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Full Length Research Paper

Effect of *Rhizobium* inoculation and NP fertilization on growth, yield and nodulation of soybean (*Glycine max* L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan

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This research experiment was conducted to examine the effect of *Rhizobium* inoculation (RI) and P fertilization (P) on nodulation, growth and yield characteristics of soybean grown in the presence of starter N fertilizer (N). Treatments included: i) 02 levels of RI (RI_0, RI_1), ii) 02 levels of P (P_0, P_1 that is, P_2O_5 @ 90 kg ha^{-1}) iii) 03 levels of N (N_0, N_1 and N_2 that is, N at of 25 and 50 kg N ha^{-1}) iv) 03 replication. Results of the experiment revealed that total number of nodules increased from 73 in the un-inoculated control to 125 and 95 following the application of RI and P representing 70 and 30% increase over control. N supply did not affect the number of nodules; however, combination of RI and P with 25 kg N ha^{-1} ($RI_1P_1N_1$) produced the highest number of nodules (152). RI, P, N and their combinations increased shoot and root biomass. Seed yield in the control was 767 kg ha^{-1} that significantly increased to 1081, 907 and 940 kg ha^{-1} following the application of RI, P and N demonstrating a 41, 18 and 23% increase over control. The highest seed yield of 1208 kg ha^{-1} was recorded in the combine treatment of $RI_1P_1N_1$ indicating 57% increase over control. Relative increase in dry matter yield due to RI, P and N was 63, 46 and 49%. Seed protein content in different treatments ranged between 33 - 40% while oil content ranged between 13 - 18%. Application of RI, P and their combinations increased protein content by 6 - 22% while increase in oil content was 12 - 35%. Concentrations of N and P in plants and their uptake was significantly increased and relative increase in N uptake due to RI, P and K was 77, 21 and 31% , respectively, while the corresponding increase in P uptake was 79, 92 and 56%. It was found that the efficiency of RI and P fertilization increased substantially with the application of 25 kg N ha^{-1} but the efficiency decreased when N supply increased from 25 kg N ha^{-1} to 50 kg N ha^{-1}. The results demonstrate the potential benefits of using *Rhizobium* inoculation and P fertilization with reduced level of N as starter fertilizer in order to achieve plant-growth promotion, increased nodulation and seed yield of soybean.

Key words: *Glycine max* L., nodulation, N fertilization, P application, soybean, *Rhizobium* inoculation, soybean.

INTRODUCTION

Forages and grain legumes are considered important for maintaining soil fertility and crop productivity in degraded soils (Hafeez et al., 2001). Legumes played a critical role in natural ecosystems, agriculture, agro-forestry, where their ability to fix atmospheric N_2 in symbiosis make them excellent colonizer of low -N environments (Graham and Vanace, 2003). Biological nitrogen fixation (BNF) capacity

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of legumes is a vital process for sustainable crop-land management and is an effective and efficient source of N supply to plants under favorable atmospheric and environmental conditions (Hungria and Vargas, 2000; Chen et al., 2002). The efficiency of the legumes is affected by various factors, e.g. soil moisture, available N and the presence of efficient and competitive rhizobial strains (Thies et al., 1995; Palmar and Young, 2000). With regard to their potential for N_2 fixation, presence of native rhizobia in soil or the development and application of rhizobial inoculants is important (Giller and Cadisch, 1995). Inoculation is a significant technology for the manipulation of rhizobia for improving crop productivity and soil fertility (Keyser and Li, 1992). It can lead to the establishment of large rhizobial population in the rhizosphere and improved nodulation and N_2-fixation even under adverse soil conditions (Peoples et al., 1995).

Generally it is considered that inoculation with rhizobia should be performed in two different situations: (1) in soils which are depleted or contain a low indigenous rhizobial population and (2) when there is an established but inefficient rhizobial population (Araujo et al., 1994). In Pakistan, soils have either nil or very low viable count of effective rhizobia and low nitrogen-supplying capacity because of low organic matter content (0.3-1.0%) (Achakzai, 2007). Therefore, there is need to inoculate legumes that had not been previously grown in a particular area as previously done in many parts of the world (Hafeez et al., 2000, 2001; O' Hara, 2001; Bruno et al., 2003; Naeem et al., 2004).

