Influence of Mineral Phosphate Solubilizing Gram-positive Bacteria on Growth and Yield of Cowpea (*Vigna unguculata*)

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Abstract- A pot experiment was conducted in glass house conditions for 120 days to study the effects of phosphorus solubilizing Gram-positive bacteria on plant growth, nutrient uptake and yield of cowpea (*Vigna unguculata*). Among all twelve bacterial treatments, the strain *Bacillus spp* (B3) has shown the greatest shoot length 67.70 cm, root length 60.5 cm and total dry weight 4.57 g plant^{-1} after 45 days of sowing. Similarly, in the same treatment the shoot length, root length and total dry weight were found 162.36 cm, 60.5 cm and 128.49 g plant^{-1}, respectively at harvesting and total yield was obtained 23.34 g plant^{-1}, which was significantly greater than all other treatments and uninoculated control treatments containing single super phosphate (SSP) and rock phosphate (RP) as phosphorus sources (7.93 g plant^{-1} and 3.81 g plant^{-1} respectively). The strains *Bacillus thurengensis* (T1 and T6) also have indicated a significant increase in above parameters i.e root length, shoot length, total dry weight and yield. In *Bacillus thurengensis* (T1 and T6) the total yield was observed 19.50 g plant^{-1} and 18.03 g plant^{-1} respectively. Besides these above strains a significant increase in growth and yield of cowpea plants was observed in all the Gram–positive phosphorus solubilizing bacteria inoculation compared to uninoculated control.

Index Terms- Bacillus spp, Phosphorus solubilization, Cowpea

I. Introduction

Pulses are an integral component of Indian agriculture and are an important source of dietary protein for the peoples more so for a large vegetarian population of India. Among the pulse crops, cowpea (*Vigna unguiculata* L. Walp.) has greater potential in productivity due to its robust nature of growth as compared to any other pulse crop.). In India, cowpea occupies an area of 3.9 m.ha with a production of 2.2 m.t. having a productivity of 567 kg/ha (Ganiger *et al.*, 2002). Pulse crops are known to respond well to phosphatic fertilizers, since phosphorus plays an important role in root growth, nodulation and nitrogen fixation. Phosphorus is considered to be the kingpin nutrient in pulse crop growth having a direct role in better root development, flower primordia initiation, stimulation of growth and formation of seeds. The chemical fertilizers applied to soil easily get fixed and phosphorus becomes unavailable to plants. It is therefore necessary to go for alternative ways to supplement phosphorus to crops. The soil microorganisms have great potential to solubilize inorganic and organic phosphorus compounds and increase plant available phosphorus (Ganiger et al., 2002). The phosphate solubilization by Gram-negative bacteria and their usefulness in increasing crop growth and yield is well documented (Almas and Mohammad 2006; Fankem et al., 2008). However, much work has not done on role of Gram-positive phosphorus solubilization bacteria and its effect on crop plants. Hence present investigation was carried out to test the beneficiary effect of Gram-positive P-solubilizing bacteria on plant growth and yield of cowpea.

II. Materials and Methods

A pot experiment was conducted with the inoculation treatments consisting of nine Gram-positive P-solubilizing bacteria (*Arthrobacter spp, Bacillus spp, Micrococcus spp and Bacillus thuringensis* strains) and a reference strain *B. polymyxa* (Table 1). Apart from these inoculated pots two un-inoculated controls were prepared with single super phosphate and rock phosphate as a phosphorus source. The three replicates for each treatment were maintained. Autoclaved black soil composition with pH 7.8, total N 163.2 kg ha^{-1}, total P_2O_5 10.20 kg ha^{-1} and available K_2O_5 320.0 kg ha^{-1} was used for the study. Pot size of diameter 169cm having capacity for 10kg soil was employed in the present experiment. Cowpea seeds of variety C-152 were collected from Department of Genetics; University of Agricultural Sciences, Dharwad was used in present investigation. All the treatments supplied with recommended dose of fertilizer (N: P: K: 25:50:25 kg ha^{-1} respectively). Nitrogen in the form of urea and potassium in form of murate of potash were applied to all the treatments at recommended levels. Phosphorus in the form of single super phosphate was applied only to SSP control, where all other treatments received phosphorus form of rock phosphate at recommended level.

