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Efficiency of Plant Growth Promoting Rhizobacteria for the Enhancement of *Cicer arietinum* L. Growth and Germination under Salinity

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Abstract: Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. Ten isolates of bacteria designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2 were successfully isolated and characterized. Subsequently to investigate the effect of PGPR isolates on the growth of *Cicer arietinum*, a pot culture experiment was conducted. Prior to seeds grown in plastic pots, seeds were treated with PGPR isolates and seedlings were harvested after 21 days of inoculation. Isolates PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induce the production of indole acetic acid, whereas only PGT3 isolate was able to solubilize phosphorus. Most of isolates resulted in a significant increasing of shoot length, root length and dry matter production of shoot and root of *Cicer arietinum* seedlings. Application of PGPR isolates significantly improves the percentage of seed germination under saline conditions. The present study, therefore suggest that the use of PGPR isolates PGB4, PGG2 and PGT3 as inoculants biofertilizers might be beneficial for *Cicer arietinum* cultivation.

Key words: *Cicer arietinum* · Indole acetic acid · NaCl · PGPR

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are group of bacteria that actively colonize plant roots and increase plant growth and yield. The mechanism by which PGPR promote plant growth are not fully understood, but are thought to includes the ability to produce phytohormones, asymbiotic N_2 fixation against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and fungicidal compounds [1]. Significant increases in growth and yield agronomical important crops in response to inoculation with PGPR have been reported [2]. Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogen and pests. Moreover, PGPR mediate biological control indirectly by eliciting induced systematic resistance against a number of plant diseases [3]. Under salt stress, PGPR have soon positive effect in plants on such parameters as germination rate, tolerance of drought, yield and plant growth [4].

Cicer arietinum is the most important staple food in several developing countries and chemical fertilizers is the most important input required for *Cicer arietinum* cultivation. In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know now to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can be contribute to the improvement of *Cicer arietinum* growth. Thus the aim of this study was to determine the effect of PGPR strains that are compatible with *Cicer arietinum*. We also investigated the influence of PGPR with salinity on seed germination.

MATERIALS AND METHODS

Ten grams of rhizosphere soil were taken in to a 250 ml of conical flask and 90 ml of sterile distilled water was added to it. After serial dilution upto 10^{-7} an aliquot of this suspension was spread on the plates of Luria Bartany (LB) agar medium. After 3 days of incubation at

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28°C bacterial colonies were streaked to other LB agar plates and incubated at 28°C for 3 more days. Typical bacterial colonies were observed over the streak. Well isolated colonies were picked up and different characteristics of colonies such as shape, size, elevation, surface, margin, color, order and pigmentation etc. were recorded. A loopful of bacterial culture from each isolates was diluted into a test tube containing 1 ml sterile distilled water and was vortexed. A loopful was then taken on a glass slide and smeared. The slide was air dried and fixed by heating on a Bunsen flame. The slide was flooded with crystal violet solution for 3 min. The slide was washed gently in flow of tap water and air dried. The slide was observed under microscope and recorded the shape. Motility of bacteria was observed by hanging drop method. A loopful of 2-day-old bacterial culture was suspended in 1 ml of nigrosin solution. A drop of suspension was taken on a cover slip. The cover slip was hanged on a hollow slide with vaseline. The slide was then observed under microscope to test the motility of bacteria.

The culture of 10 isolates were streaked on LB agar plates and incubated at 10, 20, 28, 37 and 45°C. The bacterial isolates were designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2. A single colony of bacterial culture was grown on LB liquid medium. A loopful of the respective culture was transferred to the 100 ml of conical flask then incubated for 7 days on a rotary shaker. The IAA production and phosphate solubilization were then examined according to the method given by Bric *et al*. [5] and Pikovaskya, [6] respectively. Seeds of C*icer arietinum* L. cv. Avrodhi procured from Directorate of Extension, AAI-DU, Allahabad were soaked in H_2SO_4 for 5 min and washed with sterile water three times. *Cicer* seeds were treated with bacterial isolates for 30 min. The seeds were then saturated with three different NaCl solution which were derived from sterile distilled water by adding 0(control), 3, 6 and 12 mM NaCl with the electrical conductivities (Ecs) were 0, 3.44, 12.28 and 18.40 dsm^{-1} respectively only for determination of seed germination percentage. Germinated seeds were recorded and discarded at 24 h interval over 10 days [7].