Phosphorus (P) and nitrogen (N) play specific role in symbiotic N_2-fixation through their effects on nodulation and N_2-fixation process (O' Hara et al., 2002). N_2-fixation is very sensitive to P deficiency due to reduce nodule mass and decreased ureide production (Sinclair and Vadez, 2002; Vanace, 2001). Symbiotic nitrogen fixation has a high P demand because the process consumes large amounts of energy (Schulze et al., 2006) and energy generating metabolism strongly depends upon the availability of P (Israel, 1987; Plaxton, 2004). Several reports have documented that nodules are a strong P sink and nodule P concentration normally exceeds that of roots and shoots (Sa and Israel, 1991; Drevon and Hartwig, 1997). Similarly, mineral N fertilization is a crucial factor in oil seeded legume production (Rathke et al., 2005). Although the N requirement of soybean can be met by both mineral N assimilation and symbiotic N_2 fixation (Harper, 1974), yet soybean has a relatively high N demand crop. Most studies on the effect of fertilizer-N on soybean growth and N_2 fixation showed that N fertilization increased growth but reduced N_2 fixation through a reduction in the number, weight and activity of nodules (Chen et al., 1992; Starling et al., 1998). How-ever, this effect depended on a variety of factors such as amount of N applied and type of soil, climate and farming system. A few studies showed that application of reduced amount of N as starter fertilizer could improve growth, N uptake and fixation by soybean (Gulden and Vessey, 1998; Ray et al., 2006).

Among grain legumes, soybean (*Glycine max* (L) Merril) is economically important crop that is grown in diverse environment throughout the world. It could be used in a wide range of products from hand lotion to diesel fuel. However, soybean is primarily used for high protein meal and secondarily as oil crop and accounts for 30% of world's processed vegetable oil (Graham and Vanace, 2003). Introduction of soybean in the areas like the state of Azad Jammu and Kashmir could change the agriculture pattern and trend of local farming community because of its potential to restore soil fertility by N_2 fixation. Moreover, its establishment in area will appear as a mean of improving productivity if properly managed, that is, inoculation and fertilization.

The aims of the present study were to examine the growth, yield and nodulation potential of soybean under the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir and the response of soybean to the application of *Rhizobium* inoculation and NP fertilization.

MATERIALS AND METHODS

Site description

The study was conducted in the experimental farm of the Faculty of Agriculture, Rawalakot, Poonch Division, (situated 110 km northeast from Federal Capital, Islamabad, Pakistan), Azad Jammu and Kashmir. The study area lies between 33−36^{o} N latitude and longitude 73-75°E with an altitude of 1633 m above sea level. Submountainous topography of valleys and stretched plains with temperate sub-humid climate are the characteristic of the area. Precipitation depends on season and falls irregularly while frequent intense storms occur during monsoon season. Average rainfall varies from 500-2000 mm annually. Summer is mild with mean annual temperature of 27°C while fairly cold temperature even below freezing point prevails during winter. Rainfed cropping system governs agriculture and maize (*Zea mays* L.) is the major crop of the region. Seasonal vegetables are cultivated as kitchen gardening while fruit orchards occupy noticeable area. A detailed soil survey and profile study of the area has yet to be done.

Field manipulation, experimental description and treatments

The field experiment was initiated during summer (Kharif, 2003). The soil of the experimental site was silt loam in texture with an organic matter of 0.64%, pH of soil paste 7.05, 1.10 g kg^{-1} total N, 2.6 mg kg^{-1} available P and 54 mg kg^{-1} available K in the top 15 cm depth. The meteorological data of the experimental area during the experimental period was collected and presented in Figure 1. The actual plot was ploughed thoroughly twice with tractor and divided into sub-plots in accordance with the treatments. The net plot size was 2 × 2 m^2. The field was appropriately leveled for smooth and proficient distribution of fertilizer/water. The experiment was designed in a randomized complete block design (RCBD) with three replications. There were three (3) levels of nitrogen including control (0, 25 and 50 kg N ha^{-1} designated as N_0, N_1, N_2), two levels of phosphorus (0 and 90 kg P_2O_5 ha^{-1} denoted as P_0, P_1) and two levels of rhizobial inoculation (RI) (uninoculated and inoculated hereafter RI_0, RI_1). There were three replications of each treatment. Altogether there were 36 combinations representing the individual effect of N, P, RI and their interactions. Urea was used as N source