Cowpea seeds were dressed with respective cultures using the seed bacterization method (Weller and Cook, 1983). Bacterial cultures growth was scraped and thoroughly mixed with four percent sterile carboxy methyl cellulose (CMC) suspension was used to dress cowpea seeds. *Bradyrhizobium spp* was given as common seed inoculum for all treatments following same procedure described same as earlier. Cowpea seeds were surface sterilized with sodium hypochlorite (4%) for 25 minutes and then thoroughly rinsed twice with sterile water. The seeds were coated with CMC based culture suspension was air dried for 2hrs by placing in a laminar airflow chamber (LFC) and used for sowing. Biocoated seeds were sowed in all the pots and three plants per pot were maintained. Regular equal watering (Sterile water) was done to meet the growth requirements of the crop. Observations were taken two times during crop period i.e. at 45DAS and at harvest. Root length and shoot length were measured in centimeters manually. Plant weight was estimated on fresh dry weight basis. Average number of pods per plant, test weight and average grain yield per plant were determined. Test weight was recorded by manually counting one hundred seeds randomly from each replication and their weight expressed in grams per hundred seeds. The P content in plant samples was estimated by Vanadomolybdate yellow colour method (Jackson, 1973).

Table 1. Treatment details of the greenhouse experiment involving efficient Gram-positive P-solubilizing bacteria

Treatment No.	Treatment details
T1	SSP (RDP as single super phosphate and no inoculation)
T_2	RP (RDP as rock phosphate and no inoculation)
T_3	*Arthrobacter spp*. (A1) + RP
T_4	*Arthrobacter spp*. (A2) + RP
T_5	*Bacillus spp*. (B3) + RP
T_6	*Bacillus spp*. (B7) + RP
T_7	*Bacillus spp*. (EB10) + RP
T_8	*Micrococcus spp*. (M8) + RP
T_9	*Micrococcus spp*. (M9) + RP
T_{10}	*Bacillus thuringiensis* (T1) +RP
T_{11}	*Bacillus thuringiensis* (T6) + RP
T_{12}	*Bacillus polymyxa* (Standard strain) + RP

Statistical Analysis

The data obtained from the experiments were subjected to statistical analysis for completely randomized design and interpretation of the data was done out in accordance with Panse and Sukhatme, 1985. The level of significance used in the F and' t' test was $P \leq 0.01$.

III. RESULTS AND DISCUSSION

In present investigation, a significant increase in shoot and root length of cowpea plants were observed at 45 DAS (Days After Sowing) and at harvest. The treatment inoculated with *Bacillus spp* had indicated a significant increase in root, shoot and dry weight among the inoculated strains. In *Bacillus spp* (B3) inoculated plants were observed vigorous growth. The shoot length, root length and fresh dry weight of plant was found 67.70 cm, 28.67 cm, 4.57 g plant^{-1} at 45 DAS and at harvest 162.36 cm, 96.33 cm, 139.1 g plant$^{-1,}$ respectively (Table 2).where as other inoculated pot plants such as *B. thuringensis* T1 and T6 were also indicated a significant increase in shoot, root and total dry weight over control.

Increasing shoot and root growth is due to production of growth promoting substances like IAA and GA in rhizophere soil by phosphorus solubilizing bacteria (Chaykocskaya, 2001 and Sahara et al., 2002). The total dry weight plant^{-1} was recorded maximum of 4.57 g plant^{-1} and 13.91g plant^{-1} with inoculation of *Bacillus spp* at 45 DAS and after harvest respectively. The results were significantly superior over rest of inoculated strains. All the bacterial inoculated treatments showed higher dry matter production by luxurious growth and development of crop plant due to increased availability of Pi (Jain and Singh, 2003) and production of phytohormones such as indole acetic acid (Arshad and Frankenberger, 1998) in rhizosphere soil. However, as seen from the observations, it seems to be no relationship between the growth promoting substances produced and the Pi released by the bacteria. It is obvious, therefore, that growth promoting substances and Pi producing facts acts independent of each other influencing plant growth and yield. Thus the observation of Arshad and Franknberger (1998) by use of phosphorus solubilizing bacteria can increase the growth of plants by producing growth promoting substances apart from increasing availability phosphorus in rhizosphere soil finds support.

Uptake of phosphorus was analyzed on fresh dry weight basis of the plant. The samples which indicated more amount of phosphorus it means they have observed or uptake more phosphorus from the experimental soil. The plants inoculated with phosphorus solubilizing Gram-positive bacteria were also showed significant increase in P uptake at 45 DAS and at harvest (Table 3). The *Bacillus spp* inoculated cowpea plants recorded more total phosphorus uptake than *Arthrobacter spp* and *Micrococcus spp.* The pots inoculated with *Bacillus spp* (B3) showed a significantly highest total P uptake in cowpea plant i.e., 20.41 mg plant^{-1} at 45 DAS and 189.82 mg plant^{-1} at harvest. However, the reference strain *B. polymyxa* also showed a significant increase in P uptake over control. The *Micrococcus spp* (M8 and M9) inoculated pots was recorded low total P uptake which differ non significantly with other inoculated strains.