An amount of 0.3 kg sand was placed into a pot. Ten PGPR inoculated seeds were sown at 4 to 5 cm depth of sand in each plastic pot. The *Cicer* plants were harvested after 21 days of seed sowing through separating of plants from soil. Shoot length (cm $plant^{-1}$) and root length (cm $plant^{-1}$) of each plant were recorded after drying in an oven for 1 day at 70°C. The data was analyzed statistically by Student's t-test. The significance of differences between mean values was evaluated by DMRT (Duncan's New Multiple Range Test).

RESULTS AND DISCUSSION

Isolation and Characterization of PGPR: Ten bacterial isolates were successfully isolates from the rhizosphere of *Cicer arietinum*. They were designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2. As shown in Table 1, the morphological characteristics of PGPR isolates widely varied. The isolates were found to be first growers. All the isolates produced round shape and raised colonies having smooth shiny surface with smooth margin. They differed in color but all were odorless and no pigmentation was observed in the colonies of LB agar plates. Diameters of the colonies of isolates varied from 0.2 to 2.0 mm. PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms.

Table 1: Morphological characteristics of 3-days old colony of PGPR isolates

Isolates	Shape	Size (mm)	Elevation	Surface	Margin	Colour	Odour	Pigmentation
PGB1	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Off whitish	Odourless	None
PGB2	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Pinkish	Odourless	None
PGB3	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Brownish	Odourless	None
PGB4	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Yolk brown	Odourless	None
PGB5	Round	1.9-2.0	Raised	Smooth shiny	Smooth	Brownish	Odourless	None
PGT1	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Yellowish	Odourless	None
PGT2	Round	1.5-2.0	Raised	Smooth shiny	Smooth	Yolk yellowish	Odourless	None
PGT3	Round	0.2-0.5	Raised	Smooth shiny	Smooth	Whitish	Odourless	None
PGG1	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Yellowish	Odourless	None
PGG2	Round	0.5-1.0	Raised	Smooth shiny	Smooth	Off white	Odourless	None

Table 2: Cell shape motility and Gram reaction of PGPR isolates

Isolates	Cell shape	Motility	Gram reaction
PGB1	Rod	Motile	Gram positive
PGB2	Ellipsoidal	Motile	Gram positive
PGB3	Rod	Motile	Gram positive
PGB4	Rod	Motile	Gram positive
PGB5	Rod	Motile	Gram positive
PGT1	Ellipsoidal	Motile	Gram positive
PGT2	Rod	Motile	Gram positive
PGT3	Rod	Motile	Gram positive
PGG1	Rod	Motile	Gram positive
PGG2	Rod	Motile	Gram positive

Table 3: Growth of PGPR isolates at different temperature conditions

	Temperature				
Isolates	10°C	20°C	28°C	37°C	45°C
PGB1	+	++	++	+	-
PGB2	+	++	++	+	-
PGB3	+	++	++	++	+
PGB4	++	++	++	++	++
PGB5	+	++	++	+	-
PGT1	+	++	++	+	-
PGT2	+	++	++	-	-
PGT3	-	++	++	+	-
PGG1	+	++	++	+	-
PGG2	-	++	++	++	-

- = No growth; + = weak growth and ++ = good growth

Microscopic Observation of PGPR Isolates: Microscopic observations were performed to examine some characteristics of PGPR isolates such as shape, Gram reaction and motility (Table 2). Eight isolates were rod shaped while PGB2 and PGT1 showed ellipsoidal shape. All the isolates were motile and Gram positive in reaction. It was also found to be that growth of isolates on LB agar plates varied in temperature (Table 3). The growth of all isolates was good in the temperature range of 20 to 28°C. In addition, PGB3 and PGB4 isolates were found to grow at 45°C.

Production of IAA and Solubilization of Phosphorus: As shown in Table 4, isolates PGG2, PGB4, PGT3, PGT2, PGT1 and PGG1 induced the IAA production. On the contrary, PGT3 was found to be a medium producer of IAA in comparison to the weak producer isolates PGT1, PGT2 and PGG1. On the other hand only PGT3 isolates had ability to solubilize the phosphorus. It has been reported that IAA production by PGPR can vary among different species and it is also influenced by culture condition, growth stage and substrate ability [8]. PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to *Cicer arietinum* that represent a possible mechanism of plant growth promotion under field condition [9]. In comparison to non-rhizospheric soil, higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere.

