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Nodulation, yield and quality of soybean as influenced by integrated nutrient management

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Abstract: A field experiment was conducted at the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh to study the integrated use of phosphate solubilizing bacteria (PSB), *Bradyrhizobium* and P on nodulation and sustainable soybean production. Significant differences among the different treatment combinations in terms of yield and yield contributing characters were observed. Integrated nutrient management with application of 60% of the recommended dose of phosphorus (RP), phosphate solubilizing bacteria (PSB) and biofertilizer (*Bradyrhizobium*) has significantly increased plant height, number of nodule per plant, nodule dry weight per plant, pods per plant, grains per pod, grain yield, oil and protein contents. Coinoculation of *Bradyrhizobium* seemed to help reduce the P requirement in soybean cultivation. Overall results indicate that the application of integrated nutrient management of biofertilizer (*Bradyrhizobium*) with recommended dose of P would produce the best quality of soybean with higher nodulation and yield.

Key words: Nodulation, Integrated nutrient management, Inoculation.

Introduction

Soybean (*Glycine max* L. Merril) is a very important recognized oil seed and protein crop in the world. It is a good source of protein, unsaturated fatty acids, minerals like Ca and P including vitamins A, B and D that meet different nutritional needs (Rahman, 1982).The seed contains about 40-45% protein, 18-20% edible oil and 20-26% carbohydrate (Gowda and Kaul, 1982).The multipurpose use of soybean is gradually increasing day by day in our country.

Although soybean cultivation in Bangladesh is quite limited, there is an ample scope of increasing its cultivation through use of integrated nutrient management. In Bangladesh, about five 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). Like other legume crops, it has the capacity to fix atmospheric nitrogen through root nodule bacteria. *Bradyrhizobium* can fix atmospheric nitrogen (about 300 kg^{-1} ha^{-1} Yr^{-1}) in symbiosis with soybean (Keyser, 1992).Thus it helps to increase the soil fertility and economic crop production not only for itself but also for the next cereal or non-legume crops grown in rotation thereby reducing the requirement of external use of nitrogen fertilization. Therefore, the present piece of research work was undertaken to study the effect of integrated nutrient management on the nodulation, growth, yield and yield contributing characters of soybean.

Materials and Methods

The experiment was conducted at the net-house of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh. The initial soil sample was collected from 0-15 cm depth from 10 different locations in the field with the help of auger. The composite soil sample was air dried, ground to pass through a 10 mesh (2 mm) sieve and stored in polythene bag for mechanical and chemical analyses. The experimental plot was properly prepared through several ploughing and cross ploughing to obtain good tilth. All weeds and stubbles were removed from the field and bigger clods were broken by laddering and mallet.

Treatments and replication of the experiment: The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The variety of the crop was Shohag (PB-1) recommended by the National Seed Board (NSB) of Bangladesh for cultivation. The experimental area was divided into eight unit plots with raised bands as per treatments. Thus the total number of unit plots was 24. Treatments were randomly distributed within the blocks as follows: T_0 - Control (No fertilizer and inoculants) , T_1 - Phosphate solubilizing bacteria (PSB), T_2 - *Bradyrhizobium* , T_3 - PSB + *Bradyrhizobium*, T_4 - 100% recommended phosphorus (RP), T_5 - *Bradyrhizobium* + 100% RP, T_6 -60% RP, T_7 - 60% RP +PSB and T_8 - 60% RP + PSB + *Bradyrhizobium*, respectively.

Inoculation and sowing of seeds

At first inoculated seeds were sown according to the experimental design for treatments T_0, T_4 and T_6. Prior to this, seeds were taken in small polythene bags equal in weight for each pot and mixed with sugarcane molasses (@ 30g molasses kg^{-1} seeds). Then the inoculum (*Bradyrhizobium* sp.) was mixed with the seeds @ 50 g inoculum kg^{-1}seeds for the treatments T_2, T_3, T_5 and T_8. Seeds were mixed thoroughly with PSB broth for treatments T_3 and T_8. Then *Bradhyrhizobium* inoculum was mixed thoroughly with these seeds. Only PSB broth was mixed thoroughly with the seeds for the treatments T_1 and T_7. Ten seeds were sown in each pot. For each inoculation, separate plastic bag was used and care was taken to avoid contamination of the inoculated and uninoculated seeds. Seeds were sown at 3-4 cm depth of soil. Row to row distance was 30 cm and seed to seed distance within a furrow was 5 cm. Irrigation and weeding was done as and when necessary during the period of experimentation.

