JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2539-2545

Effect of Cowpea *Bradyrhizobium* (RA-5) and *Burkholderia cepacia* (RRE-5) on Growth Parameters of Pigeonpea under Salt Stress Conditions

Dilruba A. Bano*, R.K. Singh, Showkat A. Waza and N.P. Singh

Department of Genetics and Plant Breeding,
Institute of Agricultural Sciences, BHU, Varanasi-221005, India.

(Received: 16 April 2015; accepted: 30 May 2015)

An experiment was conducted to evaluate the effects of single inoculation of cowpea *Bradyrhizobium* (RA-5) and dual inoculation of Cowpea *Bradyrhizobium* (RA-5) and PGPR *Burkholderia cepacia* (RRE-5) on growth parameters of pigeonpea under various salt concentrations at different time intervals. Results indicated that growth parameters of the dual inoculated plants were significant and more desirable over the respective single inoculated ones. Increased salt concentrations impose undesirable effects on the growth parameters. Best growth was observed in treatment containing dual inoculation of RA-5 + RRE-5 with no salt. Very poor growth was observed in single inoculated plants applied with 200 mM saline solution. Dual inoculated plants significantly reduced the accumulation of sodium (Na^+) in their leaves than the respective single inoculated plants.

Key words: RA-5, RRE-5, pigeonpea, growth, salt, DAS.

Salinity is one of the serious abiotic stresses that causes reduction in plant growth and productivity in irrigated areas of arid and semi-arid regions of the world (Cicek and Cakirlar, 2002; Parida and Das, 2005). Plant productivity is considerably reduced under saline conditions due to osmotic inhibition of water uptake by roots or specific ion effects (Mayak *et al*., 2004). Salinity increases the uptake of Na^+ or decreases the uptake of P and K^+ which lead to nutritional imbalances in the plant system (Yildirim and Taylor, 2005).

In order to improve the plant growth under salinity stress conditions, and for sustainable crop production, it is necessary to improve salt stress tolerance in crops. Inoculating seeds and seedlings with Plant Growth Promoting Rhizobacteria (PGPR) is an alternative option to reduce salt stress effects in crop plants (Dixon *et al*., 1993). It has been demonstrated that some PGPR are able to produce polysaccharide products, binding Na^+ in the root zone and hence reducing the effects of salt stress on plant (Tank and Saraf, 2010). Moreover, PGPR and *Rhizobium* co-inoculation may be of a greater value under salt stress conditions. Co-inoculation of *Rhizobium* and PGPR has potential to enhance nodulation and nitrogen fixation in roots of several legumes in addition to mitigation of salinity effects.

Therefore, keeping in view the mitigation of salt stress, present investigation was carried out to evaluate the effect of single inoculation of Cowpea *Bradyrhizobium* (RA-5) and dual inoculation of RA-5 + RRE-5 (*Burkholderia cepacia*) on plant growth parameters under various salt concentrations at different time intervals.

* To whom all correspondence should be addressed.
E-mail: dabgpb@gmail.com

MATERIALS AND METHODS

The present study was conducted during 2012-14 at the Microbial Genetics Laboratory of the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP, India. Two bacterial strains *i.e.*, cowpea *Bradyrhizobium* (RA-5) and PGPR strain *Burkholderia cepacia* (RRE-5) were used for inoculation to evaluate their effect on growth parameters of pigeonpea under salt stress conditions. These strains were obtained from the Microbial genetics laboratory of the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU. Seed of pigeonpea cv Bahar used were also obtained from the same department.

Bold and healthy seeds were surface sterilized in 0.2 % (w/v) mercuric chloride for 2 minutes, rinsed with sterilized water and germinated in autoclaved petriplates paved with blotting paper at the bottom. The petriplates were then kept in incubator at 28^0C and timely supplemented with deionised water for proper germination. After 3-5 days, germinated seeds were transferred to twenty four plastic pots each filled with oven dried sterilized sand. Each pot had a hole on the bottom allowing adequate drainage. The pots were arranged in a complete randomized design with three replications for eight treatments.

