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Research Paper

Response of soybean (*Glycine max*) to phosphorus with or without biofertilizers

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ABSTRACT

A field experiment was conducted in *kharif* 2008 and 2009 to assess the effect of phosphorus fertilization in conjunction with biofertilizers, viz. Phosphorus solubilizing bacteria (PSB) i.e.*Pseudomonas striata* and vesicular arbuscular mycorrhizae (VAM) on soybean (*Glycine max* (L.) Merrill). The 10 treatments comprised of combinations of 50% and 100% of recommended dose of P, 2 sources of P, *viz.* [di-ammonium phosphate (DAP) and rock phosphate (RP) in conjunction with PSB and VAM, one absolute control and another control (no P) with PSB+VAM were tried in a randomized block design and were replicated thrice. Maximum yield (1.56 t/ha) was obtained with RP application at recommended dose along with biofertilizers. Yield attributes and yield were at par when full dose of P was applied in conjunction with biofertilizers irrespective of the source of P fertilization indicating increased efficiency of RP when supplied in conjunction with biofertilizers even in neutral soils. Soil application of biofertilizers showed the highest protein content in soybean seed (38.89%), which signified the fact that qualitative improvement is possible with combined application of fertilizers and biofertilizers. Higher nutrient uptake by the crop was also recorded in biofertilizer applied plots. Maximum net returns (₹15,953) was obtained with full dose of DAP with biofertilizers.

***Key words* :** Phosphorus solubilizing bacteria, Phosphorus sources, Rock phosphate, Soybean, Vesicular arbuscular mycorrhizae

Soybean is a rich source of protein and oil and its seeds also contain high levels of essential amino acids, minerals, phospholipids and vitamins (Medina-Juárez *et al*., 1998). It contains a class of phytochemicals called flavonoids which are thought to have certain health benefits and Rave potential to prevent some diseases such as heart disease, osteoporosis and certain cancers (Aparicio *et al*., 2003). Being a legume crop, soybean also fixes atmospheric nitrogen in the soil to an extent of 65 to 100 kg/ha through symbiotic association with *Rhizobium* bacteria (Rao, 2007). Soybean is the most important *kharif* oilseed crop. It occupies about 9.51 million ha with a production of about 9.91 million tonnes with an average yield of 1,041 kg/ha (Economic Survey, 2009-10). It is quite low as compared to the productivity of soybean in other leading producers like USA, Brazil, Argentina, China etc. Some of the major factors for low productivity are limiting moisture conditions as it is mostly grown under rainfed conditions during *kharif* season coupled with pests, diseases and weeds. Besides these, the imbalanced nutrition is also found to be one of the major limiting factors for poor yield.

Phosphorus is a key element involved in various functions in growth and metabolism of soybean. It is frequently a major limiting nutrient for plant growth in most Indian soils. Only a part of the phosphorus supplemented through fertilizers is utilized by the plants and a large portion of it is converted into insoluble fixed forms; the recovery efficiency of phosphorus in crops is generally 10-30% (Swarup, 2002). Research in the past clearly demonstrated that rock phosphate (RP), although a low cost fertilizer is not an effective P source in neutral and alkaline soils and its application is recommended for acidic soils (Casanova *et al*., 1993). Phosphorus solubilizing bacteria (PSB) can play an important role in increasing P availability by solubilizing the fixed P and supplying it to plants in a more available form (Khan *et al.,* 2007). Increased yield of soybean through inoculation with vesicular arbuscular mycorrhizae (VAM) has been reported (Kelly *et al*., 2001). It has

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been reported that PSB interacts with VAM, may release some phosphate ions in discrete soil microhabitats, by better exploiting the sparingly soluble P-sources (Azouni, 2008). However, the feasibility that such interactions really help in cycling of soil P in neutral and alkaline soil is a topic of interest which needs further research. Therefore, the present investigation was carried out to find out the effect of PSB and VAM in influencing the availability of P fromdi-ammonium phosphate (DAP) and RP, and their retrospective on growth, yield, nutrient uptake and economic benefits of soybean crop.

