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. S0, S1, S2, S3); ii) phosphorus (field only, 0, 50, 100 kg ha\(^{-1}\) i.e. T0, T1, T2) and iii) two soils (pot only) i.e. autoclaved (A) and non-autoclaved (A). A soybean cultivar NARC-I 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 and A 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 S3T1. Shoot dry weight increased significantly from 11.8 g plant\(^{-1}\) in the control soil to 15.6 g plant\(^{-1}\) in S3T1. 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 (S3) was more effective than the strains S1 and S2. Results also indicated that high application of P (100 kg P\(_2\)O\(_5\) 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;
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* (Okerere et al., 2004; Egamberdiyeva et al. (2004a; 2004b). Ashraf et al. (2002) reported a specific combination of soybean genotype with rhizobium strains resulting in a fold increase in the amount of N2 fixed and grain yield ha-1. Hafeez et al. (2001) reported that inoculation with rhizobia should be performed 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 P2O5 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 sieved 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 + S379 designated as S0, S1, S2 and S3, 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 P2O5 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 (S1), S379 (S2) and the mixture of S377+S379 (S3). 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
plot were carefully uprooted at full flowering (R2) stage for the study of growth, root development and nodulation potential of soybean while yield traits were determined at R7 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 S0, S1, S2 and S3, respectively; Phosphorus levels = three i.e. P2O5 at the rate of 50 and 100 kg ha−1 and a control without P application; designated as T1, T2, and T0, 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 (R2 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 A1, S0 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 *Bradyrhizobium japonicum* strains accelerated nodules formation and number increased to a maximum of 25. Strain S3 produced significantly (P
≤0.05) greater number of nodules than the nodules produced by S1 and S2. Nodules dry weight also showed similar pattern of changes that observed for nodules number. In non-autoclaved soil, inoculation with \textit{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 S0. The significant increase in nodule number and mass both in autoclaved and non-autoclaved soils following inoculation indicated that inoculation with \textit{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 cropped with soybean, inoculation is an essential practice for maximum \textit{B. japonicum} following inoculation indicated that inoculation with \textit{rhizobium} strains and phosphorus fertilization. Robson and O'Hara (1981) concluded 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 \textit{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 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 \(\times\) S was also significant (\(p=0.044\)) for this parameter. Mean values for plant height are given in Table 2 showed that S2 and the mixture of S1+S2 (S3) when combine with T significantly reduced plant height inoculation with \textit{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).

Sterilization of soil showed small effect on plant growth and development. The shoot dry weight in non-autoclaved soil without \textit{B. japonicum} was 5.7 g plant\(^{-1}\) compared to 4.0 g in autoclave soil (Fig. 2). \textit{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).

\section*{2. Growth and nodulation in field trial}

Keeping in view the above findings, a field trial was conducted to examine the efficiency of \textit{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 \textit{Trifolium subterraneum} L.) by stimulating host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation and function.

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\begin{center}
\textbf{Table 1.} Analysis of variance (\(p\)) for growth, nodulation and yield characteristics of soybean in response to the application of \textit{rhizobium} strains and phosphorus fertilization.
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\begin{tabular}{lcccccccccc}
\hline
\textbf{Source} & \textbf{Degree of freedom} & \textbf{Plant height} & \textbf{Plant dry weight} & \textbf{Root length} & \textbf{Root dry weight} & \textbf{Nodules number} & \textbf{Nodule fresh weight} & \textbf{Nodule dry weight} & \textbf{No. of pods} & \textbf{100-seed weight} & \textbf{Seed yield} \\
\hline
\textbf{Phosphorus (T)} & 2 & 0.118 & 0.351 & 0.004 & 0.341 & 0.012 & 0.371 & 0.337 & 0.112 & 0.558 & 0.162 \\
\textbf{Strains (S)} & 3 & 0.034 & 0.062 & 0.002 & 0.054 & 0.002 & 0.002 & 0.073 & 0.275 & 0.049 & 0.007 \\
\textbf{T\(\times\)S} & 6 & 0.044 & 0.034 & 0.032 & 0.330 & 0.001 & 0.001 & 0.102 & 0.214 & 0.023 & 0.002 \\
\hline
\end{tabular}
\end{center}
relative to the control and S1. Interactive effect of T×S indicated that height of plant increased significantly with the combinations of S1T1, S1T2 and S2T2. 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 effects indicated that combination of S1T1 and S2T1 significantly increased dry weight of plant by 27 and 52% over control i.e. S0T0 (Table 2). Egamberdieva 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, S1 significantly increased root length while S2 and the mixture of S1+S2 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 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$^{-1}$) |
|-------------------|----------------------|-------------------------------------|
|                   | $T_0$ | $T_1$ | $T_2$ | $T_0$ | $T_1$ | $T_2$ | $T_0$ | $T_1$ | $T_2$ | $T_0$ | $T_1$ | $T_2$ |
| S0                | 63.6  | 75.8  | 70.5  | 70.0  | 11.8  | 15.9  | 13.6  | 13.8  |
| S1                | 69.5  | 72.5  | 71.5  | 71.2  | 12.9  | 13.5  | 12.5  | 13.0  |
| S2                | 66.3  | 64.0  | 70.8  | 67.0  | 14.5  | 12.8  | 15.0  | 14.1  |
| S3                | 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 ($p\leq0.05$) for phosphorus (T) = NS
LSD ($p\leq0.05$) for strains (S) = 3.91
LSD ($p\leq0.05$) for combination (T x S) = 6.76

