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Effect of plant growth-promoting rhizobacteria (PGPR) on growth and physiological parameters in chickpea

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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. Isolates of PGPR designated as *Pseudomonas aeruginosa* strain (2CpS1) is taken as PGPR. Subsequently, a pot experiment was carried out at wire house of the Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi with chickpea variety (JW-14), four treatments and three replications during rabi season 2011-2012. Isolate of PGPR induced production of plant hormones (indole acetic acid), phosphate solubilization and ammonia production to enhanced plant growth. Isolate resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings under salinity condition. Therefore, present study suggests that *Pseudomonas aeruginosa* strain (2CpS1) may be used as bio-fertilizers to enhance the growth and productivity of chickpea.

Keywords: Indole acetic acid, *Pseudomonas aeruginosa*, PGPR, Phosphate solubilization

Introduction
Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. A large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and Serratia have reported as PGPR to enhance plant growth[1]. The direct growth promotion of plants by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The indirect plant growth promotion occurs by PGPR due to preventing deleterious
effects phytopathogenic microorganisms. PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms.
They help in increasing nitrogen fixation in legumes, help in promoting free-living nitrogen-fixing bacteria, increase supply of other nutrients, such as phosphorus, sulphur, iron and copper, produce plant hormones, enhance other beneficial bacteria or fungi,control fungal and bacterial diseases and help in controlling insect pests.
Chickpea (*Cicer arietinum*) is the most important staple food in several developing countries and chemical fertilizers are the most important input required for chickpea 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 could contribute to the improvement of chickpea growth.
Thus the aim of this study was to determine the effect of plant growth promoting rhizobacteria for improvement of germination and plant growth of chickpea (*Cicer arietinum)* under salinity condition.

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Materials and Methods

Morphological and Growth Parameters

Bacterial culture and Isolation- The soil used for bacterial isolation was collected from high altitude location (31.01ºN latitude and 78.45ºE longitude) in Patalbhuwaneshwer district, of Uttarakhand state. Sampling were done during prevailing atmospheric temperature was 8 ºC. The samples were transported to the laboratory in temperature controlled conditions. The collected soil was serially diluted in sterile physiological saline, spread plated in triplicate on nutrient agar [2] and incubated at 4 ºC for 48 h. A Yellowish, irregular, medium oval colony appeared to be predominant in the isolation plates was purified and maintained on nutrient agar slants and 20% glycerol at -80 ºC. The cell cultures of the bacterium have been deposited in the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India (Accession number HQ222366).

Phosphate solubilization by bacteria. Isolate was first screened on Pikovskaya's agar plates for phosphate solubilization as described by [3]. Bacterial cultures were inoculated on centre of agar plate through inoculation loop under aseptic condition. Inoculated plates were incubated for 3 days at 30 °C. Halo zone was obtained on Pikovskaya's agar plates. This halo zone showed positive phosphate solubilization ability.

Chickpea seed germination and plant growth. Disease free and healthy seeds of chickpea (*Cicer arientinum* L.) cultivar JG-14, semi-erect having 92- 95% viability were obtained from the Department of Genetics and Plant Breeding, Jawaharlal Nehru krishi Vishwa Vidyalaya, Jabalpur.

Seed Treatment: The method of Weller and Cook [4] was followed for seed bacterization; chickpea seeds (cv. JW 14) were surface sterilized with 1% NaOCl for 3–5 minutes and subsequently washed in sterilized distilled water 3–4 times and air dried. Cells of 2CpS1 were grown in King's B broth (protease peptone 20g+ $K_2HPO_4.3H_2O$ 1.908g+ $MgSO_4.7H_2O$ 1.5 g+ glycerol 15mL+ distilled water 985 mL) for 24 h at 28± 1 °C under shaking conditions and finally cells in the exponential phase were centrifuged at 7000 rpm for 15 min at 4 °C. The supernatant was discarded and pellets were washed with sterilized distilled water and resuspended to obtain a population of 10^7 cfu mL^{-1}. This suspension was mixed with 1% caboxy methyl cellulose (CMC). Surface-sterilized chickpea seeds of uniform size were then bacterized by dipping for 2 h into the bacterial suspension followed by air drying at room temperature under aseptic conditions. Care was taken to avoid clumping of seeds. Seeds coated with only a slurry of CMC without bacteria served as control (Sharma *et al*., 2008). 6-8 seeds were sown in each pot of size 20 x 20 cm. Half of the pots were sown with treated seeds with *Pseudomonas aureginosa*, whereas, remaining pots were sown with non treated seeds. After germination a population of four plants per pot were maintained. The pots were kept under net house condition. The experiment was setup in 3 replication with 4 treatments. All seeds were germinated. After 21days, chickpea plants were harvested. Shoot and root length were recorded in centimeter of each plant. Then, plants were dried in an oven at 65 °C for 3 days. After this shoot and root dry weight were recorded in gram.