Collection of data

Plant height: The heights of randomly selected three plants from each plot were recorded in centimeter from the base of the plant to the tip of the tallest leaf and expressed as mean values. The plant heights were measured at an interval of 20 days after sowing (DAS), e.g., 45 DAS, 70 DAS, 95 DAS and 115 DAS afterwards with an interval of 25/20 days.

Nodule number and nodule dry weight per plant: One plant was randomly selected from each plot and was carefully uprooted to study the nodule number and nodule dry weight. Soil from the root was removed carefully and the nodules were separated and counted and sun dried for two days. Sun dried samples were then put in a paper packet and oven dried for 72 hours at 65-70^{o}C. After oven drying, nodules were weighed in an electric balance. The nodule number and nodule dry weight plant were measured at 20 DAS, 45 DAS, 70 DAS, 95 DAS and 115 DAS afterwards with an interval of 25/20 days.

Harvesting and recording of plant data: Three plants from each plot were harvested, threshed, cleaned and processed for analysis. All pods were collected, counted and then averaged to find out pods plant^{-1}. Randomly 10 pods were collected from each plant and number of seeds were counted and then averaged to find out the seeds pod^{-1}. Seeds of each plant were weighed and seed yield was recorded in gram and expressed in seed yield plant^{-1}. By subtracting the seed weight from the weight of whole plants including seeds, the stover weight was obtained.

Seed oil content: The oil content of soybean seed was extracted by Folsch method (Folsch *et al*., 1957) by using chloroform: methanol in 2:1 ratio in a beaker with stirring. The extractant was removed by heating and oil obtained was expressed in percentage.

Protein content: Protein content was computed by multiplying N content in soybean seed determined by microkjeldahl assay by a conventional factor of 6.25 (Jackson, 1973).

Chlorophyll content in leaves: Total chlorophyll content was measured following the method of Arnon (1949). Fresh leaf samples (0.05g), collected at mid flowering stage, were macerated in mortar and pestle with 80% acetone. It was then collected in a test tube and centrifuged at 3000 rpm for 10 minutes. The volume was made to 10 mL with 80% acetone and the absorbance was measured at 663 nm and 645 nm in a spectrophotometer.

Results and Discussion

Plant height: Plant height of soybean (var. PB-1) responded positively due to the integrated use of Phosphate Solubizing Bacteria, *Bradyrhizobium* and P (Table 1). The highest plant height (26.52 cm) was observed at 115 DAS in the treatment T_8 followed by the treatment T_7 (26.47 cm) and the lowest plant height (21.10 cm) was recorded in the control treatment. But the treatment T_8 and T_5 gave statistically identical results in respect of plant height. The result revealed that biofertilizer alone or in combination with 12 mg P kg^{-1} soil (60% RP) enhanced the crop growth which was identical with 20 mg P kg^{-1} soil (100% RP). This study is similar to the study of Jain and Trivedi (2005) and Soomro *et al.* (2005). They observed that Phosphate Solubilizing Bacteria and *Bradyrhizobium* inoculated soybean plants with low level of P gave highest plant height over uninoculated plant.

Table 1. Integrated use of Phosphate Solubilizing Bacteria, Bradyrhizobium and P on plant height at different DAS of soybean (var. PB-1)

Treatments	Plant height (cm)				
	20DAS	45DAS	70DAS	95DAS	115DAS
T_0	4.97f	8.67e	14.02d	21.02e	21.10e
T_1	6.15cd	10. 50cd	16.17bc	23.92bcd	24.07bcd
T_2	5.67de	10.47cd	16.20bc	23.45cd	23.57cd
T_3	6.30bc	11.35bc	16.20bc	24.75abc	25.52abc
T_4	6.80ab	11.50bc	16.52ab	25.77ab	25.90ab
T_5	6.97a	12.15abc	17.35ab	26.17a	26.23a
T_6	5.37ef	9.47de	14.52d	22.37de	22.50de
T_7	7.05a	12.45ab	17.75a	26.40a	26.47a
T_8	7.40a	13.80a	17.82a	26.40a	26.52a
CV (%)	6.34	9.85	5.26	5.72	5.2

