Application of *Bradyrhizobium japonicum* and Phosphorus Fertilization Improved Growth, Yield and Nodulation of Soybean in the Sub-humid Hilly Region of Azad Jammu and Kashmir, Pakistan

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Abstract : Two separate experiments (pot and field) were conducted to examine the response of soybean to *Bradyrhizobium japonicum* and phosphorus (P) fertilization. Different treatments were i) *Rhizobium* strains (0, S377, S379, and the mixture of S377+S379 i.e. S_0, S_1, S_2, S_3); ii) phosphorus (field only, 0, 50, 100 kg ha^{-1}i.e. T_0, T_1, T_2) and iii) two soils (pot only) i.e. autoclaved (A_1) and non-autoclaved (A_0). A soybean cultivar NARC-1 was tested for estimating growth traits, nodule number and mass, root development and yield traits. In the pot experiment, total number of nodules both in the A_0 and A_1 were negligible but increased significantly following the application of *Bradyrhizobium japonicum.* In the field experiment, number of nodules increased from 6 in the control treatment without strains to a maximum of 86 in S_3T_1. Shoot dry weight increased significantly from 11.8 g $plant^{-1}$ in the control soil to 15.6 g $plant^{-1}$ in S_3T_1. Root length was increased but root mass was unaffected. Soybean seed yields ranged between 615 and 1003 kg ha^{-1} against 543 kg ha^{-1} in the control soil indicating a maximum of 85% increase over control. Shoot dry weight and seed yield had significant correlation with nodulation (R^2=0.91). The results of experiments revealed significant positive effects of rhizobium inoculation and P fertilization on growth, nodulation and yield of soybean and, generally, mixture of strains (S_3) was more effective than the strains S_1 and S_2. Results also indicated that high application of P (100 kg P_2O_5 ha^{-1}) reduced the efficiency of inoculants for nodule mass and seed yield.

Key words : *Bradyrhizobium japonicum*, *Glycine max* L., Inoculation, Nodulation, *Rhizobium*, Strains.

Soil erosion is a major threat to sustainable agriculture in the hilly and mountainous areas of Azad Jammu and Kashmir (AJK), removing the upper most fertile layer of the soil, depleting the fertility and leaving the soil poor in physical conditions. An extensive survey of soils in and around Rawalakot showed a severe deficiency of most of the major nutrients especially the N (Malik et al., 2000). Meeting the plant demand for N in a deficient soil is normally achieved by the use of chemical fertilizers. However, the high cost of mineral N fertilizers and their unavailability at the time of requirement are the two major constraints responsible for low fertilizer N input. Furthermore, the hilly and slopping landscape of AJK with high rainfall during both the major cropping seasons (rabi and kharif i.e. winter and summer season) may cause inefficient utilization of an expensive input because of surface runoff and leaching losses. This emphasizes the importance of developing new production methods that are sustainable both agronomically and economically. Introduction and exploitation of legumes are potentially of great practical importance in hill farming system, which can increase soil fertility and plant productivity (Döbereiner, 1992; Sessitsch et al., 2002) thereby increasing the farm income of the farmers.

Among legumes, soybean [*Glycine max* (L.) Merrill] is an important N_2-fixing crop, cultivated throughout the world. Soybean is the world's leading source of vegetable oil and its seed contains about 20% oil on a dry weight basis provides approximately 30% of the world's supply of oil. Soybean seeds contain about 40% proteins provides approximately 60% of the world's supply of vegetable protein. There is extensive evidence that soybean could reduce the fertilizer N requirement of a following crop (Beauchamp et al., 1996) and a soybean N credit of about 45 kg N ha^{-1} has been used in different parts of the world (Kurtz et al., 1984; Bundy et al., 1993).

Soybean obtains nitrogen directly from the soil and indirectly form symbiotic fixation when nodulated with effective strains of *B. japonicum.* In soils not previously cropped with soybean or soils in nontraditional areas of soybean production seldom contain sufficient population of naturalized *rhizobium japonicum* to ensure satisfactory nodulation (Rennie et al., 1982;

