A field experiment was conducted to evaluate the effect of different phosphorus levels viz. 0, 25, 50, 75 and 100 kg ha\(^{-1}\) and inoculation with \textit{Rhizobium japonicum}. In all the phosphorus levels oil content (%) in soybean seed were found non significant. While in case of plant height and number of pod bearing branches per plant, the two higher phosphorus levels i.e. 75 and 100 kg ha\(^{-1}\), gave significantly better results as compared to other doses of phosphorus. While in all other parameters i.e. number of pods per plant, pod length, number of seeds per pod, biological yield, harvest index and oil yield, 100 kg P\(_2\)O\(_5\) ha\(^{-1}\) gave significantly better results as compared to all other doses of phosphorus. Inoculation with \textit{Rhizobium japonicum} improved soybean yield and yield components as compared to non-inoculated seed.

**Keywords**: Soybean; phosphorus, inoculation, yield, oil, protein

**INTRODUCTION**

Pakistan is facing an acute shortage of edible oils. The indigenous edible oil production does not match the growing demand of population. The total availability of edible oils in 2006-07 was 2.79 million tons. Local production stood at 0.85 million tons which accounts for 28% of total availability. The remaining 72% was made available through imports (Govt. of Pakistan, 2008a). Edible oil in fact is the largest food item being imported in the country. The import bill of edible oil was about Rs.18.43 billion in 2001-02 and has risen up to Rs. 109.13 billion in 2007-08 (Govt. of Pakistan, 2008b). In Pakistan, more than 50% of domestic oil production comes from seed of cotton, which is not a true oilseed crop, and contribution of world’s known oilseed crops like soybean is very less.

Soybean is perhaps one of the oldest food crop of the world and ranks first among the oilseed crops in the world. It contains 18-22% cholesterol free oil having 85% unsaturated fatty acids and 40-44% proteins (Govt. of Pakistan, 2002). Compared to other protein-rich foods such as meat, fish and eggs, soybean is far the cheapest. It can be sown as a sole crop, intercropped, or mixed with maize or sorghum in irrigated and rainfed areas in all provinces of Pakistan. It improves soil fertility by fixing atmospheric nitrogen through root nodules.

Soybean is negligible crop in Pakistan, wherever its grown per hectare yield is very low. Efforts have not been concentrated for its success in Pakistan. Among various factors that can contribute to soybean success, phosphorus and inoculation had quite prominent effects on nodulation, growth and yield parameters (Kumaga and Ofori, 2004). Phosphorus has important effects on photosynthesis, nitrogen fixation, root development, flowering, seed formation, fruiting and improvement of crop quality (Brady, 2002). Symbiotic nitrogen fixation is definitely beneficial to agriculture. It is major source of fixed nitrogen in agricultural soils. Responses by soybean to inoculation with \textit{Bradyrhizobium japonicum} expressed in terms of both the proportion of nodules formed by the inoculum and seed yield (Simanungkalit \textit{et al.}, 1995). Inoculation of soybean seeds with proper bacterial strains increased seed production by 70-75% (Simanungkalit \textit{et al.}, 1996). Ten years of soybean inoculation evaluation consisting of 66 field trials and over 7000 research plots indicate that inoculating soybeans is a very profitable practice. The average yield increase over ten years has returned a profit of over 300 percent (Beuerlein, 2004). Inoculation of soybean seeds with rhizobase inocula of vesicular-arbuscular mycorrhizal (VAM) fungi–\textit{Glomus macrocarpum} and \textit{G. warcupii} and root nodule bacterium, \textit{Bradyrhizobium japonicum}, showed an increase in VAM infection and root nodulation over the control. An increase in fresh weight, dry weight and seed weight was also observed as compared to control (Jalaluddin, 2005). Field studies in Northern Michigan showed 45% and 23% soybean yield increases in 2004 and 2005 where inoculant was used and field had not seen a previous soybean crop. Soybean plant coloration differences were obvious in this study, as inoculated plots appeared much greener (Thelen and Schulz, 2009).

