World Journal of Agricultural Sciences 4 (S): 835-838, 2008
ISSN 1817-3047

Yield and Nutrient Uptake of Soybean *(Glycine max* (L) Merr) as Influenced by Phosphate Solubilizing Microorganisms

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Abstract: Phosphorus (P) is a plant nutrient, which is rapidly made immobile and less available for plant use after addition to the soil as a soluble fertilizer. Although the presence of soil microorganisms capable of solubilizing phosphate has been known for many years, their isolation and use as crop inoculants have met with only limited success. Hence a Pot experiment was conducted using Phosphate Solubilizing Microorganisms (PSM) isolated from the rhizosphere soil to investigate their effect on releasing unavailable ‘P’ and improving growth parameters, with a particular focus on soybean crop. Yield and nutrient uptake, nodulation efficiency and the available ‘P’ content in soil were significantly enhanced showing the positive effect of microbial inoculation. Inoculations along with rock phosphate(RP) application further increased the yield and were comparable with the treatments receiving superphosphate(SP) alone, indicate the possibility of replacing the SP with RP.

Key words: Phosphate solubilizing microorganisms · rhizosphere · rock phosphate · superphosphate

INTRODUCTION

Phosphorus (P) is the major plant growth limiting nutrient despite being abundant in soil in both inorganic and organic forms. The ‘P’ content in average soil is about 0.05% (w/w) but only 0.1% of the total ‘P’ is available to plants because of its low solubility and its fixation in soil [1]. To circumvent the problem of ‘P’ deficiency, chemical fertilizers are added to the soil. The utilization efficiency of phosphatic fertilizers by plants are only 20 to 25% largely due to its chemical fixation in soil [2]. The situation is becoming more complicated because the natural reserves as the raw materials for the manufacture of phosphatic fertilizers are limited and are depleting fast.

There has been long standing interest to manipulate soil microorganisms to improve ‘P’ nutrition of plants. Phosphorus biofertilizers in the form of microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization [3]. Apart from fertilization and enzymatic decomposition of organic compounds, microbial P-mobilization would be the only possible way to ensure plant available ‘P’ [4]. Several studies have been conducted in India using various phosphate solubilizing microorganisms (PSM) as bioinoculants in wheat, rice, potato, Bengal gram and other crops [5] and emphasized the need for evolving efficient strains adapted to the local conditions to exploit the full potential of the technology for crop production. The present investigation deals with the inoculation effect of the rock phosphate(RP) solubilizing microorganisms isolated from rhizosphere soil on nutrient uptake and yield of soybean.

MATERIALS AND METHODS

Phosphate solubilizing microorgnaisms were isolated from rhizosphere soil by using Pikovskaya’s medium [5]. The phosphate solubilization efficiency of different isolates were quantified by phosphomolybdate method [6] and secreened two fungi identified as *Aspergillus niger* and *Penicillium vermiculosum* and two bacteria identified as *Bacillus Sp* and *Pseudomonas stutzeri* for further studies. Identification of bacterial isolates were done by the Institute of Microbial Technology, Chandigarh, India and Fungal isolates were identified by the division of pathology, Indian Agricultural Research Institute, New Delhi.

Inoculation study: A pot culture experiment was conducted under completely randomized design to assess the effect of bacterial and fungal isolates on yield and

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Table 1: Yield and nutrient uptake of soybean as influenced by PSM inoculation