Table 4: Production of indole acetic acid and solubilization of phosphorus by PGPR isolates

Isolates	IAA production	Phosphorus solubilization
PGB1	-	Not solubilizing
PGB2	-	Not solubilizing
PGB3	-	Not solubilizing
PGB4	+++	Not solubilizing
PGB5	-	Not solubilizing
PGT1	+	Not solubilizing
PGT2	+	Not solubilizing
PGT3	++	Solubilizing
PGG1	+	Not solubilizing
PGG2	+++	Not solubilizing

- = No production; + = weak producer; ++ = medium producer and +++ = good producer

Table 5: The effect of PGPR on germination rate of Chickpea under salinity

	NaCl (mM)			
Isolates	Control	3	6	12
Control	69.30	48.57	45.19	31.72
PGB1	70.90	51.38	42.59	34.88
PGB2	71.42	62.57	48.37	35.11
PGB3	74.42	70.67	55.66	34.12
PGB4	75.01	68.51	51.19	36.87
PGB5	79.12	65.53	50.42	32.18
PGT1	80.00	58.37	43.34	34.51
PGT2	80.68	58.86	45.09	36.92
PGT3	82.72	79.80	50.31	28.74
PGG1	85.62	75.34	41.31	35.12
PGG2	88.72	75.87	42.77	39.34

Seed Germination: The first observation of this study was that increasing NaCl concentration decreased the germination percentage in *Cicer arietinum* seeds (Table 5). When NaCl treatments compared to each other it was seen that the effect of PGPR on germination percentage varies with bacterial isolates. The effect of PGPR on germination rate of seeds under saline conditions was statistically significant ($p<0.05$). Shannon and Grieve, [10] reported that salinity showed the

Table 6: The effect of PGPR isolates on growth of Chickpea seedlings

Isolates	Shoot length (cm plant^{-1})	Shoot dry weight (mg plant^{-1})	Root length (cm plant^{-1})	Root dry weight (mg plant^{-1})
Control	10.30	7.20	4.10	5.60
PGB1	12.30	7.60	4.50	6.40
PGB2	12.50	8.60	4.30	6.60
PGB3	11.80	8.00	4.40	6.40
PGB4	13.80	9.40	5.30	6.80
PGB5	10.90	7.80	4.60	6.50
PGT1	12.60	7.60	4.50	6.20
PGT2	12.00	8.20	4.40	6.60
PGT3	13.10	9.20	4.80	6.00
PGG1	12.70	7.60	4.50	6.50
PGG2	13.20	9.60	5.10	7.10

germination rate and at low concentration the only was on germination rate and not total percentage of seeds. The results of our study clearly showed that PGPR improved germination percentage and rate according to the control in spite of the use of high concentrations of NaCl. Nelson, [11] noted that PGPR were able to exert a beneficial effect upon plant growth such as increasing the germination rate.

Length and Dry Weight of Shoot and Root: The PGPR isolates significantly affected the length of *Cicer arietinum* seedlings. Results reveal that the shoot length increased in PGPR treated plants over uninoculated control. The highest shoot length 13.80 cm was recorded in PGB4 isolate which was statistically similar to isolates PGT3 (13.10 cm plant^{-1}) and PGG2 (13.20 cm plant^{-1}). A significant increase in shoot dry matter of *Cicer arietinum* seedling was observed in response to PGPR isolates. The highest shoot dry matter was recorded in isolate PGG2 (9.60 mg plant^{-1}) followed by PGB4 (9.40 mg plant $^{-1}$) and PGT3 (9.20 mg plant^{-1}). Root length ranged from 4.10 to 5.30 cm plant^{-1}. The isolate PGB4 produced the highest root length (5.30 cm plant^{-1}), in comparison to other isolates PGB5 and PGT3 also showed superior root length respectively (Table 6). A significant variation in root dry weight was observed in response to different PGPR isolates. In this study, the effectiveness of PGPR isolates on shoot length, root length and dry weight of shoot and root were investigated. Most of the isolates significantly increased shoot length, root length and dry matter production of shoot and root of seedlings.

Our results suggested that PGPR are able to enhance the production of IAA, solubilization of phosphorus and resistance to pathogen and pests, thereby improving growth of *Cicer arietinum* plant. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable *Cicer arietinum* cultivation in India and other developing countries. Further investigations, including efficiency test under green house and field conditions needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on plant growth and development.

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