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Induction of Growth and Physiological Parameters in Chickpea (*Cicer arietinum* L.) by Plant Growth-promoting Rhizobacteria under Salinity Stress

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An experiment was conducted to study the effect of plant growth promoting rhizobacteria (PGPR) on the growth, dry matter production and physiochemical parameters in a chickpea cultivar (JW-14) under salinity stress. The PGPR- *Pseudomonas aeruginosa* (strain 2CpS1) was applied through seed treatment. Seed treatment was observed to affect growth significantly by increasing the plant height, root length, leaf area, total dry matter, total chlorophyll content, relative water content under both normal and salinity conditions with respect to control. A significant decline in the cell membrane injury (%) was recorded under both normal and salinity treatments, when treated with *Pseudomonas aeruginosa* (strain 2CpS1), with respect to control.

Keywords: *Pseudomonas aeruginosa*, Salinity, Cell membrane injury, Chickpea.

Gram or Chickpea (*Cicer arietinum* Linn.), a member of family Fabaceae, is an ancient self pollinated leguminous crop, diploid annual (2N=16) grown since 7000 B.C., in different areas of the world (Tekeoglu *et al*., 2000) but its cultivation is mainly concentrated in semi-arid environments (Saxena, 1990). It is ranked 3rd after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). It is cultivated on 11.98 million ha across the world producing 10.91 million tons with productivity of 9112 kg/ha (FAO 2010). India is the largest producer in the world accounting for 66% of the total world production. It is cultivated over an area of 8.21 million hectares giving 7.48 million tons (FAO 2010) with an average yield of 9111 kg/ha. It is a major source of protein in human diet and animal feed. Roy *et al*. (2001) reported, protein content of 19-21% and carbohydrates content of 60% from chickpea. A comparison of amino acids content of various dietary proteins reveals that chickpea protein is comparable to beef or fish . It provides an excellent quality of dietary protein. Soil salinity is a major abiotic stress, adversely affects physiological and metabolic processes, leading to dimin-ished growth and yield (Abbaspoor *et al*. 2009). Salinity af-fects the availability of nutrients and water. Moreover, it induces osmotic stress; the physiological drought, which typically reduces the growth and photosynthesis in plants (Munnes and Tester, 2008). Growth reduction due to sa-linity is attributed to ion toxicity and nutrient imbal-ance, which causes not only high sodium (Na^+) and

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chlo-ride (Cl^-) accumulation in plants, but also antagonistically affects the uptake of essential nutrient elements such as potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) in competition with Na^+ and also nitrate (NO^{3-}) in contrast with Cl^- (Sairam *et al*., 2004.) Salt stress in addition to the known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress (Esfandiari *et al*., 2007). Rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). PGPR are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoot growth(Lucy *et al*. 2004). Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes (Yildirim E *et al*.2005). PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms.(Hamdia *et al*. 1978). 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 (Joseph *et al*.2011). There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for various crops.

MATERIALS AND METHODS

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. 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. The seeds were subjected to seed treatment by the plant growth promoting rhizobacteria, *Pseudomonas aeruginosa* (strain 2CpS1) following the method in which the population of the rhizobacterial strain to be obtained was 10^7 cfu ml^{-1}. Seeds were surface sterilized with 1% NaOCl for 3–5 minutes and subsequently washed in sterilized distilled water 3–4 times and air dried. Care was taken to avoid clumping of seeds. Seeds coated with only a slurry of CMC without bacteria served

Table 1. Effect of PGPR *Pseudomonas aeruginosa* (2CpS1) on growth and morphological parameters in chickpea under salinity stress.(28 DAST)

Treatment	Plant Height (cm)	Root length (cm)	Leaf area (cm^2 $plant^{-1}$)	Root dry matter (mg $plant^{-1}$)	Shoot dry matter (mg $plant^{-1}$)	Total dry matter (mg $plant^{-1}$)
T_1	17.17	19.90	59.97	63.11	305.70	368.35
T_2	20.83	27.13	61.78	68.77	387.17	455.96
T_3	16.03	18.83	48.83	54.88	258.95	313.83
T_4	19.31	24.04	53.39	64.91	370.36	470.67
Sem±	1.3	2.93	3.83	2.95	26.73	24.59
C.D at 5%	3.01	6.76	8.83	6.81	61.64	56.72

