Screening the Effect of Phosphate Solubilizing Bacteria on Black Gram (*Vigna mungo* L.)

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Abstract: ***Phosphorus is a plant macronutrient that plays a significant role in plant metabolism, ultimately reflected on crop yields. Phosphorus solubilizing bacteria (PSB) play important role by enhancing its availability to plants through release from inorganic and organic soil P by solubilization and mineralization. In the present study, 6 Phosphate Solubilizing Bacterial strains were isolated on the Pikovskayas agar medium from soil samples of black gram field collected at Perambalur District, Tamilnadu, India. The PSB strains were identified as Azotobacter sp., Rhizobium sp. Bacillus sp., Pseudomonas sp., Bacillus sp. and Rhizobium sp. Out of 6 PSB strains 2 strains PSB4 (2.47) and PSB5 (2.37) were showed highest phosphate solubilization Index (PSI). In pot culture experiment, PSB strains 4 and 5 applied black gram plants were significantly enhanced the morphometric data's compared with other PSB strains and control. The PSB4 and PSB5 applied plants were recorded higher shoot length (35.2 cm and 34.0 cm) and root length (15.1 cm and 14.3 cm), number of pods (63.1 and 58.2) and yield (33.8 g and 32.9 g) on 90 DAS. Followed by PSB3 and PSB6 were moderately enhanced plant growth and yield.***

Keywords: Phosphorus, Pikovskayas medium, Phosphate solubilizing bacteria, black gram and Pot culture

1. Introduction

Phosphorus is a bio critical element in short supply in nature. Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell enlargement and several other processes in the living plant (Gyaneshwar *et al*., 2002). In India, majority of the phosphorus provided in the form of chemical fertilizer which abundant uses decreases the fertility of soil after long period of time. In nature, wide range of microbial bio-solubilization mechanisms exist which is necessary to maintain global cycle (Whitelaw, 2000).

Many microorganisms are able to solubilize unavailable forms of phosphates by excreting organic acids. These microbes present in the different forms and numbers in the soil (kucey, 1983). A large number of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus Rhizobium* and *Serratia* have reported to enhance plant growth by with their different plant growth promoting activities including phosphate solubilization (Kumar *et al*., 2012).

Phosphate solubilizing bacteria (PSB) are the bacteria that possess the capability to change the insoluble form of phosphorus into soluble one. Phosphorus is one the most essential element for plant growth second only to nitrogen in requirement for plants. Phosphorus plays a significant role in physiological and biochemical plant activities (Abd-alla, 1994).

Approximately 95-99% of soil phosphorus is present in the form of insoluble phosphates and cannot be utilized by the plants (Vassileva *et al*., 2001). Seed or soil inoculation with phosphate solubilizing bacteria is known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Yadav and Dadarwal, 1997). Application of biological fertilizers such as biological phosphate fertilizers improves soil fertility (Abdol Amir Yousefi *et al.*, 2011).

Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement, seed formation and its content is higher in seeds than in any other part of the plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants (Amit Sagervanshi *et al*., 2012). The inoculation of P-solubilizing microorganisms (PSM) is an efficient technique because it can increase P availability in the soil (Sanjotha *et al*., 2016).

2. Materials and Methods

Collection of sample

The rhizospheric soil sample was collected from black gram field at Perambalur district, Tamilnadu, India. Collected soil samples were filled in sterile poly propylene bag and brought to the Microbiology laboratory, PG Extension Centre, Bharathidasan University, and Perambalur for further analysis.

Isolation of Phosphate solubilizing Strains

For isolation of Phosphate Solubilizing Microbes, 1g rhizosphere soil was suspended in 100 ml of distilled water. An aliquot (100 µl) from decimal dilutions was inoculated on Picovskayas medium by pour plate technique and incubated at 30° C. Colonies showing phosphate solubilizing zone around them were considered as phosphate solubilizing microbes (PSM). Single colonies appearing on Picovskayas agar plates were transferred in liquid broth of Picovskayas and on agar slants for further study.

Identification of PSB

The isolated bacteria were identified by morphological, physiological characteristics in which Gram staining, shapes, motility and biochemical characters including sucrose, lactose fermentation, starch hydrolysis, Gelatin hydrolysis and Nitrate reduction etc.