Keeping in view the above discussion, a field study was conducted with a view to evaluate the effect of \textit{Rhizobium} inoculation and varying levels of phosphorus on growth and yield of soybean, under agro-climatic conditions of Faisalabad.
MATERIAL AND METHODS

Experimental design and preparation of plot

A field experiment was conducted to study the effect of different phosphorus levels viz. 0, 25, 50, 75 and 100 kg P$_2$O$_5$ ha$^{-1}$ and seed inoculation on yield of soybean. The experimental soil contained 0.935% organic matter, 0.046% nitrogen, 7 ppm available phosphorus and 148 ppm available K with pH 7.5. Experiment was laid out in randomized complete block design with factorial arrangement using three replications and net plot size of 1.8 m × 5.0 m. Seedbed was prepared by 2-3 times ploughing followed by plankings. The sowing was done on July 22, 2005 with the help of manual drill using seed rate of 100 kg ha$^{-1}$ at a row to row distance of 30 cm.

Fertilizer application and other cultural practices

A starter dose of N fertilizer @ 30 kg ha$^{-1}$ and prescribed doses of phosphorus were applied at sowing as Urea (46-0-0) and DAP (18-46-0). Plant population was maintained by thinning at four to six leaf stage (i.e. 15 days after germination) to maintain plant to plant distance of 5 cm. Hoeing was done twice to keep crop free of weeds. All other cultural practices were kept normal and uniform for all the treatments.

Inoculation

The inoculant (Rhizobium japonicum) was arranged from Ayub Agricultural Research Institute (AARI) Faisalabad. Inoculation was done by adopting standard procedures just before drilling and a control (no inoculation) was also maintained.

Data recording and analysis

Fully matured crop was harvested on Nov. 2, 2005. Ten plants were selected at random from each plot, their height was measured from the base to the top growing point at maturity with the help of a meter rod and average height was calculated. Number of pod bearing branches and number of pods per plant was recorded from ten randomly selected plants from each plot and then averaged. Pod length of ten randomly selected pods from each plot was measured with the help of a scale and the average length was calculated. These selected ten pods were threshed manually to get average number of seeds per pod. Three samples each of 1000-seeds were taken at random from the seed lot of each plot after threshing, weighed and then average was calculated. Sun dried biomass in each plot was threshed manually to record the seed yield per plot. Grain yield achieved from ten randomly selected plants were also added to yield of respective plots. Thus obtained seed yield per plot was converted to kg ha$^{-1}$. Seed samples were randomly taken from each plot. Oil content of seed was estimated by the NMR (Nuclear Magnetic Resonance) test (Robertson and Morrison, 1979) and nitrogen content was determined by Kjeldhal method and multiplied by a factor (6.25) to get seed protein percentage (A.O.A.C., 1990).

Then the data were analyzed statistically by using Fisher’s analysis of variance technique and least significant difference (LSD) test at 5% probability level was applied to compare treatments’ means (Steel et al., 1997).

RESULTS AND DISCUSSION

The growth behavior of a crop plant is measured in terms of plant height. The comparison of plant height of inoculated and un-inoculated seed depicts that plant height of inoculated seed was significantly more than that of un-inoculated seed (1). Among various phosphorus levels the maximum plant height was obtained by using 75 kg phosphorus ha$^{-1}$ which was statistically at par with P$_4$ (100 kg P$_2$O$_5$ ha$^{-1}$) (Table 1). Thus nitrogen fixation by Rhizobium and applied phosphorus balanced the nutrition of plants and growth was accelerated. Tomar et al. (2004) and Rani (1999) showed that increased levels of phosphorus had positive effects on plant height while Hernandez and Cuevas (2003) reported that inoculation increased the plant height. Increased plant height due to phosphorus and inoculation were also reported by Menaria et al. (2003).

Pod bearing branches are considered to be the major contributor to seed yield of legumes. In general, the plants from inoculated seed produced more pod bearing branches than the plants from seed which was not treated with inoculum (Table 1). Among the phosphorus levels 100 kg P$_2$O$_5$ ha$^{-1}$ (P$_4$) produced significantly maximum number of pod bearing branches per plant which was, however statistically at par with 75 kg P$_2$O$_5$ ha$^{-1}$ (P$_3$). Similar findings were also reported by Umale et al. (2002) that inoculation as well as phosphorus levels have significant effects on fruit bearing branches per plant of soybean.

The productive potential of soybean is ultimately determined by number of pods per plant which is a main yield component. Both the inoculation and different levels of phosphorus showed a significant effect on the number of pods per plant (Table 1). Inoculated seed produced significantly more pods per plant than un-inoculated seed. Increased number of pods per plant due to inoculation was also reported by Sable et al. (1998) and Hernandez and Cuevas (2003). Among different levels of phosphorus 100 kg P$_2$O$_5$ ha$^{-1}$
Soybean in relation to inoculation and P

Gave more number of pods per plant. These results support the findings of Mohan and Rao (1997) and Rani (1999) who reported that higher number of pods per plant was produced when higher doses of phosphorus were applied.