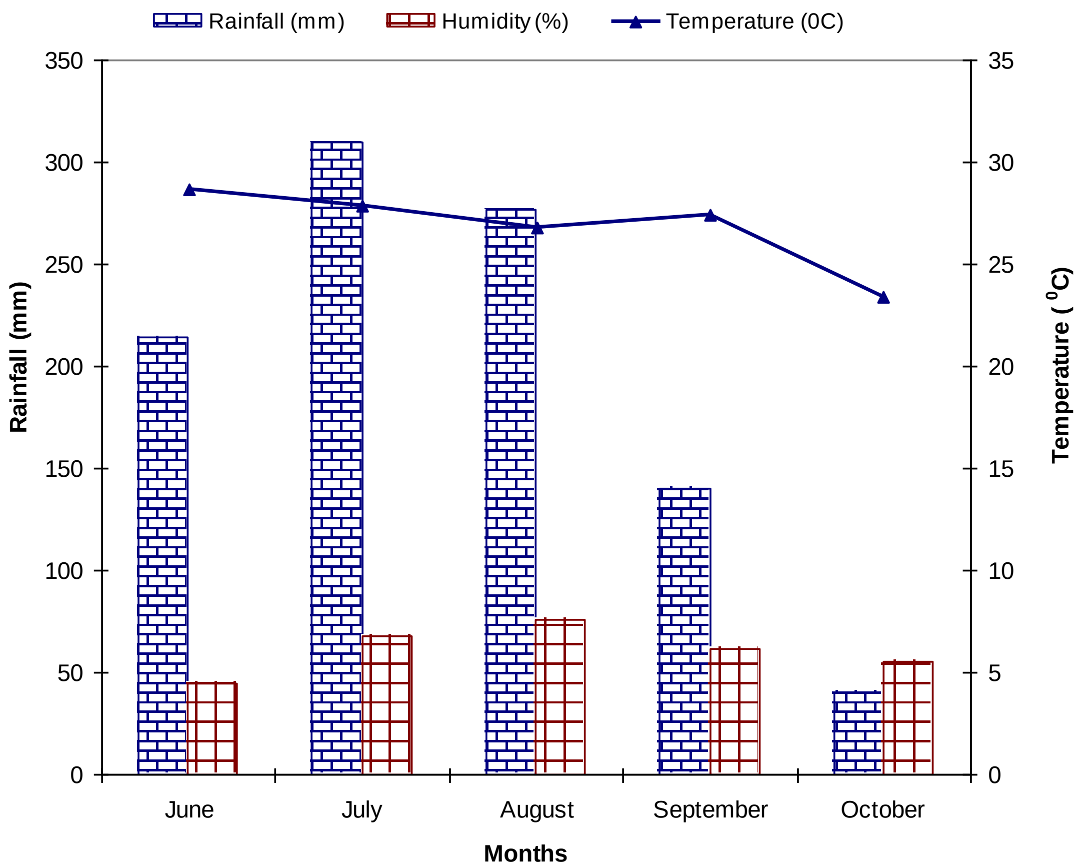

Figure 1. Meteorological data recorded during the experiment.

while P was applied as single super phosphate. Both fertilizers were broadcast by hand and incorporated immediately before planting into their respective plots.

In all inoculated treatments, seeds were treated with peat inoculant containing TAL-377 strain of *Bradyrhizobium japonicum*, obtained from Soil Biology and Biochemistry, section, NARC, Islamabad. A 10% (w/v) sucrose solution was used to ensure adhesion of the inoculant, applied at 300 ml, 50 kg^{-1} seeds. For the control treatments, seeds received only sucrose solution. Seed inoculation took place at sowing and consisted of applying the sucrose solution to the seeds, followed by the peat inoculant. After mixing, seeds were allowed to air-dry in the shade for 15 min and sown within a maximum of 4 h.

Soybean cultivar NARC-1 was used in the experiment. Seeds were obtained from Oil and Seed Department, National Agricultural Research Centre (NARC), Islamabad. Seeds were hand planted in lines on 16 June 2003. Each plot contained four planting lines and the space between rows was 30 cm. On the planting row, soybean was seeded at a 5 cm distance within-row. A total of twelve treatments were used in the experiment. These were the combination of different levels of N fertilizer with inoculation of *B. japonicum* (TAL-377) and fertilizer P. All standard local cultural practices were accomplished throughout the growth period. Manual weeding was done when required.

Nodulation

Plant samples were taken from five randomly selected plants in the central rows at R_2 stage. Plants were carefully uprooted with the help of spade by digging soil core of 150 mm around the plant and 200 mm in depth. Each core contained primary roots of two to three plants. The plants were then placed into plastic buckets full of water to loosen the adhering soil. After 15 - 20 min the adhering soil was carefully removed. Thereafter, plants were separated into roots and shoots by cutting from the first unable node. The roots were brought into the laboratory, followed by washing of roots under running water with a screen underneath to catch the detached nodules. Care was taken to avoid damage to nodules. Nodules from roots were picked and following data were recorded: (1) Total number of nodules $plant^{-1}$, and (2) dry weight of nodules $plant^{-1}$.