Analysis of Phosphate Solubilizing Activity

From the isolates, larger halo zone producing strains were selected for further study. The qualitative analyses of phosphate solubilizing activity of the selected isolates were conducted by plate screening method.

Qualitative method

All the suspected colonies were screened for phosphate solubilization on Pikovskaya's medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centre of Pikovskaya's plate and incubated at 37° C. Diameter of clearance zone was measured successively after 24 hours, up to 7 days. The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter *i.e.* clearance zone including bacterial growth and the colony diameter.

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily identify as PSB.

Effect of *Rhizobium* strains on *Arachis hypogaea* L.

The experiment was conducted in a green house with opening windows at the research and development station of Sri Amman Biocare, Biofertilizer production unit, Thanjavur, Tamilnadu, India. The agricultural field top soil (0-20 cm) was collected from Thirukkanurpatti. Soil was sterilized at 121° C and 15 lbs for 1 h intermittently for 3hours (to ensure complete sterilization) and 10 kg of soil was filled in each pot. A randomized block design with the treatment in split arrangement was used. There were three replications with each phosphate solubilizing bacterial strains (PSB). Totally six PSB strains treated and one control set up also maintained. The healthy seeds of black gram were chosen and sowed in pots, each pot have 10 seeds. The 1 ml of (10^9 cells) PSB strains was introduced in appropriate pots without PSB strains served as control. The water was irrigated in pots once in a week and morphometric data's (shoot length, root length, number of pods and yield) were studied and recorded.

Results and Discussion

The collected soil samples were evaluated for Phosphate solubilizing bacteria in Pikovskayas agar medium. A total of 6 Phosphate Solubilizing Bacterial colonies were isolated on the Pikovskayas agar medium, containing insoluble tri-calcium phosphate (TCP) from agricultural soil. The colonies showing clear halo zones around the microbial growth were considered as phosphate solubilization were shown. Similarly, Patel and Parmer, (2013) reported that 11 isolates were found good phosphate solubilizer from rhizosphere soil of sunflower field at Surat, Bharuch district of Gujarat. The bacterial isolates were further characterized by a series of morphological, physiological and biochemical tests and identified as *Azotobacter* sp., *Rhizobium* sp. *Bacillus* sp., *Pseudomonas* sp., *Bacillus* sp. and *Rhizobium* sp. (Table 1). In the present study correlated with Deepika *et al.* (2013), the isolated PSB microorganism were identified as *Bacillus* sp, *Neisseria* sp, *Klebsiella* sp, *Enterobacter sp, Pseudomonas* sp., *Proteus* sp.

From the isolates, halo zone producing strains were selected for further study. The qualitative as well as quantitative analyses of phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method. All the selected isolates were found to be potent phosphate solubilizers showing clear zone around their colonies. Out of 6 bacterial isolates 2 isolates PSB4 (2.47) and PSB5 (2.37) showed highest phosphate solubilization Index (PSI) (Table 2). Similarly, Karpagam and Nagalakshmi (2014) reported that out of 37 microbial isolates 8 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 3.0.

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available for root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Ahmad Ali Khan *et al*., 2009).

Inoculation of plants with PSMs generally results in improved plant growth and yield, in particular, under glasshouse conditions (Ahmad Ali Khan *et al.*, 2009). In the present study, inoculation of phosphate solubilization bacterial strains significantly enhanced the black gram shoot length, root length compared with control on 30, 60 and 90 day after sowing (DAS) in pot culture experiments. Phosphate solubilization bacterial strains 4 and 5 (PSB4 and PSB5) applied plants were significantly enhanced the morphometric data's compared with other PSB strains and control. The PSB4 and PSB5 applied plants were recorded higher shoot length (35.2 cm and 34.0 cm) and root length (15.1 cm and 14.3 cm), number of pods (63.1 and 58.2) and yield (33.8 g and 32.9 g) on 90 DAS. Followed by PSB3 and PSB6 were moderately enhanced plant growth and yield (Table 3 and Fig.1). Similarly, Seed or soil inoculation with PSB is known to improve the solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield (Abd-Alla, 1994; Mehta and Nautiyal, 2001).

Table 1: Morphological and biochemical characteristics of bacterial isolates from root nodules of *Arachis hypogaea* L.