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Hatam and Abbasi, 1994). In such soils, inoculation with rhizobium strains at planting should be an essential production practice (Gidden et al., 1982) to enhance soybean quality and production (Wiersma and Orf, 1992). Nodule number, nodule dry weight and soybean shoot yield were increased when seeds were inoculated by *Bradyrhizobium* (Okereke et al., 2004; Egamberdiyeva et al. (2004a; 2004b). Ashraf et al. (2002) reported a specific combination of soybean genotype with rhizobium strains resulting in many fold increase in the amount of N_2 fixed and grain yield harvested. Hafeez et al. (2001) reported that inoculation with rhizobia should be perform in two different situations: (i) in soils which are depleted or contain a low indigenous rhizobial population, and (ii) when there is an established but inefficient rhizobial population. Among essential nutrient elements, influence of phosphorus (P) on symbiotic nitrogen fixation in leguminous plants has received considerable attention. Tsvetkova and Georgiev (2003) reported that P deficiency treatments in soybean decreased the whole plant fresh and dry mass, nodule weight, number and functioning. Similarly, significant increase in soybean growth, 100-grain weight and grain yield ha^{-1} in response to added levels of 90 and 100 kg P_2O_5 ha^{-1} was reported by several workers (Hussain et al., 1981; Taj et al., 1986; Jamro et al., 1990). However, Howard et al. (1990); Misra et al. (1990) and Kumar and Rao (1991) stated that various levels of added P did not significantly affect the growth and yield of soybean.

The introduction and exploitation of soybean and the presence of indigenous and efficient rhizobial population in the State of Azad Jammu and Kashmir is not studied so far. The objective of this work was to examine the effect of rhizobium inoculation and P fertilization on the growth, yield and nodulation potential of soybean in sub-humid region of Azad Jammu and Kashmir.

Materials and Methods

1. The study site

The study site is located in an experimental farm of the University of Azad Jammu and Kashmir, Faculty of Agriculture Rawalakot in the north–east of Pakistan under the foothills of great Himalayas. The area lies between the altitude of 1800–2000 m above sea level and latitude 33–36°. The topography is mainly hilly and mountainous with valleys and stretches of plains. The area is characterized by a temperate sub-humid climate with annual average rainfall ranging from about 500–2000 mm, most of which is irregular and falls with intense storms during monsoon and winter. The monthly mean temperature ranges from a minimum of 0°C to a maximum of 22°C accompanied by a severe cold and snow fall in winter. The soil used in the study (0-15 cm) is silt loam in texture and have organic carbon 9.5 g kg^{-1}, total N 1.02 g kg^{-1}, available P 2.5 mg kg^{-1}, available K 54 mg kg^{-1} and pH 6.7.

2. Pot experiment

Pot experiment was carried out during the 2nd week of July, 2003 in the greenhouse Faculty of Agriculture Rawalakot Azad Jammu and Kashmir. The temperature of the greenhouse during the study ranges from 20 –24°C. Thoroughly cleaned earthen pots of 38 cm height and 18 cm depth were used for the experiment. The hole at the bottom of each pot was partially plugged with pebbles (wrapped in blotting paper). A composite soil sample from the top 15 cm depth was obtained from the research field of the Faculty, air dried and sieve through 4 mm mesh. Half of the soil sample was sterilized at a temperature of 121°C for a period of 30–40 minutes using autoclave while the remaining half was left as original. Pots were grouped into two sets; one half for autoclaved soil and the other half for non-autoclaved/normal soil. The treatments were: three rhizobium strains i.e. control, S377, S379 and mixture of S377+379 designated as S_0, S_1, S_2 and S_3, respectively; two soils i.e. non-autoclaved (A0) and autoclaved (A1). The variety used was NARC-1. The choice of strains was depend on their availability and collected from the Soil Biology and Biochemistry section, National Agriculture Research Centre (NARC), Islamabad Pakistan. The pots were labeled according to their respective treatments, and arranged in a Completely Randomized Design (CRD) of two factors (soil treatment and inoculation) with three replicates. There were 4 treatments and three replicates for both sets (2 soils) comprised a total of 24 pots. About 12 kg soil was filled in the pots. A basal dose of 50 kg P_2O_5 ha^{-1} as Single Super Phosphate (SSP) was incorporated well into the soil before sowing. The soil was moistened with water and maintained at 60% of its water holding capacity.

Soybean seeds for inoculated treatments were moistened with the slurry of cool concentrated sugar solution. Thereafter, the seeds were coated with the peat based inoculants of *B. japonicum* strains S377 (S_1), S379 (S_2) and the mixture of S377+S379 (S_3). This process was carried out in shade following the method of FAO (1984). The inoculum was used at the rate of 10 g of peat based inocula for 1 kg seed (Egamberdiyeva et al., 2004a). Fresh inoculum was obtained from Soil Biology and Biochemistry Section, National Agriculture Research Centre (NARC) Islamabad, Pakistan while certified soybean seeds were collected from Oil and Seed Department, NARC, Islamabad. In each pot, eight healthy and uniform seeds of soybean were sown at a depth of 3 cm. Pots were kept under shade to reduce evapotranspiration during the course of germination. After complete germination, plants were thinned to 6 plants per pot. All pots were equally irrigated when needed. Two to three plants from each

