

Soybean Response to *Bradyrhizobium japonicum*, Phosphate Solubilizing Bacteria and AM Fungi in Mollisol

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Received 16 January 2017; Accepted 18 February 2017; Published online 9 March 2017

Abstract A field experiment was conducted during kharif 2004 to study the effect of co-inoculation of *B. japonicum*, PSB and AM fungi microbial biomass carbon, nutrient uptake and yield of soybean (*Glycine max* L. merril) var Pk-416 on Tarai Mollisols. Thirteen treatment consisting inoculation of *B. japonicum*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) either alone or in combinations with 40 and 60 kg P₂O₅ per hectare were included in the study. Maximum N uptake (66.01 kg/ha) in shoot was recorded with AMF inoculation + 40 kg P₂O₅ per hectare, which was significantly higher than *B. japonicum*

inoculation alone. The highest N content of 1.73% in shoot was recorded by co-inoculation of AMF + PSB with 40 kg P₂O₅ per hectare and lowest with uninoculated control treatment (1.2%). The maximum P content (0.46%) in shoot was recorded with the treatment of *B. japonicum* + PSB + 40 kg P₂O₅ per hectare which was significantly higher than microbial inoculation alone or in combination. Microbial biomass carbon was significantly increased with AM fungi inoculation. P did not show any significant effect on microbial biomass carbon. The highest grain yield of 2,296 kg per hectare was recorded with *B. japonicum* inoculation + 60 kg P₂O₅ per hectare which was 9.4 and 5.1% more than *B. japonicum* inoculation alone and 60 kg P₂O₅ per hectare. Either alone or inoculation in combinations with 40 and 60 kg P₂O₅ per hectare did not show any significant effect on straw and test weight of grain.

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Keywords *B. japonicum*, PSB, AM fungi, Microbial biomass carbon, Soybean.

Introduction

The soybean is legume crop, native to East Asia, it is popularly known as miracle crop, introduced to India during mid sixties. It is also called as Golden bean and it is the second largest oilseed crop in India after groundnut and is being grown in varied agro-climatic conditions. In recent past it has emerged as one of the important commercial crops in many countries. The commercial cultivation of soybean has been

widely adopted both in Northern and Southern India mainly in the states of Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Chhattisgarh, Uttar Pradesh and Tamil Nadu. In India, the area under soybean cultivation is to an extent of 96.23 lakh hectares with a production of 108.28 lakh tones having a productivity of 1,124 kg ha⁻¹ [1]. In Karnataka, it is being grown in an area of 1.80 lakh hectares with a production of 1.84 lakh tons with an average yield of 1,025 kg ha⁻¹ [1]. Soybean utilizes atmospheric nitrogen by its symbiotic relationship with *Bradyrhizobium japonicum* to meet a major part of its nitrogen requirement under normal conditions. Soybean harvest about 200 to 250 kg nitrogen per hectare from atmosphere, enriching the soil, thus soybean is also economically profitable as compared to cereals and other oil seed crops. Phosphate solubilization by microorganisms is an important process in natural ecosystems, especially in agricultural land. Some types of microbes such as bacteria, fungi and actinomycetes were reported active in the conversion of insoluble phosphate into soluble phosphates. Some researchers reported that bacteria were more active than other types of microbes in the conversion of P [2]. Vesicular arbuscular mycorrhiza (VAM) has an important role in helping to increase the uptake of P by plants due to the increase in absorption capacity through the external hyphae of VAN which is infect plant roots. In P deficient soil inoculation of VAM on soybean plants, with or without P fertilizer was able to increase grain yields by 20% to 50% , whereas the application of P fertilizer alone did not increase grain yield [2]. VAM inoculation on soybean enhancing nodule weight and plant N levels. It has been suggested that the relationship between mycorrhizal and *Rhizobium* have synergistic effect. *Rhizobium* requires phosphorus for its growth and survival in soil, rhizospheric colonization and energy transformation during nitrogen fixation in root nodules. Therefore the study was undertaken to evaluate the influence of *B. japonicum*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) on soybean with applied phosphorus.

