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GROWTH, YIELD, AND QUALITY OF THREE SOYBEAN VARIETIES AS INFLUENCED BY RHIZOBIUM INOCULATION IN ACIDIC SOIL OF CHATTOGRAM

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Abstract

A field experiment was conducted to assess the impact of *Rhizobium japonicum* inoculation on the growth, yield, and quality of three soybean (*Glycine max* L.) varieties (BARI 5, BARI 6 and Shohag) in the acidic valley soils of Chattogram. The experimental design followed a randomized complete block design (RCBD) with six treatments (inoculation and uninoculation for each variety), replicated thrice. Growth parameters and yield quantity and quality varied between the varieties, as well as between inoculated and uninoculated plants. Inoculated plants exhibited significantly greater plant height ($p<0.05$) compared to uninoculated plants. The highest number of plants per plot (122±4), maximum plant height (37.8±2.5 cm), and highest number of branches per plant (10.3±1.5) were observed with the BARI 5 variety inoculated with *Rhizobium,* with no significant variations between the varieties. Yield attributes such as number of pods per plant, 100-seed weight, and yield per plot were markedly improved in inoculated treatments, particularly in BARI 5 and Shohag, which showed yield increases of 59.6% and 38.4%, respectively. Seed protein and oil contents also varied among the varieties and between inoculated and uninoculated plants. Inoculated plants exhibited elevated seed protein (up to 38.8% in BARI 6) and oil content (22.6% in BARI 5), along with higher concentrations of reducing and total soluble sugars. Nutrient analysis revealed significantly increased nitrogen, phosphorus, and potassium accumulation in roots, shoots, and seeds under inoculation, especially in BARI 6 and Shohag. The findings suggest that *Rhizobium* inoculation not only improved plant vigor and productivity and also enhanced seed nutritional quality, supporting the viability of expanding soybean cultivation in the previously uncultivated acidic soils in southeastern Bangladesh.

Keywords: Acid soil, Rhizobium, Soybean varieties, Soybean protein

1. Introduction

Soybean (*Glycine max* L. Merr) is highly valued for its substantial protein content, approximately 40% (Li-Juan and Ru-Zhen, 2010). Soybean seeds also contain around 20% oil, 30% soluble and insoluble carbohydrates, 14-24% fat, and are rich in calcium, phosphorus, and vitamins (Rotundo and Westgate, 2009). Notably soybean oil is free from cholesterol.

Soybeans can serve as feed for poultry and fish. Soybean plants are capable of fixing up to 200 kg/ha/yr of atmospheric nitrogen via their nodules, thereby reducing the need for nitrogen application in subsequent crops. Certain strains of *Bradyrhizobium japonicum* effectively fix atmospheric nitrogen and are considered effective bacterial inoculants. In soils where soybeans have not been previously cultivated, compatible populations of bradyrhizobia are rarely present (Abaidoo *et al.,* 2007). Hence, inoculation of soybean seeds with an appropriate inoculant can significantly boost soybean yield.

Rhizobium inoculation can result in the establishment of a substantial rhizobial population in the plant rhizosphere, improved nodulation, and enhanced N_2 fixation, even under unfavorable soil nitrogen conditions (Peoples *et al.,* 1995). In Bangladesh, soybean oil is the most widely used edible oil, but domestic farmers meet only 5% of demand (Hossain, 2021), likely due to a preference for other crops. Despite this, soybeans are highly promising: they offer high protein content (Salam and Kamruzzaman, 2015), require less fertilizer, and thus lower input costs. An evaluation of this crop suitability has not been conducted for the valley soils within the southeastern Brown Hill Soil region which encompasses the Department of Soil Science's crop field at the University of Chittagong. The soils in this region have moderate fertility and are strongly acidic. Since soybeans have not been previously cultivated here, the soils may not contain suitable *Rhizobium* strains. Considering these factors, a field study was undertaken to test soybean cultivation in the valley soils of the southeastern Brown Hill Soil region in Bangladesh. The primary aim of this study was to evaluate the effects of *Rhizobium* inoculation on the growth, yield, and quality of three soybean varieties released by the Bangladesh Agricultural Research Institute (BARI).

2. Materials and Methods

2.1 Collection of seeds and soil sample

Seeds of three soybean varieties, namely BARI 5, BARI 6, and Shohag, along with *Rhizobium japonicum* inoculant, were collected from the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Dhaka. A ***composite*** soil sample was collected from the site of the field experiment conducted in the valley soils of Chattogram.