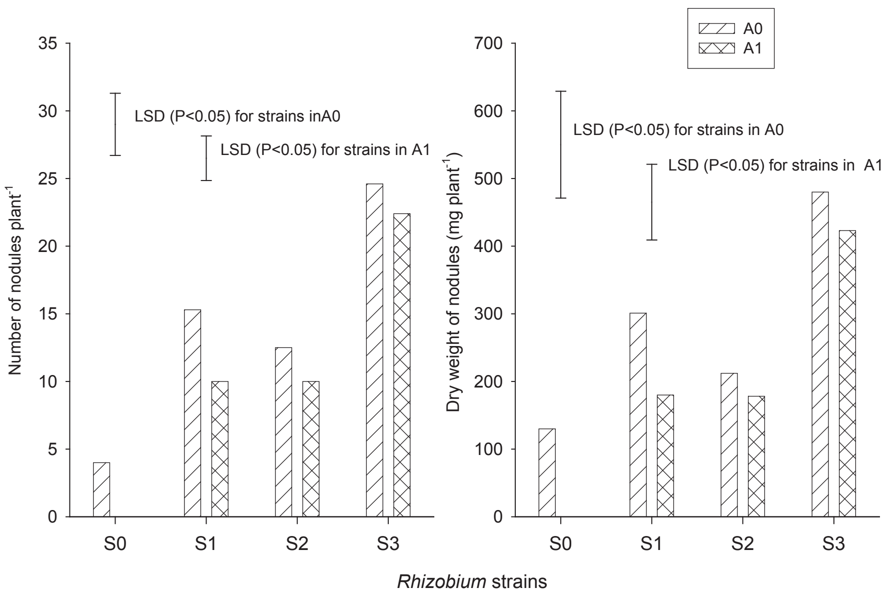

Fig. 1. Efficiency of *Bradyrhizobium japonicum* on nodulation potential i.e. nodules number and nodule dry weight of soybean grown in autoclaved (A1) and non-autoclaved (A_0) soils. Vertical lines on each bar represents LSD ($P \leq 0.05$) among different strains in A_0 and A_1 soils. S_0=no strains; S_1=Strain 377; S_2=Strain 379; S_3=combination of 377+379.

plot were carefully uprooted at full flowering (R_2) stage for the study of growth, root development and nodulation potential of soybean while yield traits were determined at R_8 stage.

3. Field trial

A field experiment was carried out in arable field at Research Farm, Faculty of Agriculture, Rawalakot Azad Jammu and Kashmir. Before sowing, experimental field ploughed twice with tractor for proper tilt. The treatments in this experiment were: Rhizobium strains=4 i.e. S377, S379, combination of S377+S379 and control, designated as S_0, S_1, S_2 and S_3, respectively;; Phosphorus levels=three i.e. P_2O_5 at the rate of 50 and 100 kg ha^{-1} and a control without P application; designated as T_1, T_2, and T_0, respectively; soybean variety=1 i.e. NARC-1; replications=3. The plots of the field were prepared according to these treatments i.e. phosphorus was arranged in the main plot while strains were applied in the sub-plots. The experiment was laid out in Randomized Complete Block Design with split-plot arrangements. Plots size was 1.5 m×1.5 m and row to row distance was maintained 40 cm. Just before sowing, phosphorus was well incorporated into the respective plots in the form of single super phosphate (SSP). Seeds of soybean cultivar NARC-1 were inoculated just before sowing according to the method described in pot experiment. After maintaining proper moisture, seeds were sown by hand in each plot and planted at a depth of 3–4 cm in rows in the beginning of June 2004. After germination, plant population was maintained by thinning and gap filling. A set of three plants from each plot was randomly selected at flowering (R_2 stage) for nodulation potential (number of nodules, fresh and dry weight of nodules) and shoot and root characteristics (shoot height, shoot dry weight; root length, root dry weight). At the end of growing season, the yield attributes i.e. number of pods, 100-seeds weight and seed yield was recorded. During the field experiment the temperature ranges between 16–24ºC while the amount of rainfall during the experiment was 550 mm.

4. Statistical Analysis

Growth, nodulation, and yield parameters were recorded and then analysed statistically using Randomized Complete Block Design (RCBD) with split-plot arrangements (Steel and Torrie, 1980). When analysis of variance showed significant treatment effects, the LSD test was applied to make comparisons among the means at the 0.05% level of significance (Steel and Torrie, 1980).