Four of the treatments each with three replications were applied with separate NaCl solutions of 0mM, 50mM, 100mM and 200mM concentration along with nutrient solution and inoculated with single bacterial strain *i.e.*, cowpea *Bradyrhizobium* (RA-5). Other four treatments each with three replications were also applied with separate NaCl solutions of 0mM, 50mM, 100mM and 200mM concentrations along with nutrient media, but were inoculated with dual bacterial strains *i.e.*, cowpea *Bradyrhizobium* (RA-5) and PGPR strain *Burkholderia cepacia* (RRE-5). 0mM NaCl means that no salt was applied with nutrient solution for 'control' in both single and dual inoculated plants. The pots were kept in an ambient air conditioned laboratory under artificial light. Growth parameters such as plant height, chlorophyll content, number of nodules per plant, number of leaves per plant, fresh weight per plant, shoot length, root length, root and shoot dry weight and accumulation of Na^+ content were recorded. Observations were recorded at 10, 20 and 30 days after sowing (DAS). Plants were completely harvested after 30 days of sowing. Completely Randomized Design (CRD) was adopted for data evaluation using analysis of variance (ANOVA) with Microsoft Excel software package.

RESULTS AND DISCUSSION

Significant and desirable effects of dual inoculation (RA-5 + RRE-5) was observed as compared to the respective single inoculated (RA-5) treatments when studied under various salt concentrations at different time intervals *viz.*, 10, 20 and 30 DAS. The average plant height of dual inoculated saline treated plants at 20 DAS decreased from 20.18 cm to 15.67 cm when the concentration of salt solution applied was increased from 50mM to 200mM. However, the average plant height of dual inoculated control plants with no salt was observed to be 24.95 cm. The average plant height of single inoculated saline treated plants at 20 DAS decreased from 18.47cm to 15.26 cm when the concentration of salt solution applied was increased from 50mM to 200mM. However, the average plant height of single inoculated control plants with no salt was observed to be 20.85 cm. The average chlorophyll content of dual inoculated treatments decreased from 11.57 to 9.67 SPAD units and single inoculated treatment from 12.67 to 9.66 SPAD units under the similar set of conditions. However, the average chlorophyll content of dual and single inoculated control treatment was observed to be 13.16 and 12.67 SPAD units, respectively at 20 DAS.

The average number of nodules per plant of dual inoculated treatment decreased from 27.40 to 22.14 and single inoculated treatment decreased from 22.67 to 19.00, when the concentration of salt solution applied was increased from 50mM to 200mM at 20 DAS. However, the control treatment revealed the average number of nodules per plant for dual and single inoculated treatment to be 30.85 and 26.67, respectively at 30 DAS. Similarly, the average number of leaves per plant of dual inoculated treatment decreased from 9.76 to 4.76 and single inoculated treatment decreased from 7.33 to 2.67 under the above given salt

Table 1. Effect of single (RA-5) and dual (RA-5 + RRE-5) inoculation on various growth parameters of pigeonpea under salt stress conditions at 10 DAS.

