in increasing the weight of pods plant^{-1} of soybean.

Table 5. Response of soybean to *rhizobium* biofertilizer and fertilizers on the yield and yield contributing characters of soybean**.**

Treatments	Fresh wt (g) of pod plant[1]	Dry wt (g) of pod plant[1]	Yield of grain (t ha^{-1})	Yield of Stover (t ha^{-1})	Biological yield (t ha^{-1}	Harvest index (%
T_0	5.190b	4.327b	0.7967c	7.867c	8.663d	9.197d
T_1	6.197a	4.913a	1.027b	9.133b	10.16c	10.10bcd
T_2	6.197a	4.943a	1.087b	9.450 b	10.54c	10.31bc
T_3	6.403a	5.077a	1.100b	10.17a	11.27b	9.760cd
T_4	6.443a	5.143a	1.383a	10.38a	11.77ab	11.75a
T_5	6.443a	5.193a	1.310a	10.48a	11.79ab	11.11ab
T_6	6.597a	5.320a	1.450a	10.57a	12.02a	12.06a
SE(±)	0.246	0.145	0.054	0.207	0.216	0.324
LS	*	**	**	**	**	**
CV (%)	6.86	5.07	8.11	3.70	3.44	5.29

**=Significant at 1% level of probability, *=Significant at 5% level of probability, Values in a column having same letter (s) do not differ significantly at 1% level by DMRT.

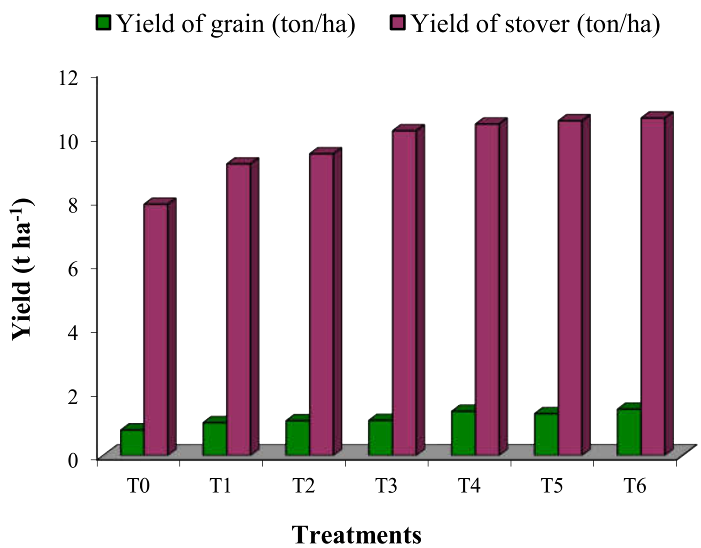

Figure 1. Effect of *Bradyrhizobium* under different P levels on the yield and yield contributing characters of soybean. T_0=Control, T_1=100% N+P, T_2=*Bradyrhizobium,* T_3=*Bradyrhizobium* +75% P, T_4=*Bradyrhizobium* +100% P, T_5= *Bradyrhizobium* +125% P , T_6= *Bradyrhizobium* +100% RFD].

Discussion

The field experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University, Mymensingh during January to May, 2015 to evaluate the response of soybean to *Bradyrhizobium* biofertilizer under different phosphorus levels. The treatments were: T_o=Control, T_1=Recommended dose of N and P (100%), T_2=*Bradyrhizobium* inoculation, T_3=*Bradyrhizobium* inoculation+75% P, T_4= *Bradyrhizobium* inoculation+100% P, T_5= *Bradyrhizobium* inoculation+125% P, T_6= *Bradyrhizobium* inoculation +N+P (100%).

The study reveals that nodule weight, growth parameters, yield, nutrient content and uptake of soybean increased significantly due to inoculation of soybean with *Bradyrhizobium* under different phosphorus levels. Similar results were reported by many workers. *Bradyrhizobium* fix atmospheric N_2 symbiotically with soybean and improves soil environment (Vance, 2001), and maximize the biomass and yield of crops (Kannaiyan, 1999). The soybean nodulating bacteria *Bradyrhizobium japonicum* can fix sufficient atmospheric nitrogen (about 300 kg ha^{-1} yr^{-1}) in symbiosis with soybean (Keyser and Li, 1999). Saha (2007) reported that *Bradyrhizobium* inoculation increased the number of dry weight of nodules of the soybean. Okereke *et al.,* (2000) concluded that inoculation with *Bradyrhizobium* alone significant increased the nitrogen content of nodules. Bhuiya *et al., (*1998) observed that soybean inoculated with *Bradyrhizobium* inoculation increased significantly the percent N of the plant.