Results and Discussion

1. Pot experiment

The experiment under the controlled environmental condition (pot experiment) showed complete absence of nodules in soybean roots in the autoclaved soil without rhizobium strains (at A_1, S_0 value was 0) while only four nodules were found in non-autoclaved un-inoculated control (Fig. 1) showing low density of indigenous or efficient rhizobial population in the soil. Inoculation with *B. japonicum* strains accelerated nodules formation and number increased to a maximum of 25. Strain S_3 produced significantly (*P*

≤0.05) greater number of nodules than the nodules produced by S_1 and S_2. Nodules dry weight also showed similar pattern of changes that observed for nodules number. In non-autoclaved soil, inoculation with *B. Japonicum* increased nodule dry weight by 2–4 folds over control while in autoclaved soil dry weight of nodules increased from 0 mg to more than 400 mg plant^{-1} (Fig. 1). The level of increase varied among the strains and the maximum increase was recorded in S_3. The significant increase in nodule number and mass both in autoclaved and non-autoclaved soils following inoculation indicated that inoculation with *B. japonicum* is an essential practice for maximum nodulation that would certainly affect the N_2 fixation and N uptake by soybean plants. In soils not previously cropped with soybean, inoculation is an essential production practice (Koutroubas et al., 1998). Soils in nontraditional areas of soybean production seldom contain sufficient population of naturalized *Rhizobium japonicum* to ensure satisfactory nodulation (Rennie et al., 1982; Hatam and Abbasi, 1994). In such soils, inoculation with rhizobium strains at planting should be an essential production practice (Giddens et al., 1982) to enhance soybean quality and production in addition to increase N_2 fixation (Wiersma and Orf, 1992).

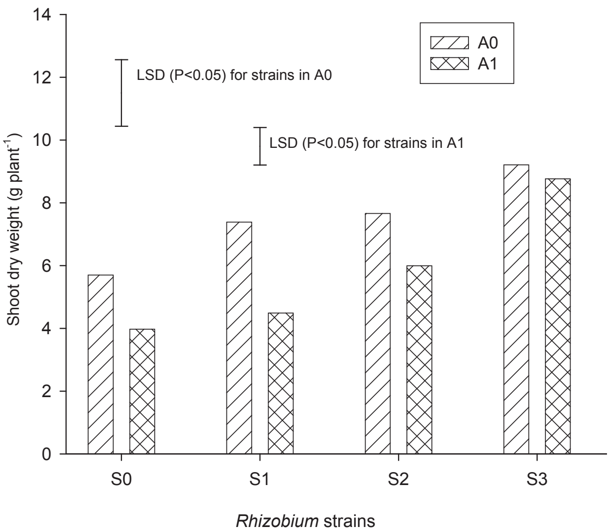

Fig. 2. Efficiency of *Bradyrhizobium japonicum* on plant growth i.e. shoot dry weight (g plant^{-1}) of soybean grown in autoclaved (A1) and non-autoclaved (A_0) soils. Vertical lines on each bar represents LSD (P≤0.05) among different strains in A_0 and A_1 soils. S_0=no strains; S_1=Strain 377; S_2=Strain 379; S_3=combination of 377+379.

Sterilization of soil showed small effect on plant growth and development. The shoot dry weight in non-autoclaved soil without *B. japonicum* was 5.7 g plant^{-1} compared to 4.0 g in autoclave soil (Fig. 2). Rhizobium inoculation increased plant dry matter to a maximum of 9.2 and 8.8 g plant^{-1} in non-autoclaved and autoclaved soil, respectively, and the difference between inoculated and un-inoculated soil was significant (P≤0.05, data not shown).

2. Growth and nodulation in field trial

Keeping in view the above findings, a field trial was conducted to examine the efficiency of *B. japonicum* on the growth, nodulation and yield of soybean. Phosphorus fertilization was also applied to examine its individual and interactive effect with strains on soybean. Tsvetkova and Georgiev (2003) reported that phosphorus deficiency treatments in soybean decreased the whole plant fresh and dry mass, nodule weight, number and functioning. Robson and O'Hara (1981) concluded that phosphorus nutrition increased symbiotic N_2 fixation in subterranean clover (*Trifolium subterraneum* L.) by stimulating host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation and function.

The overall strains effect was determined by taking the average value across the phosphorus levels, while phosphorus effect was determined by taking the average value across the different strains. Analysis of variance (p values) is given (Table 1) to summarize statistical significances of the effect of phosphorus (T) strains (S) and TxS on different growth and yield characteristics of soybean. The main effect of strains (S) was significant for plant height (p=0.034) while phosphorus application was not significant (p=0.118). However, the interaction of T ×S was also significant (p=0.044) for this parameter. Mean values for plant height are given in Table 2 showed that S_2 and the mixture of S_1+S_2 (S_3) when combine with T significantly reduced plant height

Table 1. Analysis of variance (p) for growth, nodulation and yield characteristics of soybean in response to the application of *rhizobium* strains and phosphorus fertilization.