Statistical Analysis

Simple CRD design was followed and analysis of variance was performed on the data as described by Panse and Sukhatme [5]. Critical difference (CD) values were calculated at 5 per cent probability level.

Result

Plant height, root lengh and dry matter of shoot & root

Plant height showed significant differences among different treatments at 14 and 28 days after salinity treatment (DAST) as presented in Table 1. Plant height was found maximum for T2 [*Pseudomonas aeruginosa* (2CpS1) treatment + No NaCl treatment] and the least plant height was recorded in T3 [No *Pseudomonas aeruginosa* (2CpS1) treatment + 150 mM NaCl treatment] at both the growth stages. There was significant increase in plant height recorded on treatment with *Pseudomonas aeruginosa* under both normal and saline conditions. There were 20.60 and 21.93 per cent increase in plant height as compared to control at 14 and 28 DAST respectively due to plant growth promoting rhizobacteria (PGPR) treatment. Similarly, under saline conditions, PGPR promoted plant height and there was 20.65 and 21.17 per cent increase in plant height as compared to T3 [No *Pseudomonas aeruginosa* (2CpS1) treatment + 150mM NaCl treatment] at 14 and 28 DAST respectively. Plant height for all the treatments were more at 28 DAST as compared to 14 DAST. The mean plant height recorded at both the stages was highest for treatment T2 followed by T4, T1 and T3.

A significant difference for root length was observed at 14 and 28 DAST (Table 2). Maximum increase in root length was observed in T2 [*Pseudomonas aeruginosa* (2CpS1) treatment + No NaCl treatment] (33.04% and 39.20%) at 14 and 28 DAST, respectively as compared to T1 [control]. There was significant reduction in root length in T3 [No *Pseudomonas aeruginosa* (2CpS1) treatment + 150 mM NaCl treatment] as compared to control. PGPR treatment resulted in 23.08 and 31.97 per cent increase in root length in T4 as compared to T3. Among the growth stages, 28 DAST had greater root length as compared to 14 DAST for all the treatments. The mean root length recorded at both the growth stages was highest for T2 followed by T1, T4 and T3.

A significant difference for root dry matter was observed at 14 and 28 DAST (Table 3). Maximum increase in root dry matter was observed in T2 [*Pseudomonas aeruginosa* (2CpS1) treatment + No NaCl treatment] (8.52 and 9.35 per cent) at 14 and 28 DAST, respectively as compared to T1 [control]. There was significant reduction in root dry matter in T3 [No *Pseudomonas aeruginosa* (2CpS1) treatment + 150 mM NaCl treatment] as compared to control. PGPR treatment resulted in 25.48 and 12.54 per cent increase in root dry matter in T4 as compared to T3. Among the growth stages, 28 DAST had greater root dry matter as compared to 14 DAST for all the treatments. The mean root dry matter recorded at both the growth stages was highest for T2 followed by T1,T4 and T3. Shoot dry matter showed significant differences among different treatments at 14 and 28 DAST as presented in Table 4. Shoot dry matter was found maximum for T2 [*Pseudomonas aeruginosa* (2CpS1) treatment + No NaCl treatment] and the minimum Shoot dry matter was recorded in T3 [No *Pseudomonas aeruginosa* (2CpS1) treatment + 150 mM NaCl treatment] at both the growth stages. There was significant increase in shoot dry matter recorded on treatment with *Pseudomonas aeruginosa* under both normal control and saline conditions. There were 36.49 and 18.55 per cent increase in shoot dry matter as compared to control at 14 and 28 DAST respectively due to plant growth promoting rhizobacteria (PGPR) treatment. Similarly, under saline conditions, PGPR promoted shoot dry matter and there was 46.40 and 40.13 per cent increase in shoot dry matter as compared to T3[No *Pseudomonas aeruginosa* (2CpS1)

treatment + 150 mM NaCl treatment] at 14 and 28 DAST respectively. Shoot dry matter for all the treatments was more at 28 DAST as compared to 14 DAST. The mean shoot dry matter recorded at both the stages was highest for treatment T2 followed by T4, T1 and T3.

Table 1: Effect of *Pseudomonas aureginosa* on plant height (cm) in chickpea under normal and saline conditions at two growth stages.