The greater pod length was recorded in the plants from inoculated seed compared to un-inoculated seed. The similar results were given by Patel and Patel (1991) who also showed significant increase in pod length due to inoculation in gram. Phosphorus levels differed from each other with respect to pod length of soybean. Significantly longest pods were observed when 100 kg P2O5 ha-1 was applied (Table 1).

The number of seeds per pod is perceived a significant constituent that directly imparts in exploiting potential yield recovery in leguminous crops. Highest number of seeds per pod was recorded in plants from inoculated seed as compared to plants from un-inoculated seed. The similar results were given by Patel and Patel (1991) and Hernandez and Cuevas (2003) who also showed significant increase in number of seeds per pod when inoculation and different levels of phosphorus were used.

Thousand (1000) seed weight is also an important yield contributing component. It reflects the magnitude of seed development which ultimately reflects the final yield of a crop. Interactive effect of the factors under study (I x P) on 1000-seed weight was significant (Table 2). The highest 1000-seed weight was achieved in I1P4 (inoculation and 100 kg P2O5 ha-1) which was statistically at par with I0P4 (no inoculation and 100 kg P2O5 ha-1) which gave 1000-seed weight of 137.3 g. The lowest 1000-seed weight of 107.3 g was observed in I0P0 (no inoculation and no phosphorus) which was statistically at par with I1P0 (inoculation and no phosphorus). Similar achievements on 1000-seed weight with seed inoculation and phosphorus application were reported by Sharma and Namdeo

| Table 1. Effect of phosphorus levels and Inoculation on growth, yield and quality of soybean |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Phosphorus levels (kg ha-1)     | Plant height (cm)               | No. of pod bearing branches per plant | No. of pods per plant | Pod length (cm) | No. of seeds per pod | Seed yield (kg ha-1) | Oil content (%) | Oil yield (kg ha-1) |
| P0 (0)                          | 43.96 c                         | 2.70 d                             | 37.16 e               | 3.11 d          | 2.26 e               | 1223.9 e           | 19.2              | 234.9 e           |
| P1 (25)                         | 47.01 b                         | 3.47 c                             | 38.57 d               | 3.40 c          | 2.40 d               | 1638.7 d           | 19.24             | 315.3 d           |
| P2 (50)                         | 47.72 b                         | 4.30 b                             | 39.85 c               | 3.70 b          | 2.49 c               | 1745.9 c           | 19.22             | 335.7 c           |
| P3 (75)                         | 51.07 a                         | 4.70 a                             | 40.53 b               | 3.84 b          | 2.63 b               | 1828.5 b           | 19.27             | 352.4 b           |
| P4 (100)                        | 51.02 a                         | 4.94 a                             | 42.68 a               | 4.09 a          | 2.74 a               | 1963.8 a           | 19.35             | 380.1 a           |
| LSD (5 %)                       | 1.101                           | 0.025                              | 0.561                 | 0.172           | 0.038                | 32.59              | NS                | 6.721             |

Inoculation

I0 (no inoculation)              | 47.05 b                         | 3.81 b                             | 38.69 b               | 3.55 b          | 2.47 b               | 1643.5 b           | 19.25             | 316.4 b           |
| I1 (Inoculation)               | 49.26 a                         | 4.23 a                             | 40.83 a               | 3.71 a          | 2.54 a               | 1716.8 a           | 19.27             | 331.0 a           |
| LSD (5 %)                       | 0.696                           | 0.159                              | 0.355                 | 0.109           | 0.024                | 20.61              | NS                | 4.251             |

Any two means sharing same letters within a column did not differ significantly at 5 % level of probability. NS = Non-significant

| Table 2. Effect of phosphorus levels and Inoculation on 1000-seed weight and protein content of soybean |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| I0P0 = No inoculation + no phosphorus | I0P1 = Inoculation + no phosphorus | I0P2 = No inoculation + 25 kg P2O5 ha-1 | I0P3 = No inoculation + 50 kg P2O5 ha-1 | I0P4 = No inoculation + 75 kg P2O5 ha-1 | I1P0 = Inoculation + no phosphorus | I1P1 = Inoculation + 25 kg P2O5 ha-1 | I1P2 = Inoculation + 50 kg P2O5 ha-1 | I1P3 = Inoculation + 75 kg P2O5 ha-1 | I1P4 = Inoculation + 100 kg P2O5 ha-1 |
| 1000-seed weight (g)            | 107.30 f                        | 111.2 e                           | 119.40 d               | 133.20 b        | 137.30 a             | 109.60 ef           | 117.30 d           | 122.60 c           | 132.10 b           |
| Protein content (%)            | 37.83 e                         | 38.95 d                           | 39.70 c                | 40.51 b         | 41.33 a              | 39.07 d            | 39.72 c            | 40.38 b            | 41.21 a            |
| LSD (5 %)                       | 1.111                           | 0.034                              | 0.070                 | 0.034           | 0.053                | 0.045              | 0.033              | 0.039              | 0.050             |