Source	Degree of freedom	Plant height	Plant dry weight	Root length	Root dry weight	Nodules number	Nodule fresh weight	Nodule dry weight	No. of pods	100-seed weight	Seed yield
		———	———	———	———	p values	———	———			
Phosphorus (T)	2	0.118	0.351	0.004	0.341	0.012	0.371	0.337	0.112	0.558	0.162
Strains (S)	3	0.034	0.062	0.002	0.054	0.002	0.002	0.073	0.275	0.049	0.007
T×S	6	0.044	0.034	0.032	0.330	0.001	0.001	0.102	0.214	0.023	0.002

Table 2. Effect of *Rhizobium* strains *Bradyrhizobium japonicum* and phosphorus fertilization on the growth components of soybean i.e. height, and dry weight of plant grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Rhizobium strains	Height of plant (cm)				Dry weight of plant (g plant^{-1})			
	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*
S_0	63.6	75.8	70.5	70.0	11.8	15.9	13.6	13.8
S_1	69.5	72.5	71.5	71.2	12.9	13.5	12.5	13.0
S_2	66.3	64.0	70.8	67.0	14.5	12.8	15.0	14.1
S_3	67.5	64.8	68.0	66.8	14.7	15.6	12.9	14.4
mean	66.7	69.3	70.2		13.5	14.5	13.5	

LSD ($p \leq 0.05$) for phosphorus (T) = NS
LSD ($p \leq 0.05$) for strains (S) = 3.91
LSD ($p \leq 0.05$) for combination (T x S) = 6.76

LSD ($p \leq 0.05$) for phosphorus (T) = NS
LSD ($p \leq 0.05$) for strains (S) = NS
LSD ($p \leq 0.05$) for combination (T × S) = 2.9

S_0 = no strains; S_1 = Strain 377; S_2 = Strain 379; S_3 = mixture of S377 + S379 while T_0 = no phosphorus; $T_1 = P_2O_5$ at the rate of 50 kg ha^{-1}; $T_2 = P_2O_5$ at the rate of 100 kg ha^{-1}.
NS = non-significant; R. strains = *Rhizobium* strains.

Table 3. Effect of *Rhizobium* strains *Bradirhizobium japonicum* and phosphorus fertilization on the root development of soybean i.e. length, and dry weight of root grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Rhizobium strains	Root length (cm)				Dry weight of root (g plant^{-1})			
	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*
S_0	17.7	23.7	24.0	21.8	2.15	2.95	3.27	2.8
S_1	21.7	25.8	26.6	24.7	2.83	3.25	3.52	3.2
S_2	22.2	23.4	25.9	23.8	2.90	2.89	3.43	3.1
S_3	22.7	21.8	23.8	22.8	2.99	2.78	2.91	2.9
mean	21.1	23.7	25.1		2.7	3.0	3.3	

LSD ($p \leq 0.01$) for phosphorus (T) = 2.14
LSD ($p \leq 0.01$) for strains (S) = 2.65
LSD ($p \leq 0.05$) for combination (T x S) = 6.2

LSD ($p \leq 0.05$) for phosphorus (T) = NS
LSD ($p \leq 0.05$) for strains (S) = NS
LSD ($p \leq 0.05$) f for combination (T × S) = NS

S_0 = no strains; S_1 = Strain 377; S_2 = Strain 379; S_3 = combination of 377 + 379 while T_0 = no phosphorus; $T_1 = P_2O_5$ at the rate of 50 kg ha^{-1}; $T_2 = P_2O_5$ at the rate of 100 kg ha^{-1}.
ns = non-significant; R. strains = *Rhizobium* strains.

relative to the control and S_1. Interactive effect of T × S indicated that height of plant increased significantly with the combinations of S_1T_1, S_1T_2 and S_2T_2. Application of phosphorus and strains did not show significant effect on dry weight of plant (Table 1). However, interaction of T × S showed significant effect (p=0.034). Treatments effect indicated that combination of S_2T_2 and S_3T_1 significantly increased dry weight of plant by 27 and 32% over control i.e. S_0T_0 (Table 2). Egamberdiyeva et al. (2004b) found 7–23% increase in shoot dry weight in rhizobium inoculated soil compared with uninoculated control while Bai et al. (2002) and Okereke et al. (2004) reported 29 and 2–130% increase in shoot dry weight following the inoculation of *B. japonicum*, respectively. Results indicated that application of different strains in control soil without phosphorus increased both height and dry weight of plant relative to soil without strains (not significant) but application of phosphorus reduced the efficiency of strains with regard to growth characteristics. Application of phosphorus at the rate of 50 kg ha^{-1} in the control soil without strains seemed very effective for the growth of soybean. However, application of phosphorus with strains did not show consistent trend and need further study to find out consistent pattern of this combination on soybean growth.