Morphological characteristics

At pod filling stage (R_4), plants were carefully uprooted (as for nodulation) to determine the morphological characteristics of soybean. Different growth characteristics of crop like plant height, shoot dry weight, root dry weight and root length were studied. In the laboratory, plants were separated into shoot and root by cutting from the first unable node. The roots were washed with tap water. Plant height and root length was measured with the help of a meter rod. Root and shoot dry weight $plant^{-1}$ was taken after oven drying at 70°C till constant weight.

Yield and yield attributes

Yield attributes were determined at full maturity (R_8) from randomly selected ten plants from two central rows. Numbers of pods $plant^{-1}$

Table 1. Effect of *Rhizobium* inoculation and NP fertilization on nodulation of soybean (number of nodules and dry weight of nodules) grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	Number of nodules plant^{-1}	Nodules dry weight (g)
$RI_0N_0P_0$	73g	0.89b
$RI_1N_0P_0$	125c	1.36b
$RI_0N_0P_1$	95de	1.04b
$RI_0N_1P_0$	79fg	0.56b
$RI_0N_2P_0$	72g	0.73b
$RI_1N_0P_1$	140b	1.53a
$RI_1N_1P_0$	124c	1.33ab
$RI_1N_2P_0$	103d	1.14b
$RI_0N_1P_1$	100de	1.09b
$RI_0N_2P_1$	89ef	0.85b
$RI_1N_1P_1$	152a	1.52a
$RI_1N_2P_1$	95de	1.06b
LSD	11.51	0.86

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N ha$^{-1;}$ $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}. Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

were counted before picking. Pods were removed from the plants and threshed by hand. Grain yield was corrected for 13% moisture content after determining humidity level with a grain moisture tester. Total above ground dry matter yield was the sum of selected plants after sundrying in the field for more than 7 days.

Plant analysis

Total nitrogen in shoot and seed was estimated by Kjeldhal digestion, distillation and titration method (Bremner and Mulvaney, 1982). Phosphorus content in shoot and seed was estimated by wet digestion with a 2:1 mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$). The P content was then determined by the vanado-molybdate yellow color using spectrophotometer at 440 nm.

Protein and oil contents

Seeds of the selected plants were milled, air dried and sifted (2 mm mesh). Crude protein content was calculated by multiplying Kjeldhal nitrogen concentration by 6.25 (Nelson and Sommers, 1973). The oil content of seeds was determined by a Soxhlet extraction method using n-hexane as solvent at 70°C for 6 h (Anon, 1987).

Statistical analysis

The data were analyzed with analysis of variance (ANOVA) using MSTAT-C statistical software for windows (Version 1.3). Significantly different means were separated using Duncan's protected least significant differences test (LSD) at $P \leq 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Nodulation

The number and weight of nodules are commonly used as the criteria of effective complementary interaction between macro- and micro- symbionts; thereby correlate on the whole with the rate of atmospheric nitrogen fixation (Datsenko et al., 1997). Application of RI and P fertilization (alone) significantly increased nodule numbers from 73 in uninoculated control ($RI_0N_0P_0$) to 125 and 95 in RI and P treatments, respectively (Table 1). Addition of both levels of N (25 and 50 kg N ha^{-1}) alone failed to impart any significant effect on nodules numbers when compared with control. However, nodules number increased from 79 in $RI_0N_1P_0$ to 124 when N was applied with RI ($RI_1N_1P_0$). Noticeable numbers of nodules in the control treatments reflects the presence of indigenous rhizobia capable of nodule formation in the soil, although smaller in size, on lateral roots and most of them are ineffective (white in color). However, greater number of nodules due to inoculation suggested that there is better combining and symbiotic relationship between introduced rhizobia and soybean. Larger response to inoculation and higher number of nodules per plant in comparison to uninoculated treatments in a field that has no soybean cropping history was also reported by Revellin et al. (2000) and Abbasi et al. (2008). Application of P alone ($RI_0N_0P_1$) enhanced nodulation by 30% while its combination with *Rhizobium* inoculation (RI) ($RI_1N_0P_1$) resulted in 47% more nodules than P alone. Application of P with N significantly increased nodules number by 26 and 23% in the absence of RI and by 95 and 31% in the presence of RI. It has been long recognized that P in coincidence 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). The maximum nodulation were obtained when P and RI was combined with 25 kg N ha^{-1} ($RI_1N_1P_1$) resulted in 2-fold increase in nodules number compared with the control. The number of nodules in $RI_1N_1P_1$ (25 kg N ha^{-1}) was 152 those decreased to 95 when rate of N was increased from 25 to 50 kg N ha^{-1} indicating that efficiency of RI decreased with high rate of N fertilizer. Nodules mass also showed similar trend that recorded for nodules number. Combination of RI with P without N ($RI_1N_0P_1$) showed the maximum nodules mass of 1.53 g that was at par with the nodules mass of 1.52 g recorded for $RI_0N_1P_1$.