Materials and Methods

A field experiment was conducted at the Crop Research Center of Govind Ballabh Pant University of

Agriculture and Technology, Pantnagar, Uttarakhand, during *kharif* 2004 to evaluate the effect of PSB and AM inoculation on soybean *Bradyrhizobium* symbiosis on silty clay loam soil having organic carbon 0.75%, available nitrogen (N) 295.62 kg per hectare, available phosphorus (P) 19.71 kg per hectare, available potash (K) 140.12 kg per hectare and pH 7.2. Treatments consisted of inoculation of *B. japonicum*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) either alone or in combinations with 40 and 60 kg P₂O₅ per hectare applied as basal through SSP and uninoculated control treatment with three replications. The experiment was laid out in randomized block design (RBD) in plots of 3 m × 4 m size. Soybean (var PK-416) seed was treated with 20 g inoculants of *B. japonicum*, PSB (*Pseudomonas striata*) and AM fungus (*Glomus lamellosum*) per kg seed at the time of sowing. The microbial biomass C was estimated by Vance et al. method. Pour plate serial dilution method was used for estimating the population of total bacteria, fungi and actinomycetes in soil. Nitrogen and phosphorus content in plant was determined by modified Kjeldhal [3] and vanadomolybdo phosphoric yellow color method in nitric acid system [4] respectively grinding the sample to 40 mesh. After threshing and proper cleaning, the grain yield of individual plot was recorded with single pan balance and expressed as kg per hectare after conversion.

Results and Discussion

Nitrogen and phosphorus content in plant

The highest nitrogen content of 1.73% (Table 1) was recorded by co-inoculation of PSB and AMF along with application of 40 kg P₂O₅ per hectare and lowest in uninoculated control (1.2%), which may be due to more availability of energy for N₂ fixation. Inoculation of AMF and PSB significantly increased phosphorus content in plant over uninoculated control treatment probably due to phosphorus solubilizing action of PSB and phosphorus mobilizing effect of AM fungi which increased available phosphorus to the plant roots. Application of *Rhizobium*, PSB and VAM has resulted in improved nodulation in plant roots and supplying higher amount of nitrogen for growth and yield attributes which intern helped to

Table 1. Effect of *B. japonicum*, PSB and AM fungi inoculation with applied phosphorus doses on microbial biomass carbon, microbial population, N, P, content and uptake at harvest and yield of soybean.

Treatments	Nutrient content in shoot (%)		Nutrient uptake at harvest (kg/ha)		Microbial biomass carbon (μ g/g soil)
	N	P	N	P	
Uninoculated control	1.20	0.29	36.35	5.61	252.05
<i>B. japonicum</i> alone	1.61	0.30	41.25	8.25	321.08
<i>B. japonicum</i> + AMF	1.66	0.34	59.83	7.31	302.81
<i>B. japonicum</i> + PSB	1.64	0.38	57.48	7.28	274.78
AMF + PSB	1.63	0.31	56.21	6.29	251.72
<i>B. japonicum</i> + SSP @ 60 kg P ₂ O ₅ ha ⁻¹	1.55	0.34	62.03	7.51	248.27
<i>B. japonicum</i> + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.55	0.45	62.03	7.51	308.29
AMF + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.68	0.38	66.01	10.51	250.79
<i>B. japonicum</i> + AMF + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.57	0.46	60.36	7.48	386.67
<i>B. japonicum</i> + PSB + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.59	0.31	60.69	10.51	349.36
AMF + PSB + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.73	0.34	63.23	9.05	360.49
SSP @ 40 kg P ₂ O ₅ ha ⁻¹	1.56	0.37	58.42	7.77	237.87
SSP @ 60 kg P ₂ O ₅ ha ⁻¹	1.59	0.21	59.82	8.42	293.19
SEm (\pm)	0.140	0.21	6.01	1.23	2.15
CD ($p = 0.05$)	NS	0.06	17.38	NS	73.08
CV (%)	8.55	15.18	10.51	16.28	14.13

realize higher growth parameter and dry matter of soybean. Similar observations were earlier reported by Thenua et al. [10]. In addition to this combination of *Rhizobium*, PSB and VAM has resulted in cumulative effects such as supply of N and P to the crop along with production of growth promoting substances like auxin, gibberlins and cytokinins. These results are in agreement with the findings earlier [6, 7, 11]. The highest uptake of P 10.51 kg per hectare (Table 1) was recorded by AM fungi inoculation with 40 kg P₂O₅ per hectare which was similar to that obtained by combined inoculation of *B. japonicum* and PSB with 40 kg P₂O₅ per hectare, possibly because of increased amount of available phosphorus by fertilizer, PSB and AM fungi to the plant and N supply by *B. japonicum*. Singh [8] also showed in his experiment results a synergistic effect of *Rhizobium*, PSB and VAM in increasing growth, root nodules dry weight, N and P uptake, grain yield and yield components of soybean. Inoculation of *B. japonicum* alone resulted numerical increase of 27% in microbial biomass carbon over uninoculated control treatment by AM fungi alone inoculation significantly increased microbial biomass carbon over uninoculated control treatment, *B. japonicum*, PSB alone inoculation and dual inoculation of AM fungi + *B. japonicum*. Dual inoculation of