2.2 Experimental layout and field preparation

The experiment was conducted in the crop field of the Department of Soil Science, University of Chittagong. The experimental design followed a randomized complete block design (RCBD). Six treatments were applied; three varieties of soybean were either treated with or without *Rhizobium japonicum* inoculant. The varieties of soybean were BARI 5, BARI 6 and Shohag. Each treatment consisted of three replications, resulting in eighteen experimental units (6 treatments×3 replications). Three blocks represented the three replications, with each block containing six sub-plots. The size of each sub-plot was 2 m × 2.1 m, separated by an alley of 0.5 m between sub-plots.

The beds were prepared by initially applying 16 kg N/ha, 20 kg P/ha, and 40 kg K/ha to the soil. Urea, triple superphosphate, and muriate of potash were used as sources of N, P, and K, respectively. For inoculation, seeds were soaked in distilled water for 6 h before being thoroughly mixed with *Rhizobium* inoculant in the evening. The inoculant was applied at a rate of 10 g per kg of seeds. Subsequently, the seeds were spread on a newspaper and allowed to air dry for 1 h to ensure the inoculant adhered to their surfaces. Both inoculated and uninoculated seeds were sown in the beds the following morning. Sowing in the morning was chosen to avoid direct sunlight adversely affecting the quality of the inoculant. Sowing was performed on January 3, 2011, with seeds placed in lines 30 cm apart and spaced 5 cm apart within each line. Intercultural operations such as thinning, weeding, irrigation and pest control were performed as needed.

2.3 Growth and yield parameters

Data were collected for various growth and yield parameters. The growth parameters included plant height (cm), number of plants per plot, number of branches per plant, number of leaves per plant, and fresh and dry weights (g) of roots and shoots. The number of plants per plot was counted 15 days after seedling emergence. Plant height and number of leaves per plant were recorded 60 days after sowing (DAS). The number of branches per plant was recorded, and roots and shoots were harvested at 80 DAS. Fresh weights of roots and shoots were measured. The collected root and shoot samples were then dried in an oven at 70 °C for 48 h. After 48 h, the plant samples were removed from the oven, and the dry weights of roots and shoots were recorded. Yield parameters such as number of pods per plant, number of seeds per pod, 100-seed weight (g), and yield per plot (g) were recorded after the crop was harvested.

2.4 Soil analysis

Soil pH was measured using a pH meter with a soil-to-water ratio of 1:2.5. Soil texture was analyzed following the hydrometer method (Day, 1965). Organic carbon content was estimated through the Walkley and Black wet oxidation method (Walkley and Black, 1934). The organic matter content was calculated by multiplying the organic carbon content by the Van Bemmelen factor of 1.724. Total N content was determined using micro-Kjeldahl digestion and distillation techniques. Cation exchange capacity (CEC) was assessed by saturating the soil with 1N NH_4OAc (Jackson, 1973). Available soil phosphorus was extracted using Bray and Kurtz 1 reagent.

2.5 Plant analysis

Different plant parts, including roots, shoots, and seeds, were acid digested using the method, as detailed by Hossain (2016). The digestate was analyzed for phosphorus and potassium contents in these plant parts. For N determination, plant samples were subjected to digestion with H_2SO_4 and 30% H_2O_2. Nitrogen content in the plant samples was determined using alkaline distillation followed by acidimetric titration (Bremmer and Keeney, 1965). Protein content of plant materials was calculated by multiplying the nitrogen value by 6.25. Total soluble sugars were extracted according to the procedure of Cerning and Guilhot (1973). Reducing sugars were estimated using alkaline copper and arsenomolybdate as reagents, following Nelson-Somogyi's modified method (Marais *et al.,* 1966). Non-reducing sugars were calculated as the difference between total soluble sugars and reducing sugars. Oil content was determined by the Folch method (Folch *et al.,* 1957).

2.6 Data analysis

Microsoft Excel and MINITAB (version 19) were used for data analysis. Data are presented as the mean ± SD. One-way ANOVA followed by Fisher's LSD post hoc test in order to determine if there were any significant differences, if any, at $p<0.05$.

3. Results and Discussion

3.1 Growth parameters as affected by inoculation and variety

Different growth parameters of three varieties of soybean grown with or without inoculation are presented in Table 1. The growth parameters were influenced by various treatment combinations. The number of plants per plot recorded at 15 days after sowing (DAS) ranged from 96 to 122. For varieties BARI 5 and BARI 6, significant ($p<0.05$) differences were observed in the number of plants per plot between uninoculated and inoculated treatments, with higher numbers recorded for the inoculated treatments. But for Shohag, no such significant difference was found. The increased number of plants in the inoculated plots might be due to Rhizobium inoculation, which might have enhanced seed germination and seedling establishment (Oad *et al.,* 2004).