Morphological characteristics

The morphological characteristics of soybean were signifi-

Table 2. Effect of *Rhizobium* inoculation and NP fertilization on shoot and root biomass of soybean (height and dry weight of shoot, length and dry weight of roots) grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Root length (cm)
$RI_0N_0P_0$	64.69e	22.80e	2.13b	19.77d
$RI_1N_0P_0$	91.26ab	35.97abc	2.84ab	30.75bc
RI_0N_0P	82.11cd	25.50de	3.49ab	35.18ab
$RI_0N_1P_0$	78.24d	29.80bcde	2.38b	30.03c
$RI_0N_2P_0$	82.16cd	29.65cde	2.87ab	31.12bc
$RI_1N_0P_1$	88.16abc	37.64ab	2.84ab	32.44bc
$RI_1N_1P_0$	91.53ab	32.97bcd	3.17ab	33.23bc
$RI_1N_2P_0$	82.80cd	31.12bcd	2.81ab	29.97c
$RI_0N_1P_1$	78.08d	28.54cde	3.06ab	32.37bc
$RI_0N_2P_1$	85.55bcd	30.56bcde	3.04ab	31.98bc
$RI_1N_1P_1$	94.05a	40.84a	4.44a	38.86a
$RI_1N_2P_1$	83.65bcd	30.77bcd	2.91ab	30.24c
LSD	7.56	6.96	1.54	4.04

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N ha^{-1}; $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}.
Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

cantly affected by RI (Table 2). Average increase in plant height, shoot dry weight (SDW), root length and root dry weight (RDW) due to RI was 41, 58, 63 and 77% respectively, as compared to uninoculated control. Bai et al. (2002) and Okereke et al. (2004) reported 29% and 2 - 130% increase in SDW following the inoculation with *B. japonicum*, respectively. Egamberdiyeva et al. (2004a) reported that *Rhizobium* inoculation increased soybean soot DW and root DW by 7-23% and 57-78%, respectively. Sobral et al. (2004) explained that use of B*radyrhizobium* isolates were able to produce IAA, solubilize phosphate and fix nitrogen which could be used for soybean growth promotion. Similarly, application of P alone ($RI_0N_0P_1$) increased plant height, SDW, root length and RDW by 28, 11, 63 and 77% respectively, which inveterate the findings of Abbasi et al. (2008) and Mahmood et al. (2009).

Plant height and root length showed significant response to N application while SDW and RDW did not. Mrkovački, et al. (2008) reported increased growth of soybean at lower (30 kg N ha^{-1}) dose of N fertilization while Diep et al. (2002) concluded that increased level of N fertilization reduced growth of vegetable soybean. Application of P alone significantly increased root length and RDW by 33 and 64% over the control. Combination of RI and P ($RI_1N_0P_1$) increased plant height and SDW by 7 and 47% compared to $R_0N_0P_1$ while root length and RDW were almost unaffected. Similarly, RI with both N levels ($RI_1N_1P_0$) and ($RI_1N_1P_0$) enhanced soybean growth. Average over two N levels, increase in plant height, SDW, root length and RDW due to combine application of N with RI was 8, 7, 13 and 3% compared to their respective control. Kubota et al. (2008) and Diep et al. (2002) obtained increased growth of soybean fertilized with N in the presence of RI. Benefits of inoculation and N application are generally thought to be higher if N is a limiting factor, or deficient in soil (Peoples and Craswell, 1992) or because of large N demand of high yielding cultivars (Dashti et al., 1998). All the growth parameters studied during the investigation were highest in the treatment $RI_1N_1P_1$. Facilitating plant growth with application of reduced level of N as starter fertilizer at planting together with application of P for root growth and development and RI for nodulation collectively enhanced plant growth and development.

Yield and yield components

Rhizobium inoculation alone ($RI_1N_0P_0$) significantly increased numbers of pods $plant^{-1}$, dry matter yield and seed yield by 85, 62 and 41% over the control (Table 3). Reported symbiosis between soybean and *B. japonicum* could be the possible explanation to the above findings. Increased nodulation resulted in more N_2-fixation that leads to increased yield components. Increase in pods number, seed and dry matter yield due to *Rhizobium* inoculation was well documented in many parts of the world. Tien et al. (2002) reported 6 and 10%, Okereke et al. (2004) 14 - 100%, Egamberdiyeva et al. (2004a) 48 - 55% increases in seed and dry matter yield due to inoculation. Similarly, Ashraf et al. (2002), Oad et al. (2002)