	Yield (g/pot)			N uptake		P_2O_5 uptake	
Treatment	Pods weight	Straw weight	Seeds weight	Straw (mg/pot)	Seeds (mg/pot)	Straw (mg/pot)	Seeds (mg/pot)
Control	16.81	24.07	9.06	201.49	355.79	21.43	38.41
SP_{60}	31.47	33.49	18.08	630.96	1241.78	68.33	116.98
SP_{30}	24.27	30.07	14.47	345.85	920.94	46.91	83.65
RP_{60}	18.33	24.47	12.55	281.41	664.97	27.65	61.35
Bacillus Sp	18.33	26.65	11.82	252.64	671.20	78.78	61.82
Ps. stutzeri	18.74	27.14	12.67	230.61	737.45	28.22	53.46
A. niger	19.59	24.82	10.68	272.31	688.98	28.30	54.03
P.vermiculosum	19.48	27.14	11.21	236.96	731.44	37.46	46.53
Ps.striata	17.56	26.17	11.76	222.45	719.29	29.84	55.49
A.awamori	20.05	26.56	11.32	275.39	729.13	35.58	58.05
Bacillus Sp+Rp_{60}	32.15	31.53	17.39	606.26	1229.22	63.00	108.15
Ps.stutzeri+Rp_{60}	27.05	30.55	17.21	315.62	1179.40	44.30	97.41
A.niger+RP_{60}	30.68	30.24	17.81	402.19	1234.56	59.57	108.98
P.vermiculosum+RP_{60}	32.29	39.51	18.18	382.98	1189.48	66.04	113.91
Ps.striata+RP_{60}	28.27	29.44	15.10	304.11	932.12	49.16	94.52
A.awamori+RP_{60}	29.47	30.21	16.27	397.87	1150.52	60.42	90.48
SE(M)±	0.23	0.19	0.20	8.06	12.15	0.27	0.92
CD(5%)	0.81	0.69	0.71	28.24	42.55	0.96	3.24

nutrient uptake of soybean crop (variety Pusa 22). The soil used for the study had a pH of 6.1,organic carbon 1.3 and available P_2O_5 8.8kg/ha. The soil was air dried, passed through 2 mm sieve and filled in earthen pots of 30 cm diameter at the rate of 10 kg/pot. Fym @ 10 tons / ha and the required quantity of fertilizers were applied as basal dose to the pots as per the treatment schedule.

Soybean seeds were inoculated separately with each culture suspension using 40 percent gum arabic solution as an adhesive. Before that the seeds were treated with *Bradyrhizobium japonicum* strain 119 broth culture. The experiment consisting of 16 treatments (Table 1) with six replicas and three each were used for observations on 60^{th} day after sowing and at harvest, after complete maturity. The pots were kept in a glass house and were watered regularly to maintain optimum moisture level on weight loss basis.

On 60^{th} Day after Sowing (DAS) the plants were uprooted gently and the rhizosphere soil samples were collected for enumeration of PSM. The population of PSM was enumerated by the standard serial dilution and plate count method using Pikovskaya's agar. The number and dry weight of nodules were also recorded. When the plants were completely mature harvesting was done and pod and grain weight was recorded. The grain and straw collected were analyzed for total nitrogen(N) and phosphorus by the methods of Jackson [6] and N and P uptake were calculated. The soil samples collected at 60 DAS and at harvest were analyzed for available phosphorus [6].

Performances of isolated cultures were compared with the standard cultures, *Pseudomonas striata* (Strain 27) and *Aspergillus awamori* obtained form the division of microbiology, Indian Agricultural Research Institute, New Delhi. The data were subjected to statistical analysis by 'F' test and the critical difference was calculated by student's 't' test at 0.05% level of significance.

RESULTS AND DISCUSSION

Yield and nutrient uptake of soybean: The positive effect of PSM inoculations were clearly observed on straw and grain yield of soybean (Table 1). The highest straw yield (39.51 g), seed yield (18.18 g/pot) and pods yield (32.29 g/pot) were obtained with microbial inoculation of *P.vermiculosum* along with RP_{60} treatments and the results were comparable with SP_{60}. In general fungal isolates exhibit greater P solubilizing ability than bacteria [7]. The increase in yield due to PSM inoculation seems to be related to the increased uptake of N and P by plants. In addition, the microorganisms involved in P solubilization can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of trace elements and by production of plant growth

Table 2: Available P content in soil, population of PSM and Nodulation of Soybean as influenced by PSM inoculation