Plant height, measured after 60 DAS, ranged from 23.3 to 37.8 cm, with significantly ($p<0.05$) higher values in the inoculated plots for all varieties. In this study, the tallest BARI 5 variety was inoculated with *Rhizobium*. Amin *et al.* (2009) reported similar results with Shohag soybean.

The number of branches per plant was varied between inoculated and inoculated plants; however, the differences between the varieties were not significant ($p>0.05$). The number of leaves per plant varied from 23 to 35, but did not vary significantly between inoculated and uninoculated plants. The dry weight ranged from 1.76 to 2.72 g showing significant variation between inoculation and uninoculation. Inoculum caused a significant ($p<0.05$) increase in root weight only in the BARI 6 variety. The fresh weight of shoots varied from 18.2 to 24.6 g, with the dry weight of shoots ranging between 14.57 and 21.19 g, and inoculated plants exhibited higher values of dry weight per plant. Tien *et al.* (2002) grew three varieties of soybean inoculated with three different strains of Rhizobium and observed shoot dry matter yield per plant ranging from 12.4 to 16.9 g.

Ahmed et al. (2006) observed that *Rhizobium* inoculation enhanced nodulation and growth of green gram or mung bean (*Vigna radiata* L.). Oad *et al.* (2004) documented that *Rhizobium* inoculation led to increased plant height, number of branches per plant, number of nodules per plant, and seed yield.

Table 1. Growth parameters of soybean as affected by different treatments

Treatments	No. of plants/plot	Plant height (cm)	No. of branches/ plant	No. of leaves/ plant	Root dry weight (g/plant)	Shoot dry weight (g/plant)
BARI 5, uninoc	96±1b	25.0 ±1.9d	8.0 ± 1.0a	23.0 ± 2.6c	2.19 ± 0.39ab	15.57 ± 0.97c
BARI 5, inoc	122 ±4a	37.8±2.5a	10.3 ± 1.5a	28.3 ± 3.1bc	2.53 ± 0.17a	18.48 ± 0.41ab
BARI 6, uninoc	106 ± 6b	23.3 ± 1.6d	8.3 ± 1.5a	31.3 ± 1.5ab	1.76 ± 0.38b	17.41 ± 1.15bc
BARI 6, inoc	117 ±4a	36.3 ± 2.9a	10.0 ±2.0a	35.7 ± 2.1ab	2.72 ± 0.16a	21.19 ± 1.74a
Shohag, uninoc	102 ± 3b	31.0 ± 1.8cd	8.0 ± 1.0a	26.7 ± 2.5bc	1.42 ± 0.16b	14.57 ± 0.39cd
Shohag, inoc	111 ± 3b	33.3 ± 1.4ab	9.7 ± 0.6a	27.7 ±2.1bc	2.11 ± 0.20ab	17.84 ± 0.56bc

The values are given as mean ± SD. Different letters in the same column indicate significant differences between treatments at $p<0.05$.

3.2 Yield parameters as affected by inoculation and variety

The yield parameters of soybeans are presented in Table 2. The data were pooled for variety and inoculum and subjected to one-way ANOVA separately. The analysis revealed that variety did not significantly affect the number of pods per plant, the number of seeds per pod and the weight of 100 seeds. However, yield per plot was significantly affected by varietal differences ($p<0.05$). The productive potential of soybeans is ultimately determined by the number of pods per plant, which is one of the primary yield components. The number of pods per plant ranged from 19.7 to 25.3. This growth metric was found to be significantly affected by inoculum only in the BARI 5 variety ($p<0.05$). No significant differences were observed among the varieties or between the inoculation status regarding the number of seeds per pod. As for 100-seed weight, only the inoculated variety of BARI 5 exhibited better performance compared to the uninoculated ones. The other two varieties did not show significant differences between inoculated and uninoculated plants (p>0.05). Inoculation resulted in a 59.6% increase in yield for the BARI 5 variety, while the Shohag variety exhibited a 38.4% increase in yield following inoculation with *Rhizobium*. The inoculation of the BARI 6 variety did not result in a significant increase in yield compared to its uninoculated counterpart, indicating similar performance irrespective of inoculation status. Although BARI 6 displayed enhanced vegetative growth and seed quality following *Rhizobium japonicum* inoculation, the absence of a significant yield increase suggests a genotype-specific symbiotic response.