Sogut (2006) reported that inoculation with *Bradyrhizobium japonicum* strains produced significantly higher grain yield over control. Sabir *et al.* (2001) reported that the number of pods per plant, seeds per pod, 100 grain weight and seed yield were significantly increased by different phosphorus levels. Shehzadi et al. (2003) who reported that phosphorus and *Rhizobium* inoculation induced a pronounced effect on grain yield.

Saha (2007) conducted an experiment in the net house of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from November 2006 to May 2007 to investigate the effect of four selected rhizobial strains and urea-N on growth and yield of soybean. Rhizobial inoculation significantly increased N_2-fixation, N content and uptake by plant after sowing over un inoculated control.

Bhuiyan *et al.* (2007b) carried out a field experiment with five mungbean varieties with or without *Bradyrhizobium* inoculation at BAU Farm to observe shoot dry matter production and nitrogen uptake by mungbean at different growth stages. Significant influences of the mungbean varieties were observed on dry matter production and nitrogen uptake. *Bradyrhizobium* inoculant significantly increased dry matter production of mungbean varieties.

Soomro *et al.* (2005) carried out an experiment in Tandojam, Pakistan to investigate the effect of *Bradyrhizobium japonicum* inoculation on nodulation, dry matter production, N content and seed yield of soybean. They observed that inoculated plants recorded higher N_2-fixation and N content compared to the uninoculated plants. Chang *et al.* (2005) conducted an experiment in Tandojam, Pakistan in 2004 to know the effect of *Bradyrhizobium japonicum* inoculation on N accumulation in soybean at various parts (roots, nodules, stems, pods, leaves and seeds) and growth stages (budding, flowering, podding and maturity). Uninoculated plants served as the control. A higher nodulation, N content and N_2-fixation was recorded in inoculated plants compared to uninoculated ones.

Singh (2005) conducted an experiment on inoculation of soybean seeds with *Bradyrhizobium japonicum* culture and observed significantly taller plants with more nodules, pods $plant^{-1}$, grains pod^{-1} and seed weight than untreated seeds. Egamberdiyeva *et al.* (2004) carried out a field experiment to see the effect of inoculation with *Bradyrhizobium japonicum* S 2492 on soybean [*Glycine max (L.) Merr*.] growth, nodulation and yield in nitrogen deficient soil of Uzbekistan. The results revealed positive effects on growth, nodule number and yields of soybean after inoculation with *Bradyrhizobium japonicum* S 2492.

Payakapong *et al.* (2004) studied the response of five Thai soybean cultivars to four *Bradyrhizobium japonicum* strains THA 5, THA 6, USDA 110 and

SEMIA 5019 and reported that nodule occupancies by an individual strain were directly correlated with the proportions of that strain in the inoculum mixtures. The strain USDA 110 showed higher nodulation competitiveness than the other strains on three of the five cultivars. The Thai strain THA 6 appeared to be more competitive than USDA 110 on cultivar SJ 5.

E1-Dsouky *et al.,* (2003) conducted two field experiments in Aswam, Egypt during 1999 and 2000 to assess the effect of single or mixed inoculations with *Bradyrhizobium japonicum* alone or with selected rhizobacterial strains on field grown soybean (cv. Clark) and the effect of inoculants application on the nodulation, growth and yield of soybean. Results showed that nodulation and vegetative growth were greatly promoted by inoculation with *Bradyrhizobium japonicum* alone compared to uninoculated control.

Kalhapure *et al.,* (2003) conducted a pot experiment on soybean cultivars MACS-13, MACS-57, MACS-124, MACS-390 and Monetta to investigate the effect of *Bradyrhizobium japonicum* Rh_4-Digraj, Rh_5-Rahuri and Rh_8-MPKV. *Bradyrhizobium japonicum* inoculation increased the seed germination, nodulation, growth and yield of the cultivars compared to the uninoculated control.

Praharaj and Dhingra (2003) carried out a field experiment during 1992 and 1993 in Ludhiana, Punjab, India, to study the effect of *Bradyrhizobium* inoculation, N and K rates on the productivity and profitability of soybean (cv. PK 416). The treatments comprised with or without *Bradyrhizobium* inoculation; N @ 30, 60 and 90 kg ha^{-1} and K @ 0, 25 and 50 kg ha^{-1}. The highest leaf area index, number of nodules $plant^{-1}$, nodule dry weight and nitrogenase activity were recorded with *Bradyrhizobium* inoculation. Wang and Heggo (2003) conducted an experiment on soybean and found higher nitrous reductase activity as well as higher number of nodulation in *Bradyrhizobium* inoculated plant than uninoculated plant.