Increased P content in rhizosphere soil by the process of P solubilization led to increased phosphorus uptake in crop plants inoculated with Gram-positive bacteria. Similar trend of increase in crop growth and P uptake inoculated with phosphorus solubilizing bacteria was observed by other workers (Sahara et al., 2002, Tanwar et al., 2003).

The phosphorus solubilizing bacteria inoculated pots were observed increase in average number of pods per plant, test weight and grain yield over control. *Bacillus spp* (B3) inoculated pots were recorded significantly highest average number of pods per plant (12.00), grain weight (2.55 g/100grains) and grain yield per plant (23.34 g plant^{-1}).

Table 2. Inoculation effect of efficient Gram-positive phosphate solubilizing bacteria on growth parameters of cowpea plants at 45 DAS (Daya after sowing) and at harvest.

Treatments	Shoot length (cm)		Root length (cm)		Total dry matter (g plant^{-1})	
	At 45 DAS*	At harvest	At 45 DAS*	At harvest	At 45 DAS*	At harvest
SSP control	36.13	120.33	19.33	66.00	1.69	7.28
RP control	33.90	119.03	15.00	51.67	0.69	5.09
Arthrobacter spp. (A1) + RP	39.07	145.37	25.00	78.00	2.80	10.63
Arthrobacter spp. (A2) + RP	38.20	142.33	23.00	77.00	2.69	9.98
Bacillus spp. (B3) + RP	67.70	162.36	28.67	96.33	4.57	13.91
Bacillus spp. (B7) + RP	57.07	152.30	27.10	93.33	3.89	11.76
Bacillus spp. (EB10) + RP	53.40	150.27	24.66	85.33	3.43	10.65
Bacillus thuringiensis (T1) + RP	64.60	158.37	27.67	94.33	4.28	12.71
Bacillus thuringiensis (T6) + RP	56.80	153.37	24.33	92.00	3.72	12.34
Bacillus polymyxa + RP	46.10	134.33	21.00	73.00	2.28	8.98
Micrococcus spp. (M8) + RP	45.37	140.33	21.66	75.33	2.32	9.15
Micrococcus spp. (M9) + RP	43.80	138.37	20.00	73.33	2.25	8.95
SEm±	0.456	1.915	0.489	1.351	0.082	0.253
CD ((P=0.01))	1.815	7.631	1.948	5.383	0.327	0.990

Table 3. Inoculation effect of efficient Gram-positive phosphate solubilizing bacteria on phosphorus uptake and yield components of cowpea plants.

Treatments	Total P uptake (mg plant^{-1})		Yield		
	At 45 DAS*	At harvest	pods plant^{-1}	100 grain weight	Seed Yield (g plant^{-1})
SSP control	2.91	28.53	7.92	9.53	7.93
RP control	0.69	11.42	5.56	8.60	3.81
Arthrobacter spp. (A1) + RP	7.13	76.63	9.37	10.35	12.45
Arthrobacter spp. (A2) + RP	6.55	70.32	9.41	10.65	12.10
Bacillus spp. (B3) + RP	20.41	189.82	12.28	12.55	23.34
Bacillus spp. (B7) + RP	14.64	127.32	10.69	11.54	17.56
Bacillus spp. (EB10) + RP	10.60	106.38	9.32	11.33	15.33
Bacillus thuringiensis (T1) + RP	19.09	151.43	11.40	12.39	19.50
Bacillus thuringiensis (T6) + RP	15.02	133.55	10.68	11.62	18.03
Bacillus polymyxa + RP	3.95	51.07	8.54	9.51	9.70
Micrococcus spp. (M8) + RP	4.87	57.41	8.38	9.63	10.47
Micrococcus spp. (M9) + RP	4.61	55.08	8.10	9.47	9.55
SEMm±	0.24	1.40	0.238	0.159	0.283
CD (P=0.01)	0.954	5.58	0.949	0.635	1.127

The increase in the growth and yield parameters was due to phosphate solubilizing bacteria which are able to mobilize more phosphorus to crop plants and also due to production of growth promoting substances in rhizosphere soil (Prabhakharan et al., 1999, Sahara et al., 2002). The results states that, phosphorus and growth promoting substances produced by bacteria have direct influence on growth and yield of crop plants. In conclusion that present study showed Gram-positive bacteria, *Bacillus spp* (B3) proved better than the reference strain *B. polymyxa* in performance. The range of variability seen amongst isolates indicates that it is prudent and necessary to keep the isolation of beneficial bacteria a continuous program. The phosphate solubilizing bacteria can thus definitely play an essential role in promoting plants to establish and grow better with application of rock phosphate as an alternate source of phosphorus.

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