Table 3. Effect of *Rhizobium* inoculation and NP fertilization on yield and yield components of soybean [number of pods planr^{-1}, seed yield (kg ha^{-1}) and dry matter yield (kg ha^{-1})] grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	Number of pods plant^{-1}	Grain yield (kg ha^{-1})	Dry matter yield (kg ha^{-1})
$RI_0N_0P_0$	34f	767f	1149f
$RI_1N_0P_0$	63abc	1081bc	1871c
RI_0N_0P	42e	907e	1683e
$RI_0N_1P_0$	43e	940de	1708e
$RI_0N_2P_0$	49de	935cde	1706e
$RI_1N_0P_1$	57bcd	928de	1693e
$RI_1N_1P_0$	59abc	989bcde	1782d
$RI_1N_2P_0$	55cd	981bcde	1778d
$RI_0N_1P_1$	61abc	1092ab	1954b
$RI_0N_2P_1$	65ab	1052bcd	1851c
$RI_1N_1P_1$	66a	1208a	2330a
$RI_1N_2P_1$	60abc	1041bcd	1834c
LSD	7.72	116.6	50.02

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N ha$^{-1;}$ $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}.
Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

and Abbasi et al. (2008) in Pakistan, reported increase in seed yield due to *Rhizobium* inoculation. Application of P alone ($RI_0N_0P_1$) increased pod plant^{-1}, seed and dry matter yield by 23, 18 and 46% when compared with control. This might be due to adequate supply of phosphorus which in turn increased the caroxylation efficiency and increased the ribulose-1-5-diphosphate carboxylase activity, resulting in increased photosynthetic rate, growth and yield (Jacob and Lawlor, 1992). Combination of RI with P ($RI_1N_0P_1$).resulted in 67% increase in number of pods plant^{-1} with 21% increase in seed yield Gentili and Huss-Danell, (2003) and Fatima et al. (2006) concluded that combined application of P with *Rhizobium* inoculation increased growth, yield and nitrogenease activity as well as improved soil fertility for sustainable agriculture.

Average increase in number of pods plant^{-1}, dry matter yield and seed yield due to application of starter N was 35, 48 and 22%, respectively. Yinbo et al. (1997) and Starling et al. (1998) reported that only rarely could seed yield of legumes be enhanced by starter-N fertilizer application. A starter-N effect will occur only when the host plant shows poor nodulation or when ineffective rhizobia are present (Kessel and Hartley, 2000). However, results indicated positive effect of starter fertilizer on the yield components of soybean in our conditions. The maximum increase of 94, 102 and 57% for pods plant^{-1}, dry matter and seed yield were recorded in the treatment where 25 kg N ha^{-1} was combined with P and RI ($RI_1N_1P_1$) while yield components were decreased when rate of N was increased to 50 kg N ha^{-1}. Mrkovacki et al. (2008) reported maximum results for number of pods plant^{-1}, seed and dry matter yield by applying 30 kg N ha^{-1} to inoculated soybean instead of higher rates of N. Tien et al. (2002) and Diep et al. (2002) concluded that application of about 25 kg N ha^{-1} together with rhizobial inoculation seemed to be an appropriate cultivation practice.

Oil and protein content

Seed protein and oil content in the un-inoculated control was 32.7 and 13.4%, respectively (Table 4). Application of RI, P, N and their combinations increased protein and oil content ranging from 33 - 40% for protein and 14 - 18% for oil. The percent increase in protein due to RI, P and N over the control was 9, 3 and 1% while the corresponding increase in oil content was 12, 5 and 0% respectively showing that application of N had no effect on protein and oil content of soybean. The highest protein (40%) and oil content (18%) was recorded in the combined treatment of RI and P with 25 kg N ha^{-1}($RI_1N_1P_1$) demonstrating an increase of 22 and 35% over the control. Egamberdiyeva et al. (2004a,b) reported 23 and 10% increase in protein content and 19% increase in oil content of soybean following the inoculation of *B. japonicum*. Weber (1966) showed a slight increase in protein concentration and a slight decrease in oil concentration with fertilizer N applied to nodulated soybean whereas Ray et al. (2006) and Purcell et al. (2004) found that higher rates of fertilizer N significantly reduce protein contents while oil concentration was increased in soybean seed. We found 33% protein in N_1 and 35% in N_2 while