Raverkar and Tilak (2002) carried out a greenhouse pot experiment consisting of two cultivars (Kalitur and Lee), four different strains of *Bradyrhizobium japonicum* (SB 103, SB 113, SB 117 and SB 119) and an uninoculated control using unsterilized sandy loam soil. In both cultivars Vesicular Arbuscular Mycorrhiza (VAM) colonization by indigenous propagules improved significantly due to inoculation with various bradyrhizobial strains. Significant enhancement in synthesis of number of nodules, nodule dry biomass and nitrogenase activity was recorded due to bradyrhizobial inoculation. Kumar *et al.* (2001) conducted an experiment on inoculated soybean with *Bradyrhizobium* CP-3 and *Azotobacter* Mac-21 in nutrient deficient sterilized soil using earthen pots. Plants inoculated with *Bradyrhizobium* plus *Azotobacter* exhibited the highest nodule number and dry weight.

Okereke *et al.* (2000) carried out two field experiments at Akwa, Nigeria, to assess the competitiveness of foreign *Bradyrhizobium* in influencing the promiscuous soybean cultivar (TGX 536-021). Seeds were inoculated with antibiotic mutants of the bradyrhizobial strains before sowing after land preparation. Nodule number significantly increased and showed great variability in 84 days after sowing, probably due to differences in the ability of inoculant bradyrhizobia to form nodules with the soybean cultivars TGX 536-021. Islam *et al*. (1999) conducted an experiment to study the performance of some *Bradyrhizobium* inoculants on soybean at BINA experimental farm, Mymensingh. They found the lowest nodule number in uninoculated and the highest in inoculated treatments; all the *Bradyrhizobium* inoculated treatments performed better in nodulation of soybean.

Sobaran *et al*. (1999) carried out a field experiment in Uttar Pradesh, India, with one soybean variety and two levels of N (0 and 15 kg ha^{-1}), three levels of P (0, 30 and 90 kg P_2O_5 ha^{-1}), two levels of K (0 and 30 kg K_2O ha^{-1}) and uniform inoculation with *Bradyrhizobium japonicum* multi strain culture (USDA

110 + TAL-377). The results showed that application of 30 kg phosphorus or N X K interaction significantly increased the number of nodules at 60 days (flowering stage) of plant growth.

Taiwo et al. (1999) carried out an experiment during 1995-96 in Iberian, Nigeria, with soybean cv. TGX 1740-2F and 1448-2E. Seeds were inoculated with *Bradyrhizobium* strains IRJ 284 A or keptun inoculated and given 0 or 40 kg P ha$^{-1.}$Inoculation along with P fertilizer lead to increase in nodulation in both the cultivars. Bhuiya *et al.,* (1998) conducted a field experiment at Regional Agricultural Research Station, Ishwardi, Pabna to evaluate the effect of seed inoculation with 4 *Bradyrhizobium strains on* nodulation in soybean. They found that *Bradyrhizobium* inoculation significantly increased nodule number than uninoculated control.

Dashti *et al*. (1998) carried out a field experiment to evaluate the effect of seed inoculation with 2 plant growth-promoting rhizobacteria (PGPR) strains (*Serratia liquefaciens* 2-68 or *Serratia proteamaculan* 51-102) on the nodulation of soybeans. Inoculation of soybean with *Bradyrhizobium japonicum* and PGPR increased soybean nodulation. Mitra *et al*. (1998) conducted a pot experiment where inoculation of soybean seed or soil with *Bradyrhizobium japonicum* along with 0-30 kg N ha^{-1} was done. The soils in the pots were further inoculated 15 days after emergence. Seed or soil inoculation increased root nodulation and biomass yield. Seed yield was the highest in soil inoculated pots having 30 kg N ha^{-1}.

Dubey *et al.* (1997) reported that seed inoculation with different strains of *Bradyrhizobium japonicum* can enhance symbiotic traits and yield attributes. Compared with uninoculated control he found significantly increased nodulation in inoculated plots.Uslu *et al*. (1997) conducted a field trial where soybeans were inoculated with *Bradyrhizobium japonicum* or not inoculated**.** They found that nodules plant^{-1} was increased by inoculation**.** Thanausont and Vilhay (1996) observed in a pot experiment that *Rhizobium* inoculum increased nodulation and N_2-fixation of soybean**.**

Okereke *et al.* (2000) conducted two field experiments at Akwa, Nigeria. Soybean seeds were inoculated with antibiotic mutants of the bradyrhizobial strains before sowing. *Bradyrhizobium* strains increased N content, N_2-fixation and total N uptake of plants. Barakah (1999) carried out a green house experiment in calcareous loamy soil of Saudi Arabia to evaluate the response of bradyrhizobial strains on the nodulation and N_2-fixation by soybean cultivars Cabrillo and Bedford. Inoculation with different bradyrhizobial strains enhanced nodule formation, symbiotic N_2-fixation, dry matter content and nitrogen content of plants.