Analysis of variance (Table 1) showed no significant effect of rhizobium inoculation and phosphorus fertilization on root mass (dry weight) but root length was increased significantly with the application of strains (p=0.002) and phosphorus fertilization (p=0.004). The interaction of T × S also showed significant effect (p=0.032). Among strains, S_1 significantly increased root length while S_2 and the mixture of $S_1 + S_2$ were in line with the control (Table 3). On average, increase in root length resulting from rhizobium inoculation was 9% over control while phosphorus fertilization significantly increased root length by 16%. The interactive effect of T × S showed

Table 4. Effect of *Rhizobium* strains *Bradyrhizobium japonicum* and phosphorus fertilization on the nodulation potential i.e. no. of nodules, nodule fresh weight and nodule dry weight of soybean grown under field conditions at Rawalakot Azad Jammu and Kashmir.

R. strains	No. of nodules plant^{-1}				Nodules fresh weight (g plant^{-1})				Nodules dry weight (g plant^{-1})			
	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*
S_0	5.5	51.0	63.0	39.8	0.66	3.53	3.43	2.5	0.24	0.76	0.64	0.55
S_1	44.7	39.9	75.3	53.3	2.62	3.73	2.49	2.9	0.52	0.45	0.55	0.51
S_2	66.3	60.5	42.6	56.5	3.83	3.13	2.21	3.1	0.79	0.64	0.36	0.60
S_3	75.8	86.5	64.8	75.7	4.81	4.17	2.78	3.9	0.96	0.92	0.67	0.85
mean	48.1	59.5	61.4		3.0	3.6	2.7		0.63	0.69	0.56	

LSD ($p\leq0.05$) for phosphorus (T) = 10.2 LSD ($p\leq0.05$) for phosphorus (T) = NS LSD ($p\leq0.05$) for phosphorus (T) = NS
LSD ($p\leq0.01$) for strains (S) = 14.62 LSD ($p\leq0.01$) for strains (S) = 0.67 LSD ($p\leq0.05$) for strains (S) = NS
LSD ($p\leq0.01$) for combination (T×S) = 18.2 LSD ($p\leq0.01$) for combination (T×S) = 1.17 LSD ($p\leq0.05$) for combination (T×S) = NS

S_0 = no strains; S_1 = Strain 377; S_2 = Strain 379; S_3 = combination of 377+379 while T_0 = no phosphorus; T_1 = P_2O_5 at the rate of 50 kg ha^{-1}; T_2 = P_2O_5 at the rate of 100 kg ha^{-1}.
ns = non-significant; R. strains = *Rhizobium* strains.

significant increase in most of the combinations and the maximum root length of 25.8, 25.9 and 26.6 cm was recorded for S_1T_1, S_2T_2 and S_1T_2 (Table 3).

The main effect of T, S and T×S was significant for nodule number (p=0.012; 0.002 and 0.001, Table 1). Similarly, application of strains and TxS was also significant for fresh weight of nodules (p=0.002, 0.001) while T alone was not significant (p=0.371). Inoculation of soybean with *B. japonicum* strains S_1, S_2 and S_3 resulted in significant increase in nodule number and nodule fresh weight, indicating that the inoculant *Bradyrhizobium* strains were more effective than the indigenous rhizobium population infecting soybean. However, the dry weight of nodule did not show significant response to T and S (Table 1). On average, the nodule numbers in the control soil without strains were 39.8 which increased significantly to 56.5 and 75.7 following the application of S_2 and S_3. Strain S_1 did not show significant difference with the control (Table 4). Mixture of S_1+S_2 i.e. S_3 showed the maximum increase in nodule number i.e. 90% over control. Both the levels of phosphorus i.e. 50 and 100 kg P_2O_5 ha^{-1} (T_1 and T_2) significantly increased nodule number (but not fresh and dry weight) from 48.1 in the control to 59.5 and 61.4 at T_1 and T_2 level, respectively. The interactive effect of T×S showed most significant response to nodule number. Root nodules in the control soil (S_0T_0) were 5.5 in number which increased significantly to 86.5 in S_3T_1. On average, S_3 with phosphorus yielded higher number of nodules relative to S_1 and S_2. Nodule mass i.e. fresh weight increased significantly with rhizobium inoculation but dry weight did not show significant changes. Phosphorus fertilization did not show significant effect on nodule mass. The interaction of T×S significantly increased nodule fresh weight but had no significant effect on dry weight of nodules. The level of increases varied among strains. For example, nodules number, fresh and dry weight in S_3 was relatively higher than those found in S_1 and S_2, indicating the synergistic effect of two strains used in the inoculum (S377+S379). Few nodules in uninoculated plants indicated that the indigenous bradyrhizobia population was low in the soil. The presence of few effective strains assumed to be always present in a soil population. The results show that the response to inoculation by soybean cultivar may not be hindered in a field that has not been previously inoculated or used to grow soybean. Egamberdiyeva et al. (2004a) tested three different strains of rhizobium on soybean and reported an average of 44% increase in nodule numbers in plants inoculated with rhizobium strains. In an experiment in Nigeria, the percentage increase in nodule number and dry weight after inoculation of soybean cultivars with Bradyrhizobia strains ranged from 71–486% and from 0–200%, respectively (Okereke et al., 2004). A greater proportion of nodules developed on the root of soybean after inoculation and phosphorus fertilization. Growth promotion of soybean by inoculation with *Bradyrhizobium* was reported by Rahmani and Saleh-Rastin (2001) in N-deficient soil. A substantial increase in root number with phosphorus fertilization is associated with the effect of phosphorus on plant growth. Enhancement of root development with phosphorus led to a better nodules initiation. The supply of phosphorus directly and positively stimulates nodulation in red clover (Hellsten and Huss-Danell, 2000), peas (Jakobsen, 1985), and soybeans (Israel, 1987, 1993). There were also other reports suggesting that phosphorus indirectly induced nodulation by a positive effect on the plant growth (Yang, 1995; Reddell et al., 1997). Also, phosphorus may increase the nodulation and stimulate the nitrogenase activity by improving the plant growth (Gentili and Huss-Danell, 2003). However, in the present study the extent of increase in nodulation by phosphorus fertilization