Table 4. Effect of *Rhizobium* inoculation, P fertilization and varying levels of N fertilizer on protein and oil contents of soybean grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	Seed protein (%)	Oil content (%)
$RI_0N_0P_0$	32.7e	13.3bc
$RI_1N_0P_0$	35.7bc	15.0abc
RI_0N_0P	33.7cde	14.0bc
$RI_0N_1P_0$	33.1de	13.0c
$RI_0N_2P_0$	35.0bcd	14.5bc
$RI_1N_0P_1$	35.1bcd	15.9abc
$RI_1N_1P_0$	36.2b	14.0bc
$RI_1N_2P_0$	35.1bcd	13.7bc
$RI_0N_1P_1$	34.5bcde	16.7ab
$RI_0N_2P_1$	33.5cde	15.2abc
$RI_1N_1P_1$	39.7a	18.0a
$RI_1N_2P_1$	34.5bcde	16.0abc
LSD	1.98	2.94

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N $ha^{-1;}$ $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}. Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

the protein content for N_1 was 13% and N_2 was 14.5%. However, in the combine treatments of RI_1P_1 with 25 kg N ha^{-1} that is, $RI_1N_1P_1$ protein and oil content was the highest 39.7 and 18% than those decreased to 34.5 and 16% when RI_1P_1 was combined with 50 kg N ha^{-1}.

N content and uptake

Application of RI alone ($RI_1N_0P_0$) increased soybean seed N, shoot N and N uptake by 9, 122 and 76% over un-inoculated control (Table 5). Increase in N contents in seed, shoot and total N uptake due to *Rhizobium* inoculation was mainly due to significant increased in nodulation, resulted in higher accumulation of N due to atomspheric N_2-fixation. Number of nodules showed significant correlation with seed N (R^2 = 0.64), shoot N (R^2 = 0.45) and total N uptake (R^2 = 0.57). Significant increase in seed and shoot N of soybean inoculated with *B. japonicum* was previously reported by Seneviratne et al. (2000), Sarr et al. (2005) and Zhang et al. (2002). Application of P alone did not show significant response to N contents of seed and shoot but uptake of N was significantly increased by P application. Shu-Jie et al. (2007) reported that application of P increased N concentration in shoot and root and N in shoot correlated well with P supply. Combinations of P with RI significantly increased N contents in seed and shoot and also total N uptake. Addition of both levels of N (25 and 50 kg N ha^{-1}) significantly increased total N content in shoots, N uptake in seed and N uptake in shoot relative to control while N contents in seed was not affected. The maximum increase in N content and N uptake both in seed and straw was recorded in the treatment where RI and P were combined with 25 kg N ha^{-1} ($RI_1N_1P_1$). Application of *Rhizobium* inoculation and P increase nitrogenease activity, nodule mass that ultimately increased plant N content and uptake while addition of reduced level of N (starter fertilizer) fulfill the immediate need of N to plants and these combinations leading to higher N content and N uptake in plant.

Phosphorus content and P uptake

Response of P content in seed and shoot to RI was non-significant while uptake of P in seed and shoot was significantly increased by RI (Table 6). The total uptake of P was increased by 79% following the application of RI compared with the control. Higher P uptake due to *Rhizobium* inoculation was due to the ability of applied rhizobia to solubilize precipitated P components thereby increased P uptake in plants as reported by Fatima et al. (2007). The higher P concentration in plant benefits the bacterial symbiont and the functioning of its nitrogenease activity, leading to increased nitrogen fixation. Application of P alone resulted in 28 and 84% increase in seed and straw P content while P uptake was increased by 91% over the control. Phosphorus contents in seed and straw and total P uptake were further improved to a maximum of 37, 107 and 112% (over control) due to the combine application of P with RI ($RI_1N_0P_1$). Olivera et al. (2004) reported that phosphorus application to legumes increase plant biomass including nodule biomass and shoot P content due to the increased rate of nitrogen fixation. Increased P contents of straw, seed and P uptake due to combine application of P and RI was also reported by Moharram et al. (1994). P contents in seed and shoot was not affected by applying 25 kg N ha^{-1} but P uptake was significantly increased. However, application of 50 kg N ha^{-1} increased both N content and N uptake of soybean. The maximum P content and P uptake in grain and straw was recorded in $R_1N_1P_1$ while application of relatively higher rate of N that is, 50 kg N ha^{-1} significantly decreased P content and uptake by soybean.