S0 = no strains; S1 = Strain 377; S2 = Strain 379; S3 = mixture of S377+S379 while $T_0$ = no phosphorus; $T_1$ = P$_2$O$_5$ at the rate of 50 kg ha$^{-1}$; $T_2$ = P$_2$O$_5$ at the rate of 100 kg ha$^{-1}$.

NS = non-significant; R. strains = Rhizobium strains.

### Table 3. Effect of Rhizobium strains *Bradyrhizobium 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$^{-1}$) |
|-------------------|------------------|-------------------------------------|
|                   | $T_0$ | $T_1$ | $T_2$ | $T_0$ | $T_1$ | $T_2$ | $T_0$ | $T_1$ | $T_2$ |
| S0                | 17.7  | 23.7  | 24.0  | 21.8  | 2.15  | 2.95  | 3.27  | 2.8   |
| S1                | 21.7  | 25.8  | 26.6  | 24.7  | 2.83  | 3.25  | 3.52  | 3.2   |
| S2                | 22.2  | 23.4  | 25.9  | 23.8  | 2.90  | 2.89  | 3.43  | 3.1   |
| S3                | 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 ($p\leq0.01$) for phosphorus (T) = 2.14
LSD ($p\leq0.01$) for strains (S) = 2.65
LSD ($p\leq0.05$) for combination (T x S) = 6.2

S0 = no strains; S1 = Strain 377; S2 = Strain 379; S3 = combination of 377+379 while $T_0$ = no phosphorus; $T_1$ = P$_2$O$_5$ at the rate of 50 kg ha$^{-1}$; $T_2$ = P$_2$O$_5$ at the rate of 100 kg ha$^{-1}$.

NS = non-significant; R. strains = Rhizobium strains.
The main effect of T, S and T × S was significant for nodule number (p<0.01; 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 \( S.1 \), \( B. japonicum \) was not significant (p=0.371).

Strain S 1 did not show significant difference with the indigenous bradyrhizobia population infecting soybean. However, the dry weight of nodule did not show significant changes.

The main effect of T, S and T × S was significant for fresh weight of nodules (p≤0.05; 0.005 and 0.001 for T, S and T × S, respectively). Similarly, application of strains and TxS was also significant for fresh weight of nodules (p=0.001) while T alone was not significant (p=0.371). Inoculation of soybean with \( B. japonicum \) strains \( S.1 \) and \( S.2 \) resulted in significant increase in nodule number and nodule fresh weight, indicating that the inoculant \( B. japonicum \) 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 \( S.1 \) and \( S.2 \), strain \( S.1 \) did not show significant difference with the control (Table 4).

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, nodule numbers, fresh and dry weight in \( S.1 \) was relatively higher than those found in \( S.2 \) and \( S.3 \), indicating the synergistic effect of two strains used in the inoculum (\( S.77 + S.379 \)). 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.