CV - Coefficient of variation, T0 - Control (No fertilizer and inoculants) , T1 - Phosphate solubilizing bacteria (PSB), T2 - Bradyrhizobium , T3 - PSB + Bradyrhizobium, T4 - 100% recommended phosphorus (RP), T5 - Bradyrhizobium + 100% RP, T6 -60% RP, T7 - 60% RP +PSB , T8 - 60% RP + PSB + Bradyrhizobium

Number of nodules per plant: The inoculation of Phosphate Solubizing Bacteria, *Bradyrhizobium* and P had a significant effect on total numbers of nodules plant^{-1} at different days after sowing (Table 2). Overall results of nodule number plant^{-1} indicated that the highest nodule number plant^{-1} was observed in the treatment T_8 (41.25) at 95 DAS followed by the treatment T_3 (32.00). The results showed that integrated use of Phosphate Solubizing Bacteria, *Bradyrhizobium* and 60% P fertilizer substantially increased the nodule number plant^{-1}. So, Phosphate Solubizing Bacteria and *Bradyrhizobium* with reduced level of P had tremendous effect on nodulation of soybean crop. The nodule number plant^{-1} was gradually decreased after 95 DAS until 115 DAS in all the treatments. The lowest nodules plant^{-1} was recorded in 20 DAS in all the treatments. The reason of the maximum number of nodules plant^{-1} in inoculated plants was probably due to the fact that *Bradyrhizobium* helps in nodulation, while Phosphate Solubizing Bacteria increased the availability of P to the plants and helps in root elongation. This result was supported by Tomar *et al*. (2004) who observed that significantly lowest number of nodules produced in uninoculated treatments than the inoculated treatments.

Dry weight of nodule per plant: The effect of integrated use of Phosphate Solubizing Bacteria, *Bradyrhizobium* and P on dry weight of nodule plant^{-1} was significantly influenced at different days after sowing (Table 3). The highest dry weight of nodule plant^{-1} (174.35 mg) was observed at 95 DAS in the treatment T_8 followed by the treatment T_3 (170.68 mg). But the treatment T_8 was statistically identical with the treatment T_3 (170.68 mg). The uninoculated control treatment (T_0) produced the lowest dry weight of nodules plant^{-1} during the growing period of soybean. Coinoculation of Phosphate Solubizing Bacteria and *Bradyrhizobium* with 60% RP significantly produced higher nodule dry weight compared to application of 100% RP fertilizer or 60% RP fertilizer with Phosphate Solubizing Bacteria. This study was similar to the study of Balamurugan and Gunasekaran (1996). They observed that soybean inoculated with Phosphate Solubizing Bacteria and *Bradyrhizobium* with lower level of P increased nodule dry weight of soybean crop.

Yield and yield contributing characters: The effect of integrated use of Phosphate Solubilizing Bacteria, *Bradyrhizobium* and P on pods plant^{-1} was significantly varied (Table 4). The application of Phosphate Solubilizing Bacteria and *Bradyrhizobium* inoculants significantly increased the number of pods plant^{-1}. The maximum pods plant^{-1} (32.00) were recorded in the treatment T_8 followed by the treatment T_7 (27.75) and the minimum pods plant^{-1} (18.50) were in the treatment T_0. This study was similar to the study of Tomar *et al*. (2006) and Egamberdiyeva *et al*. (2004). They observed that soybean plants treated with *Bradyrhizobium*, Phosphate Solubilizing Bacteria and low level of P gave the highest number of pods plant^{-1}.

Number of grains pod^{-1} was significantly influenced by the integrated use of Phosphate Solubilizing Bacteria and *Bradyrhizobium* inoculants and P (Table 4). The treatments T_8, T_7, T_4, T_3, T_2 and T_1 (3.00) produced the highest number of grains pod^{-1} which was statistically similar also. The uninoculated T_0 (2.25) produced the lowest number of grains pod^{-1}. The application of inoculants (*Bradyrhizobium* + PSB) with 60% recommended P increased the number of grains pod^{-1} which was equivalent to higher level of recommended P (100% RP). This study was similar to the study of Jain and Trivedi (2005). They observed that the soybean plants treated with *Bradyrhizobium* and Phosphate Solubilizing Bacteria alone or in combination with different levels of P gave highest number of grains pod^{-1}.