Conclusion

The results of present investigation reveal that application of *Rhizobium* inoculation (RI) alone or in combination with P and N significantly increased nodulation of soybean. A 2-fold increase in nodules number was recorded in the combine treatment of $RI_1P_1N_1$. A substantial Increase in

Table 5. Effect of *Rhizobium* inoculation and NP fertilization on N content of plant shoot and seed and its uptake by soybean grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	Seed N content ($g\ kg^{-1}$)	Shoot N content ($g\ kg^{-1}$)	Seed N uptake ($kg\ ha^{-1}$)	Shoot N uptake ($kg\ ha^{-1}$)	Total N uptake ($kg\ ha^{-1}$)
$RI_0N_0P_0$	52.2e	20.6d	40.04g	23.66i	83.66l
$RI_1N_0P_0$	57.0bc	45.8ab	61.61b	85.70b	147.3b
RI_0N_0P	53.9cde	30.8cd	48.88f	51.38h	100.71k
$RI_0N_1P_0$	52.8de	34.5bc	49.60f	58.92g	108.52j
$RI_0N_2P_0$	56.0bcd	42.8ab	52.36	73.01	125.40h
$RI_1N_0P_1$	56.0bcd	44.9ab	51.95e	76.01d	127.96g
$RI_1N_1P_0$	57.8b	42.6ab	57.15c	75.92d	133.06e
$RI_1N_2P_0$	56.0de	43.1ab	54.90d	76.63	131.53f
$RI_0N_1P_1$	55.2bcde	38.8abc	60.27b	75.81d	136.08c
$RI_0N_2P_1$	53.6cde	36.3abc	56.38cd	67.19f	123.57i
$RI_1N_1P_1$	63.5a	47.4a	76.70a	110.44a	187.14a
$RI_1N_2P_1$	55.1bcde	42.6ab	57.35c	78.12c	135.47d
LSD	3.2	10.2	17.1	09.2	3.2

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N ha^{-1}; $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}. Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

Table 6. Effect of *Rhizobium* inoculation and NP fertilization on P content (shoot and seed) and its uptake by soybean grown under field conditions at Rawalakot Azad Jammu and Kashmir.

Treatments	P content in seed ($g\ kg^{-1}$)	P content in shoot ($g\ kg^{-1}$)	Seed P uptake ($kg\ ha^{-1}$)	Shoot P uptake ($kg\ ha^{-1}$)	Total P uptake ($kg\ ha^{-1}$)
$RI_0N_0P_0$	4.5f	1.3e	3.4d	1.4f	4.8h
$RI_1N_0P_0$	5.3cdef	1.6de	5.7b	2.9e	8.6ef
RI_0N_0P	5.8bc	2.4abc	5.2bc	4.0c	9.2d
$RI_0N_1P_0$	4.8ef	1.8cde	4.5c	3.0e	7.5g
$RI_0N_2P_0$	4.9def	1.7cde	4.5c	2.9e	7.4g
$RI_1N_0P_1$	6.2ab	2.7ab	5.7b	4.5b	10.2c
$RI_1N_1P_0$	5.1cdef	2.0bcde	5.0bc	3.5d	8.5f
$RI_1N_2P_0$	5.4bcde	2.2abcd	5.2bc	3.9cd	9.1de
$RI_0N_1P_1$	5.2cdef	2.4abc	5.6b	4.6b	10.2c
$RI_0N_2P_1$	5.6bcd	2.3abcd	5.8b	4.2bc	10.0c
$RI_1N_1P_1$	6.9a	2.8a	8.3a	6.5a	14.8a
$RI_1N_2P_1$	5.9bc	2.6ab	6.1	4.7b	10.8b
LSD	7.4	6.6	7.9	4.7	4.2

*$RI_0N_0P_0$: Control (no inoculation and no fertilizer); $RI_1N_0P_0$: *Rhizobium* inoculation; $RI_0N_0P_1$: 90 kg P_2O_5 ha^{-1}; $RI_0N_1P_0$: 25 kg N ha^{-1}; $RI_0N_2P_0$: 50 kg N ha^{-1}; $RI_1N_0P_1$: *Rhizobium* inoculation + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_0$: *Rhizobium* inoculation + 25 kg N ha^{-1}; $RI_1N_2P_0$: *Rhizobium* inoculation + 50 kg N ha^{-1}; $RI_0N_1P_1$: 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_0N_2P_1$: 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_1P_1$: *Rhizobium* inoculation + 25 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}; $RI_1N_2P_1$: *Rhizobium* inoculation + 50 kg N ha^{-1} + 90 kg P_2O_5 ha^{-1}. Values with the different letter(s) in a column are significantly different ($P \leq 0.05$).

nodulation directly affected growth enhancement, seed production and nutrient uptake due to the N_2 fixation potential of soybean. Application of P also increased nodulation, growth and yield potential of soybean. Results indicated that higher dose of N applied alone or in combinations with P and RI reduced the efficiency of *Rhizobium* inoculation for various growth and yield traits of soybean. Starter N of 25 kg ha^{-1} with RI and P resulted in the maximum increase in nodulation, growth, yield and NP uptake of soybean. The results obtained in the present work can have potential applications for increasing the productivity of soybean and enriching the soil with N through N_2 fixation under the sub-humid hilly region of Azad Jammu and Kashmir.

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