T_1 : Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T_2 : *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T_3 : No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T_4 : *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
DAST : Days after salinity treatment

Table 2. Effect of PGPR *Pseudomonas aeruginosa* (2CpS1) on physio-chemical parameters in chickpea under salinity stress.(28 DAST)

Treatment	Chlorophyll 'a'(mg g^{-1} FW)	Chlorophyll 'b'(mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Relative water content (%)	Cell membrane injury (%)
T_1	0.51	0.70	1.21	82.22	36.76
T_2	0.81	0.75	1.55	86.17	28.45
T_3	0.38	0.36	0.75	78.05	37.97
T_4	0.75	0.57	1.33	82.03	34.16
Sem±	0.07	0.12	0.16	1.84	2.57
C.D at 5%	0.16	0.27	0.37	4.25	5.93

T_1 : Control [No *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment]
T_2 : *Pseudomonas aureginosa* (2CpS1) treatment + No Nacl treatment
T_3 : No *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
T_4 : *Pseudomonas aureginosa* (2CpS1) treatment + 150 mM Nacl treatment
DAST : Days after salinity treatment

as control (Sharma *et al*., 2008). Soil to be used in the pots were dried, powdered and mixed thoroughly. Soil, sand and FYM were mixed in the ratio of 1: 3: 1 and then sterilized by using 4% formaldehyde (HCHO). The pots were washed with tap water and then sterilized by using 70% methanol and kept for drying. The pot filling was done after 5-6 days of soil and pot sterilization. Each plastic pot (20x20 cm) with the closed bottom end was filled-up with air dried soil, sand and farm yard manure. 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 and consistent care and precaution was taken. After 21 days of sowing, six pots each having *Pseudomonas aureginosa* treated seeds and non treated seeds were imposed with 150 mM NaCl treatment and similar number of pots were not given any salinity treatment. The pots having salinity treatment were poured with 100 ml of 150 mM Nacl. There were four treatments with three replications.

RESULTS AND DISCUSSION

Seed treatment with *Pseudomonas aeruginosa* (strain 2CpS1) resulted in an overall increase in the morphological, physiological and biochemical parameters in chickpea plant under salinity treatments as well as under control as indicated in Table 1. The parameters such as plant height, root length, leaf area, total dry matter, relative water content and chlorophyll content were observed to have the maximum value for the treatment T2 [*Pseudomonas aeruginosa* (2CpS1) treatment + No NaCl treatment]. Treatment with *Pseudomonas aeruginosa* resulted in significant increase in plant height (cm), Root length (cm) and Leaf area (cm^2 $plant^{-1}$) as compared to control, under both normal and saline condition. Significant increase in root dry matter (mg $plant^{-1}$), Shoot dry matter (mg $plant^{-1}$) and total dry matter (mg $plant^{-1}$) was observed as a result of treatment with *Pseudomonas aeruginosa* under both normal and saline condition. The chlorophyll 'a', chlorophyll 'b' and Total chlorophyll content (mg g^{-1} fresh weight) was recorded to be increased significantly, resulting from the treatment with *Pseudomonas aeruginosa* with respect to control, under both normal and saline condition. Relative water content (%) was observed to be increased significantly on treatment with *Pseudomonas aeruginosa* ,with respect to control, under both normal and saline condition. Treatment with *Pseudomonas aeruginosa* resulted in significant decrease in cell membrane injury (%) as compared to control, under both normal and saline condition, and it was found

to be maximum in case of saline condition (no *Pseudomonas aeruginosa* treatment+ 150mM NaCl treatment).

CONCLUSIONS

Thus, it can be concluded that seed treatment with *Pseudomanas aeruginosa* strain 2CpS1, can ameliorate the deleterious effect of salt stress by increasing plant height, root length, leaf area, chlorophyll content, relative water content and decreasing cell membrane injury in chickpea. The positive effect of PGPR treatment on these parameters was observed in comparison to control under both salt stress and normal (without salt) condition. Feasible strategy for improving the crop production, could therefore, be the application of PGPR to enhance stress tolerance. However, the study doesn't 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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