Table 2. Integrated use of Phosphate Solubilizing Bacteria, Bradyrhizobium and P on number of nodule plant-1 at different DAS of soybean (var. PB-1)

Treatments	Nodule plant-1				
	20DAS	45DAS	70DAS	95DAS	115DAS
T_0	0.00f	4.00f	5.00f	7.50f	9.25d
T_1	0.25f	14.00d	15.25cd	19.50de	11.00bcd
T_2	0.00f	6.50e	10.75e	18.25e	11.00bcd
T_3	5.00b	17.25a	21.75b	32.00b	11.00bcd
T_4	1.75e	15.00d	16.25cd	20.75de	11.0cd
T_5	3.50c	15.75bc	17.75c	26.25c	12.75b
T_6	0.00f	4.00f	5.50f	8.75f	10.00cd
T_7	3.00d	15.50cd	16.25cd	22.50d	12.50b
T_8	7.50a	24.25a	39.50a	41.25a	15.25a
CV (%)	6.34	9.85	5.26	5.72	5.2

CV - Coefficient of variation, T0 - Control (No fertilizer and inoculants) , T1 - Phosphate solubilizing bacteria (PSB), T2 - Bradyrhizobium , T3 - PSB + Bradyrhizobium, T4 - 100% recommended phosphorus (RP), T5 - Bradyrhizobium + 100% RP, T6 -60% RP, T7 - 60% RP +PSB , T8 - 60% RP + PSB + Bradyrhizobium

Grain yield plant^{-1} was significantly influenced by the integrated use of biofertilizers (Phosphate Solubilizing Bacteria and *Bradyrhizobium*) with or without P and the results have been presented in Table 4. The grain yield plant^{-1} of soybean varied from 4.79 to 9.68 g due to different treatments. The maximum grain yield plant^{-1} (9.68 g) was observed by the application of Phosphate Solubilizing Bacteria and *Bradyrhizobium* with 60% recommended phosphate (T_8). The second highest grain yield plant^{-1} (8.70 g) was recorded in the treatment T_7. The lowest grain yield plant^{-1} (4.79 g) was recorded in the uninoculated pot. It is clearly indicated that the coinoculation of biofertilizer (Phosphate Solubilizing Bacteria + *Bradyrhizobium*) with low level of P showed better performance in grain yield of soybean. This study was similar to the study of Jain and Trivedi (2005). They observed that the soybean plants treated with *Bradyrhizobium* sp. and Phosphate Solubilizing Bacteria alone or in combination with low level of P gave the highest grain yield plant^{-1}.

Influence of integrated use of Phosphate Solubilizing Bacteria, *Bradyrhizobium* and P on stover yield of soybean was also significant (Table 4). Similar to the grain yield, all the treatment gave significantly higher stover yield over the control. The stover yield due to different treatments ranged from 9.54 to 19.37 g plant^{-1}. The highest stover yield plant^{-1} (19.37 g) was obtained in treatment T_8. The second highest stover yield plant^{-1} (17.41 g) was observed in the treatment T_7. This study was supported the findings of Jain and Trivedi (2005). They observed that the soybean plants treated with Phosphate Solubilizing Bacteria and *Bradyrhizobium* alone or in combination with low level of P gave the highest stover yield plant^{-1}.

Table 3. Integrated use of Phosphate Solubilizing Bacteria, Bradyrhizobium and P on dry weight of nodule plant-1 (mg) at different DAS of soybean (var. PB-1)

Treatments	Dry weight of nodule plant-1 (mg)				
	20DAS	45DAS	70DAS	95DAS	115DAS
T_0	0.00f	5.80f	13.07e	19.12e	11.62e
T_1	0.05f	18.22d	40.42d	46.46cd	14.70 d
T_2	0.00f	10.67e	39.92d	33.63de	12.85de
T_3	2.25b	36.72b	126.32a	170.68a	31.67a
T_4	1.25d	19.07d	54.40c	54.20c	18.57c
T_5	0.72e	18.67d	52.37c	52.88c	17.40c
T_6	0.00f	9.67e	35.72d	31.34de	12.07de
T_7	1.47c	30.42c	86.60b	166.89b	21.50b
T_8	3.27a	43.87a	130.57a	174.35a	30.62a
CV (%)	12.94	9.89	12.26	10.96	9.52