MATERIALS AND METHODS

The experiment was conducted during *kharif* 2008 and 2009 at Indian Agricultural Research Institute, New Delhi. The soils of experimental field had 252 kg/ha alkaline permanganate oxidizable N, 17.5 kg/ha Olsen's available P, 170 kg/ha 1 *N* ammonium acetate exchangeable K and 0.48% organic carbon analysed as per procedures described by Prasad *et al*., 2006. The pH of soil was 7.2 (1: 2.5 soil and water ratio) in 2008. In 2009, the experimental soil had 242 kg/ha alkaline permanganate oxidizable N 17.5 kg/ha available P, 186 kg 1*N* ammonium acetate exchangeable K and 0.46% organic carbon with soil pH of 7.4. The 10 treatments consisted of 50% (16.12 kg P/ha) and 100% (32.25 kg P/ha) of recommended dose of P supplied through 2 sources, viz. DAP and RP without or with inoculation of PSB and VAM, one absolute control and the other control (no P) with application of only PSB+VAM. The treatments were tried in randomized block design and were replicated thrice. A common dose of 100% recommended dose of N (30 kg/ha) and K (40 kg/ha) were applied to all the treatments as basal dose. Culture of phosphorus solubilising bacteria i.e. *Pseudomonas striata* @ 500 g/ha and vesicular arbuscular mycorrhiza, i.e. *Glomus fasciculatum* @ 11.25 kg/ha were applied to the soil as per treatment before sowing. The gross plot size was 14 m × 3.6 m. Soybean variety 'DS 9814' was sown at an inter-row spacing 45 cm using a seed rate of 75 kg/ha. The crop was sown on 27th July in both the years of experimentation. In the first year, crop was harvested on 7th November, while in the succeeding year, crop was harvested on 4th November. The crop was raised as per the recommended package of practices. For recording biometric observations, five representative plants of soybean from each treatment were selected randomly at 30, 60, 90 DAS and at harvest. For calculating seed index, random sample of 100 seeds from each treatment was drawn from the net plot produce after harvest. The same was weighed and was expressed in grams. Data obtained were statistically analyzed by applying "Analysis of Variance" technique for randomized block design.

RESULTS AND DISCUSSION

Growth parameters

Application of biofertilizers elicited significant response in terms of LAI and total dry matter accumulation. Maximum LAI (2.37) and total dry matter accumulation (10.68 g/plant) was recorded when full dose of P was supplied through RP along with biofertilizers (Table 1). Dubey (2001) also reported higher dry matter accumulation on application of biofertilizers.

Yield attributes and yield

Significant improvement in yield attributes of soybean was recorded with application of biofertilizers (Table 1).

Table 1. Leaf area index (LAI), total dry matter accumulation and yield attributes as influenced by levels and sources of phosphorus and biofertilizers (Pooled over two years)

Treatment	LAI at 90 DAS	Dry matter accumulation at 90 DAS (g/plant)	Pods/plant	Seeds/pod	Seed index
Control	1.95	8.90	23.66	2.05	12.57
PSB+VAM	2.03	9.52	25.84	2.35	12.93
50% P as RP	2.20	9.74	25.58	2.22	12.82
50% P as RP + PSB+VAM	2.35	10.52	29.72	2.53	13.45
50% P as DAP	2.14	10.03	27.29	2.32	13.23
50% P as DAP + PSB+VAM	2.19	10.30	28.69	2.33	13.20
100% P as RP	2.29	9.93	27.67	2.22	13.06
100% P as RP + PSB+VAM	2.37	10.68	30.63	2.57	13.62
100% P as DAP	2.31	10.50	29.47	2.42	13.29
100% P as DAP+ PSB+VAM	2.30	10.58	29.66	2.52	13.56
SEm±	0.03	0.09	0.37	0.02	0.06
CD (P=0.05)	0.09	0.25	1.06	NS	0.17

PSB: Phosphate solubilizing bacteria; VAM: Vesicular arbuscular mycorrhizae; RP: Rock phosphate; DAP: Di-ammonium phosphate; DAS: Days after sowing

Application of 100% P through RP along with biofertilizers recorded the highest number of pods per plant (30.63), number of seeds per pod (2.57) and seed index (13.62). Treatments receiving P from chemical source only recorded lower values of yield attributes compared to its integration with biofertilizer. This might be the result of improved supply of nutrients by biofertilizers at the later stages of crop growth. Generally, nutrient uptake by legume crop gets reduced towards maturity. With regard to number of seeds per pod, application of 50% P through RP along with biofertilizers was found at par with 100% P through RP in combination with biofertilizers. Azouni (2008) reported significant increase in number of pods per plant and seed index with the application of single or dual inoculation of the tested strains.

Similar to growth and yield attributes, seed yield and harvest index registered maximum value with 100% RP in combination with biofertilizers (Table 2). The improved leaf area index, higher dry matter accumulation, increased number of pods per plant, number of seeds per pod and higher seed index made the treatment receiving combined application of RP (100% P) along with biofertilizers to produce the highest seed yield. Reports had shown the improvement in plant growth and yield using biofertilizers (Withers *et al.,* 2005).

Protein and oil content

Protein content and yield of soybean increased significantly with P application and biofertilizers (Table 3). Protein content was maximum (38.89%) with the application of 100% P through RP along with biofertilizers. Treatments where biofertilizers were applied in combination

Table 2. Seed yield, biological yield and harvest index of soybean as influenced by levels and sources of phosphorus and biofertilizers (Pooled over two years)

Treatment	Seed yield (t/ha)	Biological yield (t/ha)	Harvest index	Gross returns (×10³ ₹/ha)	Net returns (×10³ ₹/ha)	Net B:C ratio
Control	1.10	3.75	29.42	18.6	10.6	1.31
PSB+VAM	1.21	4.14	29.33	20.2	11.9	1.42
50% P as RP	1.23	4.08	30.17	20.6	11.9	1.37
50% P as RP + PSB+VAM	1.50	4.64	32.47	24.9	15.9	1.77
50% P as DAP	1.38	4.49	31.04	23.1	14.2	1.61
50% P as DAP + PSB+VAM	1.51	4.74	32.00	25.1	15.9	1.74
100% P as RP	1.40	4.62	30.46	23.5	14.2	1.53
100% P as RP + PSB+VAM	1.56	4.42	35.33	25.5	15.9	1.66
100% P as DAP	1.49	4.47	33.53	24.6	14.9	1.55
100% P as DAP+ PSB+VAM	1.55	4.56	34.35	25.5	15.5	1.56
SEm±	0.02	0.08	0.41	0.5	0.5	0.06
CD (P=0.05)	0.06	NS	1.18	1.4	1.4	0.16