B. japonicum + AM fungi showed numerical increase (20.1%) in microbial biomass carbon over uninoculated control treatment. However, phosphorus application @ 60 kg per hectare recorded numerical increase of 55.3% in microbial biomass carbon over 40 kg P₂O₅ per hectare. The increase in microbial biomass carbon due to microbial inoculation showed the increase in microbial cells in soil over the uninoculated control treatment. AM fungi inoculation recorded significant increase in microbial biomass carbon over *B. japonicum* and PSB alone inoculation treatments due to development of more fungal mycelium in soil. Application phosphorus with microbial inoculants recorded significant increase in microbial biomass carbon over microbial inoculation without applied phosphorus showed the effect of applied P due to more cellular components were synthesized.

Yield and yield attributes

An increase of 14.9% in the seed yield of soybean (Table 2) over uninoculated control was obtained with inoculation of *B. japonicum*. It might be due to *Rhizobium* is a symbiotic N fixer in pulses and oilseed crops and is known to produce growth promoting substances like IAA, gibberellins and cytokinins, which

Table 2. Effect of co-inoculation of *Bradyrhizobium japonicum*, PSB, AM fungi with phosphorus on yield and yield attributes of soybean.

Treatments	Grain yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)	100 grain weight (g)
Uninoculated control	1827	1905	86.01
<i>B. japonicum</i> alone	2099	2196	89.07
<i>B. japonicum</i> AMF	2116	2148	90.52
<i>B. japonicum</i> + PSB	2138	1998	86.58
AMF + PSB	2074	1971	93.01
<i>B. japonicum</i> +SSP@ 60 kg P ₂ O ₅ ha ⁻¹	2296	2148	93.55
<i>B. japonicum</i> +SSP@ 40 kg P ₂ O ₅ ha ⁻¹	2296	2240	93.41
AMF +SSP @ 40 kg P ₂ O ₅ ha ⁻¹	2044	2296	90.22
<i>B. japonicum</i> +AMF+SSP@ 40 kg P ₂ O ₅ ha ⁻¹	2155	2333	90.68
<i>B. japonicum</i> + PSB + SSP @ 40 kg P ₂ O ₅ ha ⁻¹	2106	2777	94.39
AMF+PSB+SSP @ 40 kg P ₂ O ₅ ha ⁻¹	2261	2498	87.94
SSP @ 40 kg P ₂ O ₅ ha ⁻¹	2130	2231	90.66
SSP @ 60 kg P ₂ O ₅ ha ⁻¹	2184	2259	92.09
SEm (±)	97.09	312	3.13
CD (p=0.05)	281.0	NS	NS
CV	8.09	-	5.95

help in enhancing the plant growth and yield. Many research studies have showed that dual or triple microbial inoculation is highly beneficial to crop growth in obtaining higher crop yield [2]. These findings are in corroboration with the results reported by Singh [8] who observed that the highest increased in grain yield (102%) over control (without biofertilizer) was obtained from the combination of the three biofertilizers (*Bradyrhizobium* + PSB + VAM). The highest grain yield of 2,296 kg per hectare was recorded with *B. japonicum* inoculation + 60 kg P₂O₅ per hectare which was 9.4 and 5.1% more than *B. japonicum* inoculation alone and 60 kg P₂O₅ per hectare, respectively, because of more availability of phosphorus supplied through chemical fertilizer along with N fixed by *B. japonicum*.

The applied treatments did not show significant effect on the straw yield over uninoculated control treatment. However, highest straw yield of 2,498 kg per hectare was recorded with combined inoculation

of AM + PSB with 40 kg P₂O₅ per hectare. All the treatment showed numerical increase in straw yield over the uninoculated control treatment. Alone inoculation of *B. japonicum*, AM fungi and PSB resulted in 15.2, 12.2 and 26.0% numerically higher straw yield over uninoculated control treatment. The applied treatments did not show significant effect on test grain weight over uninoculated control treatment. The test grain weight ranged from 86.01 to 95.45 g. The highest test grain weight (95.45 g) was recorded with AM fungi inoculation followed by *B. japonicum* + PSB inoculation with application of 40 kg P₂O₅ per hectare (94.39 g).

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