Table 2. Yield parameters of soybean as affected by different treatments

Treatments	**No. of pods/plant**	**No. of seeds/pod**	**100-seed wt. (g)**	**Yield/plot (g)**
BARI 5, uninoc	21.30 ± 0.93b	3.14 ± 0.31a	10.63 ± 2.09b	790.70 ± 32.10b
BARI 5, inoc	25.29 ± 0.53a	3.32 ± 0.06a	13.81 ± 1.37a	1261.90 ± 92.70a
BARI6, uninoc	19.69 ± 1.47b	3.24 ± 0.13a	12.31 ± 0.71ab	1141.70 ± 59.9a
BARI 6, inoc	24.48 ± 1.14ab	3.03 ± 0.84a	11.18 ± 0.16b	1279.50 ± 140.30a
Shohag, uninoc	21.06 ± 1.56b	3.04 ± 1.33a	11.08 ± 0.69b	828.80 ± 45.20b
Shohag, inoc	23.86 ± 1.58ab	2.95 ± 0.81a	12.14 ± 2.11ab	1146.60 ± 80.40a

The values are given as mean ± SD. Different letters in the same column indicate significant differences between treatments at p<0.05.

In a recent study, transcriptomic analysis demonstrated that different *Bradyrhizobium* strains elicit distinct nodule formation patterns—ranging from effective nitrogen-fixing nodules to non-functional pseudonodules depending on soybean genotype (e.g., USDA110 vs. LVM105 in G. max) (Zadegan *et al.,* 2024). Investigation into nodulation compatibility among diverse strains revealed that certain *Rhizobium alamii* and *Bradyrhizobium* strains exhibit highly selective symbiosis, forming effective nodules only with certain cultivars (e.g., PANNAR1644R but not others) (Ndhlovu *et al.,* 2024). Seed yield, ranging from 791 to 1280 kg/ha, was generally higher with inoculation, significantly affecting BARI 5 and Shohag varieties. Soybeans grown on soils without a rhizobia population will utilize available native soil nitrogen. If soil nitrogen levels are low due to factors like soil type or soil erosion, nitrogen deficiency symptoms may appear.

3.3 Protein, oil, and sugar contents of soybean

Table 3 represents the levels of protein, oil, reducing sugar, non-reducing sugar, and total soluble sugar. A one-way ANOVA showed that variety and inoculation had significant influence on those parameters. Seed protein content of inoculated plants ranged from 36.4% to 38.8%, whereas uninoculated plants exhibited levels between 29.3% and 35.9%. *Rhizobium* inoculation resulted in a significant increase in protein content for the BARI 5 and Shohag varieties ($p < 0.05$). Oil content across the treatments varied from 18.3% to 22.6%. Although inoculated plants generally had higher oil content across all varieties, the differences between inoculated and uninoculated groups were not statistically significant ($p > 0.05$).

Inoculated BARI 5 and BARI 6 plants also showed significantly elevated levels of reducing sugars compared to their uninoculated counterparts ($p < 0.05$). Non-reducing sugar content ranged from 7.2 to 8.6 mg/g dry weight, with no significant differences observed between the varieties ($p > 0.05$). Similarly, total soluble sugar content ranged from 11.9 to 14.4 mg/g dry weight, with no significant variation ($p > 0.05$).

Table 3. Protein, oil, and soluble sugar contents of soybean seeds as affected by different treatments

Treatments	% Protein	%Oil	Soluble sugar (mg/g dry weight)		
			Reducing	Non-reducing	Total soluble sugar
BARI 5, uninoc	29.3±3.2b	20.7±0.9ab	4.9±0.3b	7.3±0.4b	12.2±0.7b
BARI 5, inoc	36.4±0.7a	22.6±0.9a	5.4±0.3a	8.1±0.4ab	13.4±0.8ab
BARI 6, uninoc	35.9±0.5a	18.3±0.9c	4.8±0.3b	7.2±0.4b	11.9±0.7b
BARI 6, inoc	38.8±0.7a	19.6±1.3bc	5.2±0.3a	7.8±0.4ab	13.1±0.6ab
Shohag, uninoc	29.4±3.8b	18.8±0.2bc	5.2±0.2a	7.9±0.2ab	13.1±0.4ab
Shohag, inoc	36.5±1.1a	21.7±0.7ab	5.8±0.3a	8.6±0.4a	14.4±0.7a

The values are given as mean ± SD. Different letters in the same column indicate significant differences between treatments at $p<0.05$.

Shahid *et al.* (2009) reported significant differences in seed protein content, ranging from 37.8 to 41.4%, but found oil content not significantly impacted. Sugars impacted the quality and nutritional profile of soy foods, serving roles such as providing energy for fermentation and affecting sweetness in soymilk and tofu.