Any two means in a row sharing same letter did not differ significantly at 5 % level of probability

LSD (5 %) for 1000-seed weight = 2.690
LSD (5 %) for protein content = 0.2602
(1999) and Ilbas and Sahn (2005) on soybean. They showed that inoculation and phosphorus had significant effect on 1000-seed weight.

The final seed yield of a crop is a function of cumulative contribution of its various growth and yield parameters which are influenced by various agronomic practices and environmental conditions. Greater seed yield (1716.8 kg ha⁻¹) was obtained from plants of seeds which were inoculated with *Rhizobium* than those which were un-inoculated (1643.5 kg ha⁻¹). Sable et al. (1998) also reported significant increase in seed yield with inoculation. Soybean seed yield increased with each increase of phosphorus level from 0 to 100 kg ha⁻¹ and this increase was significant (0.05). Maximum seed yield (1963.8 kg ha⁻¹) was given by P₄ (100 kg P₂O₅ ha⁻¹), followed by P₃ (75 kg P₂O₅ ha⁻¹) which gave seed yield of 1828.5 kg ha⁻¹. Minimum seed yield (1223.9 kg ha⁻¹) was observed in P₃ (no phosphorus) (Table 1). Stefanescu and Palanciuc (2000), Landge et al. (2002), Umale et al. (2002), Ilbas and Sahn (2005), Tapas and Gupta (2005) and Jain and Trivedi (2005) also reported that seed yield of soybean increased with seed inoculation and higher levels of phosphorus.

Inoculation and different levels of phosphorus has non significant effects on oil contents. Similar results were recorded by Thimmegowda and Devakumar (1996) who observed that both inoculation and phosphorus had non-significant effects on oil content (%). These results are in contrast to Tomar et al. (2004) who observed that inoculation and phosphorus increased oil contents (%) of soybean. However, the results indicate that seed inoculation and the various levels of phosphorus under study had a significant effect on total oil yield of the crop as shown in Table 1. More oil yield was recorded in plants from inoculated seeds (331.0 kg ha⁻¹) than that of plants from un-inoculated seeds (316.4 kg ha⁻¹). Among different levels of phosphorus, P₄ (100 kg P₂O₅ ha⁻¹) gave maximum oil yield of 380.1 kg ha⁻¹ followed by oil yield (352.4 kg ha⁻¹) with the application of 75 kg P₂O₅ ha⁻¹ while the minimum oil yield of 234.9 kg ha⁻¹ was recorded in P₀ (no P₂O₅) (Table 1). Mehasen et al. (2002) and Badran (2003) showed that more oil yield was produced when inoculation and higher doses of phosphorus was applied. Apart from other factors, quality of a crop depends upon the protein content of seed, which is the major constituent of seed in legumes. It is evident from Table 2 that interactive effect of inoculation and phosphorus levels was highly significant on seed protein content of soybean. The maximum seed protein content of 41.42% was achieved by using 100 kg P₂O₅ ha⁻¹ and inoculation which was statistically at par with I₄P₄ (no inoculation and 100 kg P₂O₅ ha⁻¹) which gave protein contents of 41.33% and I₁P₃ (75 kg P₂O₅ ha⁻¹ and inoculation) which gave protein contents of 41.21%. Significantly lowest seed protein content of 37.83 % was observed in I₀P₀ (no inoculation and no phosphorus). Similar finding were reported by Sharma and Namedo (1999), Stefanescu and Palanciuc (2000), Mehasen et al. (2002), Bardan (2003) and Tomar et al. (2004). They showed that both inoculation and increasing levels of phosphorus have significant effects on protein contents of soybean.

**ACKNOWLEDGEMENTS**

The authors thank to Prof. Dr. M. Asghar Malik who provided the opportunity to write this research paper and Syed Muhammad Zaka for his help in analysis.

**REFERENCES**