Inoculation and Soybean Yield in Kashmir

Table 4. Effect of \( R. \) strains \( B. japonicum \) 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 \(^{-1}\) | Nodule fresh weight (g plant \(^{-1}\)) | Nodule 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 | 3.75 | 2.49 | 2.9 | 0.52 | 0.45 | 0.55 | 0.51 |
| \( S_2 \) | 66.3 | 60.5 | 42.6 | 56.5 | 3.83 | 3.15 | 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 \( (p≤0.05) \) for phosphorus (T) =10.2 LSD \( (p≤0.05) \) for phosphorus (T) =NS LSD \( (p≤0.05) \) for phosphorus (T) =NS

LSD \( (p≤0.05) \) for strains (S) = 14.62 LSD \( (p≤0.05) \) for strains (S) = 0.67 LSD \( (p≤0.05) \) for strains (S) =NS

LSD \( (p≤0.05) \) for combination (T × S) =18.2 LSD \( (p≤0.05) \) for combination (T × S) =1.17 LSD \( (p≤0.05) \) 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\(_2\)O\(_5\) at the rate of 50 kg ha\(^{-1}\); \( T_2 \) =P\(_2\)O\(_5\) at the rate of 100 kg ha\(^{-1}\).

ns = non-significant; R. strains = \( R. \) strains.
Inoculation with rhizobium strains S1 and S2 did not show significant increase in seed yield and the average values across different strains were almost similar. The percent increase in seed yield due to the inoculation of soybean by the mixture of both strains (S3) increased seed yield significantly i.e. 21% over control. Phosphorus fertilization did not show significant increase in seed yield and all combinations except S1T1, S2T2 and S3T2 showed significantly higher yield than the control (S0T0). The maximum seed yield in combine treatments were observed in S1T2 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 S3T2. Similarly application of phosphorus at the rate of 100 kg P\(_{2}\)O\(_{5}\) ha\(^{-1}\) (T2) in the soil without strains (S0) 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 T0, T1 and T2. 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 combination of both showed more significant increase in seed yield and all combinations except S1T1, S2T2 and S3T2 showed significantly higher yield than the control (S0T0). The maximum seed yield in combine treatments were observed in S1T2 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 S3T2. Similarly application of phosphorus at the rate of 100 kg P\(_{2}\)O\(_{5}\) ha\(^{-1}\) (T2) in the soil without strains (S0) 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 T0, T1 and T2. 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.

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 \((T \times S, p=0.002)\). 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 S0) gave the lowest seed yield i.e. 721 kg ha\(^{-1}\) (Table 5). Inoculation with rhizobium strains S1 and S2 did not show significant increase in seed yield over control but the mixture of both strains (S3) 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 percent increase in 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 involved in the process of increase in nodulations with phosphorus nutrition and its role in soybeans is poorly understood. Nodules spp. strains S1, S2 and S3 clearly demonstrated that rhizobium inoculation with the mixture of S1+S2 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 \((R^2=0.91)\). Un inoculated 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

### 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\(^{-1}\)) |
|------------|---------------------------|---------------------|-----------------------------|
|            | T0 | T1 | T2 | mean | T0 | T1 | T2 | mean | T0 | T1 | T2 | mean |
| S0         | 29.8 | 34.5 | 29.3 | 29.2 | 13.4 | 15.56 | 15.9 | 15.0 | 543 | 740 | 879 | 721 |
| S1         | 29.0 | 29.7 | 32.3 | 30.3 | 16.4 | 13.8 | 15.9 | 15.4 | 766 | 621 | 767 | 718 |
| S2         | 26.0 | 30.8 | 35.5 | 30.8 | 15.8 | 15.5 | 14.3 | 15.2 | 811 | 846 | 615 | 757 |
| S3         | 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 strains (S)=NS; LSD \((p \leq 0.01)\) for strains (S)=0.85; LSD \((p \leq 0.05)\) for combination (T\(\times\)S)=NS; LSD \((p \leq 0.01)\) for combination (T\(\times\)S)=1.417; LSD \((p \leq 0.01)\) for combination (T\(\times\)S)=295.

S\(_0\)= no strains; S1=Strain 377; S2=Strain 379; S3=combination of 377+379 while T0 = no phosphorus; T1 = P\(_{2}\)O\(_{5}\) at the rate of 50 kg ha\(^{-1}\); T2 = P\(_{2}\)O\(_{5}\) at the rate of 100 kg ha\(^{-1}\).

ns= non-significant; R. strains = Rhizobium strains.
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 Bradyrhizobium 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 18ºC to <15ºC. 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 varieties 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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