CV - Coefficient of variation, T0 - Control (No fertilizer and inoculants) , T1 - Phosphate solubilizing bacteria (PSB), T2 - Bradyrhizobium , T3 - PSB + Bradyrhizobium, T4 - 100% recommended phosphorus (RP), T5 - Bradyrhizobium + 100% RP, T6 -60% RP, T7 - 60% RP +PSB , T8 - 60% RP + PSB + Bradyrhizobium

Table 4. The effect of Phosphate Solubilizing Bacteria, Bradyrhizobium and P alone and in combination on yield and yield contributing characters of soybean (var. PB-1) at harvest

Treatments	Pods plants^{-1}	Grains pod^{-1}	Total grains plants^{-1}	Grain yield plants^{-1} (g)	Stover yield plant^{-1} (g)
T_0	18.50e	2.25c	41.00d	4.79g	9.54g
T_1	22.75d	3.00a	68.25c	6.18ef	12.37ef
T_2	23.50d	3.00a	70.50c	6.70de	13.39de
T_3	23.75cd	3.00a	71.25c	6.97cde	13.98cde
T_4	24.75cd	3.00a	74.25bc	5.64fg	11.29fg
T_5	24.75cd	2.75ab	70.50c	7.66c	15.48c
T_6	26.00bc	2.50bc	64.75c	7.00cd	14.80cd
T_7	27.75b	3.00a	83.25b	8.70b	17.41b
T_8	32.00a	3.00a	96.00a	9.68a	19.37a
CV (%)	6.26	11.14	11.19	9.29	9.28

CV - Coefficient of variation, T0 - Control (No fertilizer and inoculants) , T1 - Phosphate solubilizing bacteria (PSB), T2 - Bradyrhizobium , T3 - PSB + Bradyrhizobium, T4 - 100% recommended phosphorus (RP), T5 - Bradyrhizobium + 100% RP, T6 -60% RP, T7 - 60% RP +PSB , T8 - 60% RP + PSB + Bradyrhizobium

Chlorophyll content in leaf, oil and protein contents of soybean seeds: Table 5 presents chlorophyll content of leaf and oil and protein content of soybean seeds. The chlorophyll content of soybean leaves and oil content of soybean seeds were not significantly influenced by the application of integrated use of biofertilizer

(*Bradyrhizobium*) and 60% of the recommended dose of phosphorus (RP), but the protein content of soybean seed was significantly influenced by the different treatments. The highest chlorophyll content (2.03 mg g^{-1}), oil content (20.25%) and protein content (38.75%) were obtained in the treatment T_8 and the lowest chlorophyll content (1.50 mg g), oil content (13.88%) and protein content (33.18%) were obtained in control treatment (T_o). Similar results were also found by Singh and Rai (2005) in soybean.

Table 5. Effect of integrated nutrient management on chlorophyll, oil and protein content of soybean

Treatments	Chlorophyll content (mg g^{-1})	Oil content (%)	Protein content (%)
T_0	1.5	13.88	33.18
T_1	1.68	14.2	34.24
T_2	1.94	16.29	34.62
T_3	1.74	15.2	34.9
T_4	1.61	19.18	37.91
T_5	1.7	17.26	35.62
T_6	1.97	18.75	37.7
T_7	1.98	19.25	38.02
T_8	2.03	20.25	38.75
CV (%)	15.57	4.17	9.3

CV - Coefficient of variation, T0 - Control (No fertilizer and inoculants) , T1 - Phosphate solubilizing bacteria (PSB), T2 - Bradyrhizobium , T3 - PSB + Bradyrhizobium, T4 - 100% recommended phosphorus (RP), T5 - Bradyrhizobium + 100% RP, T6 -60% RP, T7 - 60% RP +PSB , T8 - 60% RP + PSB + Bradyrhizobium

The application of integrated nutrient management of biofertilizer (*Bradyrhizobium*) with 60% of the recommended dose of phosphorus (RP) as well as Phosphate solubilizing bacteria (PSB) produced the best quality of soybean with higher nodulation and yield. Biofertilizer *(Bradyrhizobium)* alone or integrated with either organic or inorganic fertilizer can be used to obtain better yield of soybean and sustained soil fertility.

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