Strain	Gram stain	Motility	Shape	Indole	MR	VP	Starch hydrolysis	H_2S	Citrate Utilization	Suspected Organisms
PSB1	_-	+	rod	+	+	+	+	-_	+	*Azotobacter*
PSB2	_-	+	rod	+	+	+	+	+	-_	*Rhizobium*
PSB3	+	+	rod	-	-	+	+	-_	+	*Bacillus*
PSB4	_-	+	rod	-	-	-	+	+	+	*Pseudomonas*
PSB5	+	+	rod	-	-	+	+	-_	+	*Bacillus*
PSB6	_-	+	rod	+	+	+	+	+	-_	*Rhizobium*

Table 2: Screening of Phosphate solubilization in Pickovskayas medium

S.No.	PSB	Colony size in mm	Halo zone in mm	Solubilization index (SI)*
1	*Azotobacter*	12	16	2.33
2	*Rhizobium*	14	17	2.21
3	*Bacillus*	15	19	2.26
4	*Pseudomonas*	17	25	2.47
5	*Bacillus*	16	22	2.37
6	*Rhizobium*	14	18	2.28

Table 3: Effect of inoculation of PSB strains on plant growth, nodulation and yield of black gram (*Vigna mungo*) under pot culture conditions

S.NO	Strain	% germinations	Shoot Length (cm)			Root Length (cm)			Number of pods	Yield (g)
			30 days	60 days	90 days	30 days	60 days	90 Days		
1.	PSB1	90	14.5	24.3	31.3	9.2	12.9	13.5	52.6	29.5
2.	PSB2	95	15.6	26.2	32.4	10.3	13.1	13.7	52.8	30.2
3.	PSB3	95	16.2	26.6	33.4	10.6	13.4	14.1	56.2	32.5
4.	PSB4	100	18	28.5	35.2	11.1	13.6	15.1	63.1	33.8
5.	PSB5	100	17.5	27.2	34.0	10.9	12.0	14.3	58.2	32.9
6.	PSB6	95	16	26.3	32.1	9.9	12.1	13.8	54.6	31.4
7	control	85	13	24	29.5	9.0	1.6	12.5	47	27.2

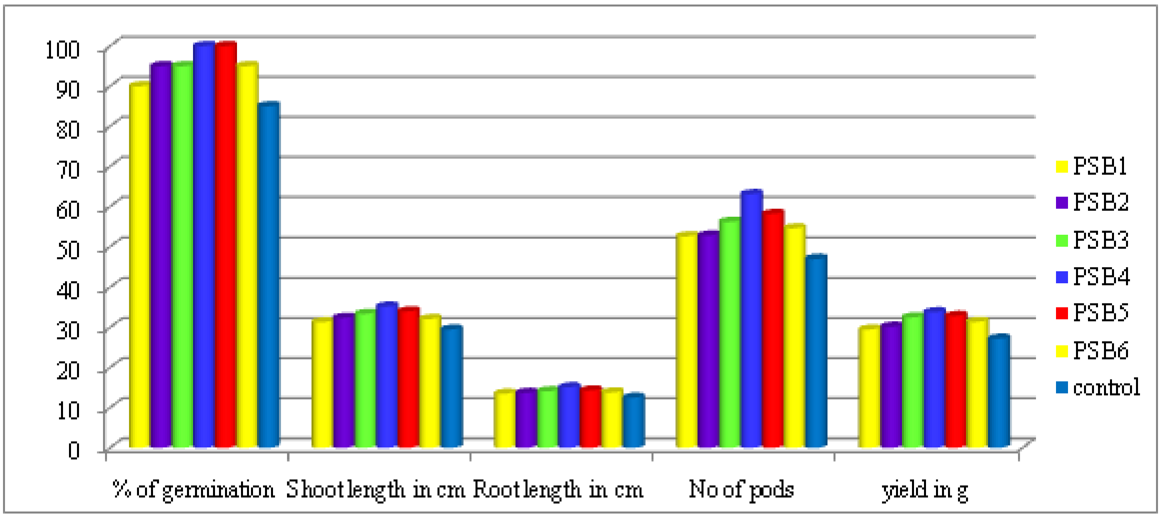

Figure 1: Effect of inoculation of PSB strains on plant growth, nodulation and yield of black gram (*Vigna mungo*) under pot culture conditions

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