Treatments	Available P (mg/kg soil) 60 DAS	Available P (mg/kg soil) After harvest	Population of PSM at 60 DAS ($NoX10^4$/g soil)	Nodulation at 60 DAS No. of nodules/pot	Nodulation at 60 DAS Dry wt. of nodules (mg/pot)
Control	11.18	11.05	11.33	88.0	107.5
SP_{60}	24.38	20.34	13.33	105.0	195.0
SP_{30}	19.94	17.90	15.33	89.0	152.0
RP_{60}	15.39	14.69	14.33	89.0	191.5
Bacillus SP	22.02	24.70	22.33	120.0	307.5
Ps. stutzeri	21.31	18.15	25.00	98.0	197.5
A. niger	22.34	21.62	16.67	102.5	231.5
P. vermiculosum	21.24	20.17	16.00	107.5	295.0
Ps.striata	18.62	21.15	20.67	95.0	143.0
A. awamori	18.22	20.56	16.67	92.0	140.0
Bacillus SP + RP_{60}	26.10	23.93	47.33	125.0	409.0
Ps. stutzeri + RP_{60}	22.77	22.21	28.67	110.5	360.5
A. niger RP_{60}	25.24	23.18	17.67	124.5	427.5
p. vermiculosum + RP_{60}	26.18	25.16	26.67	135.5	482.5
Ps. striata + RP_{60}	24.14	23.77	46.33	119.5	380.5
A. awamori + RP_{60}	26.04	25.45	29.33	124.0	401.0
SE (M)±	0.22	0.32	2.21	8.62	9.17
CD (5%)	0.78	1.13	7.75	30.22	32.15

promoting substances [8]. Beneficial effect of PSM in increasing the yield of soybean as well as in reducing the requirement of phosphatic fertilizers has already been established [9].

N and P uptake both in straw and seeds of soybean was substantially increased due to microbial inoculation and was comparable with SP_{60} treatment. N uptake was highest (1241.78 mg/pot) in SP_{60} treatment followed by *A.niger* + RP_{60} treatment (1234.56 mg/pot). The highest P_2O_5 uptake by seeds were recorded with SP_{60} treatment (116.98 mg) followed by *P.vermiculosum* + RP_{60} (113.91mg). The increase in P uptake and its consequent reflection on yield as an effect of microbial inoculations may be caused by its ability to solubilise insoluble inorganic phosphates as well as to produce a necessary phytohormone indole 3 acetic acid [10]. Besides P soulubilizers are reported to enhance root growth which in turn help in increased nutrient uptake [11] and they are also known to increase use efficiency of applied P fertilizers [12].

Effect on nodulation: It is evident from the results (Table 2) that inoculation of seeds with PSM significantly increased the number and dry weight of nodules both in the absence and presence of RP. The highest number (135.5) and dry weight (482.5 mg) of nodules were recorded in the treatment of *P. vermiculosum* with RP_{60} follwed by *A.niger* with RP_{60} (124.5 and 427.5 mg). Both these cultures performed better than the test cultures, *Ps.striata* and *A.awamori*. The results confirm the findings of Gaur [5] and Dubey [9] who were also reported increased nodulation due to PSM inoculation. It is known that every aspect of the process of formation of the N_2 fixing nodule is limited by the availability of P [13].

Available P content and PSM population in soybean rhizosphere: The highest accumulation of available P content in the inoculated series indicated that introduced microorganisms got themselves well established and proliferated. It is also evident from the highest count of P soulubilizers in the inoculated treatments compared to control (Table 2). The highest count (47.33×10^4) was observed in *Bacillus $S_{P.}$* + RP_{60} treatment. Phosphate solubilizing bacteria are common in rhizosphere [14]. Stimulation of P solubilizers in the rhizosphere of sunflower crop due to inoculation was also reported by Jones and Sreenivas [15]. The organisms were isolated from the rhizosphere of various plants and are known to be metabolically more active than those isolated from sources other than rhizosphere [16].

The available P_2O_5 content of soil with the inoculated cultures was found to be declined at harvest stage. Probably at later stages, the assimilable organic matter content might have been reduced considerably. Further, it might be due to the refixation of solubilized phosphate into insoluble compounds of Ca, Mg, Fe and Al and also due to immobilization by microorganisms during metabolic processes. The increased available P content of soil due to inoculation were reported by Banik and Dey [7] and Jisha and Alagawadi [3].

From the results, it may be concluded that inoculation of PSM along with RP has a higher impact on increasing crop yield in comparison with conventional phosphorus fertilizer. This practice can conveniently be adopted by the farmers and will be cost effective besides being ecofriendly.

ACKNOWLEDGEMENT

We are thankful to University Grants Commission (UGC), New Delhi for providing financial assistance.

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