Taiwo *et al.* (1999) conducted a field experiment in Ibadan, Nigeria. The seeds of soybean cultivars cv. TGX 1740 and TGX 1448-2E were inoculated with *Bradyrhizobium* strain IRJ-284A or not inoculated. Inoculated treatments led to increase N content in plant tissue when compared with the control. Dashti *et al.* (1998) carried out a field experiment to evaluate the effect of seed inoculation with 2 plant growth-promoting rhizobacterial (PGPR) strains (*Serralia liquefaciens* 2-68 of *Serratia proteamaculans* 1-102) on the nodulation, nitrogen fixation and total yield of soybean. They observed that total plant N and protein yield were increased due to inoculation of *Bradyrhizobium japonicum* with PGPR. Thanausont and Vilhay (1996) conducted a pot experiment and observed that the *Bradyrhizobium* inoculation increaed N_2-fixation of soybean.

Conclusion

A field experiment was conducted at the Soil Science Field Laboratory of Bangladesh Agricultural University (BAU), Mymensingh during January to May 2015 to study the response of soybean to *Bradyrhizobium* biofertilizer under different levels of phosphorus. The soil belongs to Sonatala soil series of AEZ 9 (Old Brahmaputra Floodplain). The treatments of the experiment were: T_o = Control, T_1 = Recommended dose of N and P (100%), T_2= *Bradyrhizobium* inoculation, T_3= *Bradyrhizobium* inoculation +75% P, T_4= *Bradyrhizobium* inoculation + 100% P, T_5 = *Bradyrhizobium* inoculation +125% P, T_6 = *Bradyrhizobium* inoculation + N + P (100%). The study reveals that yield and nutrient content and uptake of soybean increased significantly due to inoculation of soybean with *Bradyrhizobium* under different phosphorus levels. Soybean inoculated with *Bradyrhizobium* in combination with recommended dose of N, P, K, and S fertilizers recorded the highest grain and straw yields. It may be concluded that **s**oybean cultivation can effectively be done in the Old Brahmaputra Floodplain soils by inoculation with *Bradyrhizobium* in combination with recommended dose of N, P, K and S fertilizers

References

BARI (Bangladesh Agricultural Research Institute) (2006). Krishi Projukti Hatboi (in Bangla). 4th edn,Banglaesh Agricultural Ressearch Institute Gazipur, Bangladesh 209-211.

Bhuiyan MAH, Mian MH, Islam MS (2007b). Effect of *Bradyrhizobium* inoculation on dry matter production and nitrogen uptake in mungbean. *Bangladesh Journal of Environmental Science* 3(2): 7-16

Berg RK, Lynd JQ (1985). Soil fertility effects on growth, yield, nodulation and nitrogenase activity of Australian winter pea. *Journal of Plant Nutrition* 8: 131-145.

Bums RC, Hardy RWF (2004). Nitrogen Fixation in Bacteria and Higher plants. Springer Verlay, Berlin, Germany.

Danso et al., (1987). S.K.A. Danso, C. Hera and C. Douka, Nitrogen fixation in soybean as influenced by cultivar and *Rhizobium* strain, *Plant Soil* 99 (1987), pp. 163–174.

Dashti N, Zhang F, Hynes T, Smith DL (1998). Plant growth promoting Rhizobacteria accelerate and increase nitrogen fixation activity by field grown soybean (*Glycine max*) under short season conditions. Plant Soil 2000(2): 205- 213.

Dubey SK, Siddique SA, Shukla NP, Hasija SK (1997). Application of Rhizobium japonicum as biofertilizer for soybean (*Glycine max* L.) crop. *Advanced Agricultural Research,* India 4: 29-53.

El-Dsouky MM, Badawy FH ,Sadiek HS (2003). Response of soybean to mixed inoculatin with *Bradyrhizobium*and rhizobacteria. *Assiut Journal of agricultural science* 34 (5): 287-300.

Gowda CLL, Kaul AK (1982). Pulses in Bangladesh. BARI and FAO Publication, Gazipur, Bangladesh 338-407.