PSB: Phosphate solubilizing bacteria; VAM : Vesicular arbuscularmycorrhizae; RP: Rock phosphate;

Table 3. Protein and oil content in soybean seed, their respective yields and nutrient uptake (seed+stover) as influenced by levels and sources of phosphorus and biofertilizers (Pooled over two years)

Treatment	Quality parameters				Nutrient uptake (kg/ha)		
	Protein content (%)	Protein yield (t/ha)	Seed oil content (%)	Oil yield (t/ha)	N	P	K
Control	36.29	0.40	18.19	0.20	130.4	8.13	31.12
PSB+VAM	37.03	0.45	18.31	0.22	151.6	11.19	34.54
50% P as RP	37.17	0.46	18.04	0.22	150.6	10.92	33.82
50% P as RP + PSB+VAM	38.76	0.59	18.15	0.27	186.1	14.64	37.88
50% P as DAP	37.73	0.52	18.06	0.25	173.1	13.16	37.44
50% P as DAP + PSB+VAM	37.45	0.57	18.20	0.28	185.5	14.67	38.91
100% P as RP	37.72	0.53	18.34	0.26	180.2	14.73	39.19
100% P as RP + PSB+VAM	38.89	0.61	18.24	0.28	182.6	14.18	36.86
100% P as DAP	38.54	0.58	18.27	0.27	179.1	13.80	36.54
100% P as DAP+ PSB+VAM	38.86	0.60	18.18	0.28	185.3	14.39	38.04
SEm±	0.18	0.01	0.04	0.004	2.61	0.37	0.75
CD (P=0.05)	0.52	0.02	NS	0.01	NS	1.06	NS

PSB: Phosphate solubilizing bacteria; VAM: Vesicular arbuscularmycorrhizae; RP: Rock phosphate;

with P fertilizer recorded higher protein content in soybean seed than when applied alone. Higher P fertilization was necessary for higher protein content in seed as well as protein yields in soybean (Shah *et al*., 2002). Fattah (2002) reported increase in total protein content on application of VAM.

The increase in protein content of soybean seeds was mainly due to high N uptake which may be attributed to the beneficial effect of biofertilizers. In case of P uptake, application of 50 or 100% P through RP along with biofertilizers proved to be at par. All treatments containing P source along with the biofertilizers recorded similar results.

There was an increase in N and P content in plant due to higher availability of nutrients which has resulted in high protein content in seeds. Also, the release of organic acids by microbes that both sequester cations and acidify the micro-environment near the roots is thought to be a major mechanism of P-solubilization and subsequent uptake by plants and mycorrhizal fungi (Cunningham and Kuiack, 1992). This may be due to high P availability caused by solubilisation and mobilization by PSB and VAM, respectively. Microbial community influences soil fertility through soil processes, *viz.* decomposition, mineralization, and storage/ release of nutrients. Microorganisms enhance the P availability by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006). Oil content was found to be non significant whereas oil yield varied significantly with application of P as well as biofertilizers (Table 3).

Nutrient uptake

The total nutrient uptake was found to be higher in biofertilizer applied treatments (Table 3). Application of 100% P through RP was equally good as DAP when applied in combination of biofertilizers. Sufficient supply of N, P and K from the different sources was responsible for significantly higher nutrient uptake by the crop as compared to the control with only N and K fertilization. Mahanta and Rai (2008) reported higher content and uptake of nutrients by the crop due to inoculation with biofertilizers compared to no inoculation. Vesicular arbuscular mycorrhizae were reported to be responsible for enhanced mineral uptake. The organic acids added to the soils by microbes may have significantly increased nutrient release leading to higher uptake by the plant (Bolan *et al.*, 1994).

Economics

The cost of cultivation increased with increasing levels of nutrients as compared to control. Absolute control had the lowest cost while the highest cost (₹9,992/ha) of cultivation for soybean was with 100% P as DAP in combination with biofertilizers. Application of 50% P through DAP with biofertilizers resulted in the highest net returns (₹15,953/ha). The highest net B:C ratio (1.77) was recorded with application of 50% P through RP in combination with biofertilizers, followed by 50% P through DAP with biofertilizers. Nagaraju *et al*. (2009) reported higher net returns and B:C ratio with application of P in combination with biofertilizers. This indicates that fertilizer P application can be reduced to half, thus cutting the P input cost.

It was concluded that biofertilizers command significant response in soybean when applied along with fertilizer P. Rock phosphate in conjunction with PSB and VAM can be used as an alternative source of P to a more costly DAP in neutral soils. There was an increase in protein content with combined application of P and biofertilizers.

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