Treatment	14 DAST	28 DAST	Mean
T1	15.00	19.33	17.17
T2	18.10 (20.60)	23.57 (21.93)	20.83
T3	14.57	17.50	16.03
T4	17.58 (20.65)	21.03 (21.17)	19.31
Mean	16.31	20.36	-
SEm+	0.76	1.31	-
C.D. at 5%	1.74	3.01	-

Figure in parentheses indicate percentage increase over normal control and saline control.

DAST: Days after salinity treatment
T1: Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T2: *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T3: No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T4: *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment

Table 2: Effect of *Pseudomonas aureginosa* on root length (cm) in chickpea under normal and saline conditions at two growth stages.

Treatment	14 DAST	28 DAST	Mean
T1	18.73	21.07	19.90
T2	24.92 (33.04)	29.33 (39.20)	27.13
T3	17.33	20.33	18.83
T4	21.33 (23.08)	26.83 (31.97)	24.04
Mean	20.58	24.39	-
SEm+	1.06	2.93	-
C.D. at 5%	2.46	6.76	-

Figure in parentheses indicate percentage increase over normal control and saline control.

DAST: Days after salinity treatment
T1: Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T2: *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T3: No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T4: *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment

Table 3: Effect of *Pseudomonas aureginosa* on root dry matter (mg plant^{-1}) in chickpea under normal and saline conditions at two growth stages.

Treatment	14 DAST	28 DAST	Mean
T1	59.00	67.22	63.11
T2	64.03(8.52)	73.51(9.35)	68.77
T3	48.78	60.97	54.88
T4	61.21(25.48)	68.62(12.54)	64.91
Mean	58.25	67.58	-
SEm+	4.49	2.95	-
C.D. at 5%	10.35	6.81	-

Figure in parentheses indicate percentage increase over normal control and saline control.

DAST: Days after salinity treatment
T1: Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T2: *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T3: No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T4: *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment

Table 4: Effect of *Pseudomonas aureginosa* on shoot dry matter (mg plant^{-1}) in chickpea under normal and saline conditions at two growth stages.

Treatment	14 DAST	28 DAST	Mean
T1	275.95	335.45	305.70
T2	376.65 (36.49)	397.70 (18.55)	387.17
T3	238.67	279.23	258.95
T4	349.42 (46.40)	391.30 (40.13)	370.36
Mean	310.17	350.92	-
SEm+	32.67	26.73	-
C.D. at 5%	75.34	61.64	-

Figure in parentheses indicate percentage increase over normal control and saline control.

DAST: Days after salinity treatment
T1: Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T2: *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T3: No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T4: *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment

Discussion

The rhizosphere is the zone surrounding the root system of all plants where exudates stimulate microbial growth. It has a major influence on the health and productivity of crops. Among rhizosphere microorganisms, some can reduce plant growth by acting as pathogens, while others such as plant growth-promoting rhizobacteria (PGPR) colonize roots and can promote plant growth [6]. PGPR can promote plant growth directly through nitrogen fixation [7, 8], facilitation of nutrient uptake [7, 9], solubilization of phosphorus [10, 11], phytohormone production [12], or by lowering soil levels of ethylene [8, 13]. In addition, PGPR may indirectly promote plant growth by decreasing or preventing the effects of nonpathogenic, deleterious microorganisms through production of antimicrobial compounds [14, 15], production of siderophores which helps them compete with other microbes (including pathogens) for iron [16, 17], competition for colonization sites on the root, competition for nutrients [18, 19], or induced systemic resistance [20].

The principle rhizobacterial genera known to act as PGPR include *Azospirillum* [21], *Bacillus* [22], *Burkholderia* [23], *Enterobacter* [24], *Paenibacillus* [25], *Pseudomonas* [26], *Serratia* [27], and *Streptomyces* [22]. These rhizobacteria have been studied for their potential as biocontrol agents to control plant diseases and as biofertilizers to improve plant growth [28]. The largest number of reports of PGPR involves *Pseudomonas* spp. The pseudomonads rapidly colonize roots and produce several different antifungal metabolites and