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002). Role of soil microorganisms in improving P nutritionof plants. *Plant and Soil* 245: 83-93.

Hardarson G, Zapata F, Danso SKA (1984). Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean *(Glyine mm (L.) Merr)* cultivars. *Plant and soil* 82: *397-405.*

Harold H, Keyser, Li F (1992). Potential for increasing biological nitrogen fixation in soybean*. Plant and Soil* 141: 119-135.

Hoque MS, Jahiruddin M (1988). Response of soybean to *Rhizobium* inoculation and NPK fertilization. *Crop Research* (Hisar) 1 (1): 102-108.

Hoque MS, Solaiman ARM, Bhuiya ZH (1982). Effectiveness of locally prepared and imported inoculants of soybean in Bangladesh. *Thailand journal of Agriculture Science* 15 1270-1334**.**

Hoque MZ (1976). Soybean cultivation in Bangladesh: crop Management and Improvement. Paper presented at the First Natiional Workshop on Oilseeds and Pulses. 11-13 October, 1976, Dhaka

Kannaiyan S (1999). Bioresources Technology for Sustainable Agriculture. Associated Publishing Company, New Delhi. P. 422.

Keyser HH and Li F (1992). Potential for increasing biological nitrogen fixation in soybean. *Plant Soil* 141: 119-135.

Okereke GU, Onochie CC, Onokwo AU, Onyeagba E and Elejindu GO (2000). Response of introduced Bradyrhizobium strains infecting a promiscuous soybean cultivar. *World Journal of Microbiology of Biological* 16(1): 43-48.

Praharaj CS, Dhingra KK (2003). Productivity and profitability of soybean (*Glycine max* (L.) Merr) as influenced by *Bradyrhizobium* inoculation, nitrogen and potassium levels. *Journal of Research Birsa Agricultural University*

Sara S, Morad M, Reza CM (2013). Effects of seed inoculation by *Rhizobium* strains on Chlorophyll content and protein percentage in common bean cultivars (*Phaseolus vulgaris L*.). *International Journal of Biosciences* 3(3): 1-8.

Saha BK (2007). Nodulation yield biochemical constituents and N uptake by lentil and soybean as affected by different Rhizobial strains. M. S. Thesis, Department of Agricultural Chemistry, Bangladesh agricultural university, Mymensingh.

Singh D (2005). Effect of potassium, zinc and sulphur on growth characters, yield attributes and yield of soybean *(Glycine max* 14.). *Indian Journal of Agronomy* 4(3): 132-136.

Sobaran S, GrangwarMS, Singh HP, Singh S (1999). Nodulation and biomass production in soybean as influenced by the application of nutrients. *Journal of Potassium Research* 15 (1-4): 127-130.

Soomro FM, Sheikh SA, Jamro GH, Leghari MH (2005). Response of soyb ean to inoculation of *Rhizobium japonicum. Indus Journal of Plant Science* 4 (1): 100-101.

Shahid MQ, Saleem MF, Khan HZ, Anjum SA (2009). Performance of soybean (*Glycine max* L.) under different phosphorus levels and inoculation. *Pakistan Journal of Agricultural Sciences* 46(4): 237-241.

Solaiman ARM, Hoque MS (1983). Effect of some locally prepared *Rhizobium* inoculation on nodulation and dry matter yield of soybean. *Bangladesh journal of Agriculture* 8: 57-65.

Somasegaran P, Abaidoo RC, Kumaga F (1990). Host *Bradyrhizobium* relationship land nitrogen fixation in the Barnbarra groundnut [*Voandzeia subterranean* (L.) Thouarsnon, Cons.]. *Tropical agriculture Trinidad* 67: 137-142.

Taiwo LB, Nworgu FC, Adatayo OB (1999). Effect of *Bradyrhizobium* inoculation and phosphorus fertilization on growth, nitrogen fixation and yield of promiscuously nodulation soybean [*Glycine max* (L.) Merr.] in a tropical soil. *Crop Research Hisar* 18 (2): 169-177.

USFDA(United State Food and Drug Administration) (2000). Report and recommendations on organic farming. Prepared: USDA study team on organic farming. United States Department of Agriculture. 94p.

Vance CP (2001). Symbiotic nitrogen fixation and phosphorus acquistion, Plant nutrition in a world of decling renewable resources. P1. *Physiology* 127: 390-397

Vessey JK (2004). Benefits of inoculating legume crops with rhizobia in the northern Great Plains. *Online Crop Management doi* 10: 1094/CM-2004-0301-04 RV.