3.4 Nitrogen, phosphorus, and potassium contents in roots, shoots and seeds

Table 4 presents the concentration of nitrogen (N), phosphorus (P), and potassium (K) in the roots, shoots, and seeds of soybean. The one-way ANOVA results revealed that %N in roots ($p<0.01$), %N in shoots ($p<0.05$), and %N in seeds ($p<0.01$) of soybean varieties were significantly influenced by *Rhizobium* inoculation. Moreover, N percentage of shoots ($p<0.05$) and seeds ($p<0.01$) were significantly affected by varietal differences. Phosphorus and K contents in roots ($p<0.05$) and shoots ($p<0.05$) demonstrated significant differences among treatments due to inoculation. Varietal differences were also significant in terms of K contents in roots ($p<0.05$), shoots ($p<0.01$), and seeds ($p<0.01$). Inoculation resulted in a significant increase in nitrogen content in the roots of BARI 6 and Shohag varieties ($p<0.05$). Nitrogen content in the shoots of BARI 5, BARI 6, and Shohag varieties was significantly affected by *Rhizobium* inoculation ($p<0.05$). Seed nitrogen content was found to be significantly different between inoculated and uninoculated plants of BARI 5 and Shohag varieties ($p<0.05$).

Phosphorus content in the roots ranged from 0.16% to 0.17% in uninoculated plants and from 0.17% to 0.19% in inoculated plants. Root phosphorus content was not significantly affected by inoculation across the varieties. However, potassium content in the roots showed significant differences between inoculated and uninoculated plants of BARI 6 and Shohag varieties ($p<0.05$). No significant differences were observed between uninoculated and inoculated plants of different tested varieties regarding the potassium content of soybean seeds.

Table 4. Nitrogen, phosphorus, and potassium concentration in roots, shoots, and seeds of soybean as affected by different treatments

Treatment	Root			Shoot			Seeds		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
BARI 5, uninoc	1.28 ± 0.06bc	0.17 ± 0.01ab	0.07 ± 0.01b	2.32 ± 0.09c	0.16 ± 0.01ab	0.24 ± 0.02bc	4.69 ±0.51b	0.08 ± 0.01b	0.24 ± 0.01a
BARI 5, inoc	1.42 ± 0.06b	0.17 ± 0.01ab	0.09 ± 0.02bc	2.88 ± 0.04a	0.16 ± 0.01ab	0.26 ± 0.01ab	5.83 ± 0.12 a	0.10 ± 0.01a	0.24 ± 0.01a
BARI 6, uninoc	1.40 ± 0.07c	0.17 ± 0.01ab	0.09 ± 0.01b	2.61 ± 0.05b	0.15 ± 0.01b	0.25 ± 0.01ab	5.76 ± 0.08 a	0.09 ± 0.01ab	0.23 ± 0.01a
BARI 6, inoc	1.74 ± 0.07a	0.19 ± 0.01a	0.11 ± 0.01a	3.03 ± 0.08a	0.17 ± 0.01a	0.27 ± 0.01a	6.21 ± 0.11a	0.11 ± 0.01a	0.24 ± 0.01a
Shohag, uninoc	1.14 ± 0.10c	0.16 ± 0.01b	0.08 ± 0.01b	2.61 ± 0.03b	0.15 ± 0.01b	0.22 ± 0.01c	4.70 ± 0.61b	0.09 ± 0.01ab	0.24 ± 0.01a
Shohag, in	1.39 ± 0.13b	0.18 ± 0.01ab	0.13 ± 0.01a	2.92 ± 0.06a	0.16 ± 0.01ab	0.23 ± 0.01b	5.84 ± 0.17a	0.10 ± 0.01a	0.24 ± 0.01a

The values are given as mean ± SD. Different letters in the same column indicate significant differences between treatments at $p<0.05$.

5. Conclusions

This study was conducted to evaluate the feasibility of cultivating soybeans in the acidic valley soil of the Chittagong region in Bangladesh, where it had not been previously grown. The study indicated that seeds of inoculated plants had higher protein content compared to that of uninoculated plants in the BARI 5 and Shohag varieties. Additionally, increased reducing sugar content was observed in inoculated plants of the BARI 5 and BARI 6 varieties. *Rhizobium* application also significantly influenced the accumulation of nitrogen, phosphorus, and potassium in plant tissues, contributing to improved physiological and metabolic functions. The findings underscore the agronomic and nutritional advantages of *Rhizobium* inoculation in soybean production, particularly in acidic and biologically low-input soils. Adaptability of these BARI-released varieties, when combined with effective inoculation strategies, offers a promising avenue for expanding soybean cultivation in non-traditional agroecological zones of Bangladesh. This could contribute to national efforts aimed at enhancing oilseed self-sufficiency, improving dietary protein intake, and promoting sustainable soil fertility management through biological nitrogen fixation.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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