therefore have been widely applied as biocontrol agents [26].
Environmental stresses are limiting factors for agricultural productivity worldwide. These stresses not only decrease the yield of crops but also represent barriers to the introduction of crop plants into areas that are not suitable for crop cultivation. Abiotic stress factors include high and low temperatures, salinity, drought, flooding, ultraviolet light, heavy metals, and oxidative stresses [29]. The responses of plants to drought and salt stresses have much in common and involve a number of metabolic and physiological changes, many of which have not been fully characterized. Plant cells maintain total water potential during drought and salt stress by osmotic adjustment, a process to decrease water potential by accumulation of sugars or other compatible solutes such as proline, glycine betaine, mannitol, and sorbitol. Several transgenic plants which overproduce such solutes have shown some tolerance to drought and salt stress [30]. Osmotic adjustment helps to maintain turgor and enables the continuation of cell elongation at lower water potentials. Osmotic adjustment is a mechanism by which plants acclimate to dehydration conditions, like drought and salt stress. Plants exhibit decreased water uptake and a subsequent reduction in leaf growth rate, which results in restricted photosynthetic capacity under salinity [31]. There are overlaps in signal transduction between abiotic and biotic stresses. Studies have shown that plants resistant to one stress are often more resistant to others. This phenomenon is known as cross-tolerance [32, 33]. For example, ozone treatment triggers induced resistance of Arabidopsis to subsequent infection with *P. syringae* [34].
Application of PGPR increases plant health overall. Precisely how the interaction of plant and PGPR affects the physiology and metabolism in plants is unclear. There is much research concerning the effect of PGPR applied as biocontrol agents on a plant's resistance or tolerance to different plant pathogens. In contrast, only a few PGPR strains have been studied for their capacity to enhance plant tolerance to environmental stresses. Plants with reduced ethylene levels by PGPR with 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity showed a substantial tolerance to flooding stress [35] and metal contaminants [36, 24] .
This study was conducted with *Pseudomonas aeruginosa*, strain 2CpS1 showing ACC deaminase activity and surviving well at 8% NaCl concentration in lab on how it affects growth, physiological, biochemical and yield and yield components in wheat under both control and salinity situation imposed in pots. In our study, Pseudomonas treatment to wheat seeds showed significant increase in plant height, root length and leaf area when compared to non-treated control seeds. Results showed 8.5 %, 15.41% and 30.65% increase in plant height, root length and shoot length due to PGPR treatment across both the growth stages. Without PGPR treatment, plant height, root length and leaf area decreased drastically under salinity stress compared to no salt stress-control treatment. When 150mM NaCl was applied, wheat plants treated with *Pseudomonas aeruginosa* had significantly greater plant height, root length and leaf area as compared to the nonbacterized salt control. Seeds bacterized with *Pseudomonas aeruginosa* under salt stress had 22.03 %, 14.31% and 35.22% greater plant height, root length and leaf area compared to non-bacterized salt control. Many studies have been published showing that inoculation of PGPR can significantly increase root and shoot growth. PGPR interact directly with plant root systems and have a positive impact on root biomass, morphology and physiology [37, 38]. The association between *Azospirillum* spp. and the plant root has been extensively investigated [21] and *A. brasilense* strain Cd has been shown to alter root morphology. Inoculation of wheat with Cd enhanced cell division in the root tips and increased the size of the elongation zone [39].
Our findings are in agreement with Upadhyaya *et al*. [40]. It is reported that the inoculation with rhizobacteria containing ACC-deaminase could result in the development of much better root system, which subsequently affects shoot growth and yield positively. This view is also supported by the work of other researchers [8, 36, 41]. This is also confirmed from the finding of Nukui *et al*. [42] who observed decreased root growth after application of exogenous ethylene or ACC and also determined the promotion of root growth because of the treatment with ethylene inhibitors. Similarly, Sergeeva *et al*. [43] observed the role of ACC-deaminase in enhancing growth promotion and greater tolerance in transgenic canola plants against high salt stress. Very recently, Yue *et al*. [44] reported that PGPR strain promoted growth of cotton under salt stress.
There was non-significant increase in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content between the treatments due to PGPR treatment under both non saline control and saline condition. Under salinity and without PGPR treatment there was reduction in chlorophyll content as compared to control. Our findings are concordant with Yildirim *et al*. [45]. Previous work indicated that PGPR can increase the chlorophyll content of plants, which is not surprising, considering that some PGPR enhanced plant nitrogen uptake [7, 28].
The present study suggests that *Pseudomonas aeruginosa* strain 2CpS1, seed treatment can ameliorate the deleterious effects of salt stress by increasing plant height, root length, leaf area, chlorophyll content, relative water content and decreasing cell membrane injury in wheat plant. PGPR strain tested in the experiment affected positively these parameters and also yield and yield components compared to the control under both salt stress and salt stress absence. Earlier researchers also found that PGPRs could ameliorate the deleterious effects of salinity on growth of pepper, cotton, canola and lettuce [46, 47, 44]. Application of PGPR to enhance stress tolerance in plants could be used as a feasible strategy for improving crop production In salinity environment. The study does not provide evidence on salt stress tolerance induction at plant tissue, cell or molecular level. Thus, future line of work could be to determine the effect of different locally isolated PGPR to be tested at plant tissue, cell or molecular levels and the efficiency of these PGPRs under natural field condition at different salinity levels.

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