Table 5. Effect of *Rhizobium* strains *Bradyrhizobium japonicum* and phosphorus fertilization on the yield components i.e. no. of pods, 100-seed weight and seed yield of soybean grown under field conditions at Rawalakot Azad Jammu and Kashmir.

R. strains	No of pods plant^{-1}				100-seed weight (g)				Seed yield (kg ha^{-1})			
	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*	T_0	T_1	T_2	*mean*
S_0	23.8	34.5	29.3	*29.2*	13.4	15.56	15.9	15.0	543	740	879	721
S_1	29.0	29.7	32.3	30.3	16.4	13.8	15.9	15.4	766	621	767	718
S_2	26.0	30.8	35.5	30.8	15.8	15.5	14.3	15.2	811	846	615	757
S_3	26.3	30.0	30.0	28.8	16.8	17.1	15.6	16.5	1003	933	677	871
mean	26.3	31.3	31.8		15.6	15.5	15.4		781	785	735	

LSD ($p \leq 0.05$) for Phosphorus (T) = NS	LSD ($p \leq 0.05$) for Phosphorus (T) = NS	LSD ($p \leq 0.05$) for Phosphorus (T) = NS
LSD ($p \leq 0.05$) for strains (S) = NS	LSD ($p \leq 0.01$) for strains (S) = 0.85	LSD ($p \leq 0.05$) for strains (S) = 118
LSD ($p \leq 0.05$) for combination (T×S) = NS	LSD ($p \leq 0.01$) for combination (T×S) = 1.417	LSD ($p \leq 0.01$) for combination (T×S) = 205

S_0 = no strains; S_1 = Strain 377; S_2 = Strain 379; S_3 = combination of 377 + 379 while T_0 = no phosphorus; T_1 = P_2O_5 at the rate of 50 kg ha^{-1}; T_2 = P_2O_5 at the rate of 100 kg ha^{-1}.
ns = non-significant; R. strains = *Rhizobium* strains.

alone was substantially higher than the increases in growth indicating that some other mechanisms also involved in the process of increase in nodulations with phosphorus fertilization. It is accepted that phosphorus in conjunction with the plant demand for N controls the nodule growth and modulates the symbiotic processes of the legume and rhizobium (Wall et al., 2000; Hellsten and Huss-Danell, 2000). However, the effect of phosphorus supply on nodule development and its role in soybeans is poorly understood. Nodules number and mass were higher most of the time in the treatments where both strains and phosphorus were applied together. Mullen et al. (1988) reported that nodule numbers and nodule mass were all higher in plants grown with phosphorus nutrition and *rhizobium* inoculation. Zhang et al. (2002a) reported that in the two year field study on soybean, inoculation with *B. japonicum* increased nodule number (14 and 23%) and nodule dry weight (13 and 16%) over control without inoculation.

3. Seed yield and yield components

Analysis of variance (ANOVA) for seed yield is depicted in Table 1. Statistical analysis showed a significant difference among strains (p=0.007) and the interactions (T×S, p=0.002). However, application of phosphorus did not show significant effect. The average values of seed yield across different phosphorus levels indicated the control soil (mean of S_0) gave the lowest seed yield i.e. 721 kg ha^{-1} (Table 5). Inoculation with rhizobium strains S_1 and S_2 did not show significant increase in seed yield over control but the mixture of both strains (S_3) increased seed yield significantly i.e. 871 kg ha^{-1}. The percent increase in seed yield due to the inoculation of soybean by the mixture of two strains used in the inoculum was 21% over control. Phosphorus fertilization did not show significant increase in seed yield and the average values across different strains were almost similar. The combination of both showed more significant increase in seed yield and all combinations except S_1T_1, S_2T_2 and S_3T_2 showed significantly higher yield than the control (S_0T_0). The maximum seed yield in combine treatments were observed in S_3T_1 that was almost double than the control. However, it is worth noting that strains in the absence of phosphorus and phosphorus in the absence of strains seemed more efficient and the maximum seed yield of 1003 kg ha^{-1} was recorded in S_3T_0. Similarly application of phosphorus at the rate of 100 kg P_2O_5 ha^{-1} (T_2) in the soil without strains (S_0) yielded 879 kg ha^{-1} seed i.e. 62% higher than the seed recorded in the control soil without phosphorus. The reduced effect of inoculation under high P application was also observed in seed yield by comparing the average values at T_0, T_1 and T_2. Response of 100-seed weight to inoculation and P fertilization was similar to that observed for seed yield. However, number of pods plant^{-1} did not differ significantly among the strains and P treatments.

The results of our experiments with *Bradyrhizobium* spp. strains S_1, S_2 and S_3 clearly demonstrated that rhizobium inoculation with the mixture of S_1+S_2 significantly increased soybean seed yield (21% average) in the absence of native soil rhizobium population in the field. The significant response of the mixture of the two strains indicated the synergistic effect of the mixture of two strains used in the inoculum. These results could be explained by the reported symbiosis efficiency between soybean and *B. japonicum.* The increase in nodulation by inoculation may increase N_2-fixation that led to increase in plant dry matter and seed yield because regression analysis between nodules number and plant dry matter and nodules vs seed yield indicated significant positive correlation (R^2=0.91). Uninoculated plants had the lowest shoot dry weight and seed yield, probably because the native rhizobia was ineffective and did not fix much N_2 to encourage growth and seed yield. Egamberdiyeva et al. (2004b) reported that the yield of soybean varieties in Uzbekistan was 48% higher for inoculated than for uninoculated plants, while Okereke et al. (2004) in Nigeria reported increase in seed yields after *Bradyrhizobia* inoculation ranged between 1200 and 2180 kg ha^{-1} against the uninoculated plants, which had seed yields of 1050 kg ha^{-1}. Zhang et al. (2002b) reported that *B. japonicum* improved seed yield of soybean largely due to increase in pod and seed number. In experiments carried-out in Pakistan, a 40–50% increase in yield owing to inoculation has been found common (Ashraf et al., 2002; Oad et al., 2002). However, Achakzai et al. (2002) did not find any increase in soybean yield after inoculation. This non-significant effect of inoculation on seed yield was attributed to the complete absence of apparent nodulation in soybean plants.

The maximum seed yield recorded in the present investigation was 1003 kg ha^{-1} as compared to the potential yield of more than 2000 kg ha^{-1} reported under different conditions (Oad et al., 2002; Okereke et al., 2004). Sowing in the beginning of June is average cultivation schedule in north-east of Pakistan and seeding in the later season affect yield because of low temperature during pod filling and mature stage of soybean. During the months of September and October, temperature at Rawalakot normally fell down to 18°C to <15°C. Therefore, the sowing schedule may be one factor that affected the yield of soybean recorded in the present study. In addition some other environmental factors i.e. soil type, altitude, variety, and strains may also contributed to low yield. However, co-inoculation of plant growth promoting rhizobacteria (PGPR) with *Bradyrhizobium* has been reported to increase legume nodulation and growth even at low soil temperatures (Zhang et al., 1996; Dashti et al., 1998). In addition, introduction of temperate regions soybean cultivars in the region could fulfill the yield gap. Similarly, more strains should be tested for their efficiency.

Conclusions

The investigation presented in this study indicates that application of *B. japonicum* and P fertilization enhanced growth and yield of soybean in the State of Azad Jammu and Kashmir. The results also indicated that high application of P reduced the efficiency of inoculants for nodule mass and seed yield. As for selection of *Bradyrhizobium* spp., the mixture of two strains proved the best one and combination of different strains together might be a beneficial technique. Strategies for maximizing yield should be adopted because of relatively low yield of soybean recorded in the present study. These include use of improved/foreign verieties and strains, changing in sowing time to avoid low temperature in the later part of September and October and soil management practices.

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