Treatment	Plant height (cm)	Chlorophyll content	No. of nodules per plant	No. of leaves per plant	Fresh weight per plant (g)	Shoot length (cm)	Root length (cm)	Shoot and root dry weight (g)	Na content
No Salt + RA-5 (control)	15.06	11.49	22.39	4.33	0.67	15.17	9.81	0.11	10.06
No salt + RA-5 + RRE-5 (control)	22.40	11.86	26.11	6.03	0.73	18.33	9.98	0.34	7.45
50mM NaCl + RA-5	14.75 (-5.89)**	10.90 (-5.11)**	21.77 (-2.74)**	2.67 (-38.46)**	0.49 (-27.36)**	14.13 (-6.81)**	8.00 (-18.45)**	0.10 (-6.25)**	10.23 (1.69)**
50mM NaCl + RA-5 + RRE-5	19.67 (-12.19)**	11.75 (-0.87)**	23.70 (-9.21)**	4.37 (-27.58)**	0.67 (-8.18)**	16.67 (-9.09)**	8.67 (-13.16)**	0.27 (-21.36)**	10.18 (36.69)**
100mM NaCl + RA-5	12.30 (-18.33)**	9.52 (-17.17)**	16.52 (-26.21)**	2.67 (-38.46)**	0.35 (-47.26)**	13.95 (-8.02)**	8.00 (-18.45)**	0.07 (-31.25)**	11.40 (13.32)**
100mM NaCl + RA-5 + RRE-5	15.85 (-29.25)**	11.17 (-5.82)**	22.51 (-13.76)**	3.33 (-44.72)**	0.55 (-24.55)**	16.50 (-10.00)**	8.33 (-16.50)**	0.14 (-59.22)**	11.33 (52.13)**
200mM NaCl + RA-5	11.40 (-24.31)**	8.55 (-25.59)**	16.00 (-28.53)**	1.33 (-69.23)**	0.48 (-28.86)**	12.82 (-15.49)**	6.33 (-35.44)**	0.06 (-40.63)**	11.80 (17.30)**
200mM NaCl + RA-5 + RRE-5	14.63 (-34.69)**	11.17 (-5.82)**	22.00 (-15.73)**	3.00 (-50.25)**	0.49 (-33.64)**	13.53 (-26.18)**	7.97 (-20.17)**	0.09 (-73.79)**	11.67 (56.60)**

[Figures in brackets indicate percent change over control] * and ** indicates significant at 0.05 and 0.01 levels, respectively

Table 2. Effect of single (RA-5) and dual (RA-5 + RRE-5) inoculation on various growth parameters of pigeonpea under salt stress conditions at 20 DAS.

Treatment	Plant height (cm)	Chlorophyll content	No. of nodules per plant	No. of leaves per plant	Fresh weight per plant (g)	Shoot length (cm)	Root length (cm)	Shoot and root dry weight (g)	Na content
No Salt + RA-5 (control)	20.85	12.67	26.67	8.33	0.71	23.05	10.67	0.22	10.69
No salt + RA-5 + RRE-5 (control)	24.95	13.16	30.85	10.62	0.79	24.33	12.15	0.41	9.04
50mM NaCl + RA-5	18.47	12.00	22.67	7.33	0.68	21.01	9.67	0.19	11.67
	(-11.43)**	(-5.26)**	(-15.00)**	(-12.00)**	(-27.36)**	(-8.84)**	(-9.38)**	(-13.64)**	(9.13)**
50mM NaCl + RA-5 + RRE-5	20.18	11.57	27.40	9.76	0.53	21.33	10.00	0.38	10.76
	(-19.09)**	(-12.09)**	(-11.18)**	(-8.13)**	(-33.61)**	(-12.33)**	(-17.70)**	(-6.50)**	(19.11)**
100mM NaCl + RA-5	16.55	10.62	21.85	3.33	0.52	16.51	8.58	0.12	12.73
	(-20.61)**	(-16.13)**	(-18.05)**	(-60.00)**	(-47.26)**	(-28.36)**	(-19.53)**	(-45.45)**	(19.05)**
100mM NaCl + RA-5 + RRE-5	18.33	11.53	27.26	7.00	0.51	18.67	9.46	0.25	12.63
	(-26.51)**	(-12.39)**	(-11.65)**	(-34.11)**	(-35.29)**	(-23.29)**	(-22.14)**	(-39.02)**	(39.76)**
200mM NaCl + RA-5	15.26	9.66	19.00	2.67	0.51	15.30	8.04	0.07	14.07
	(-26.83)**	(-23.74)**	(-28.75)**	(-68.00)**	(-28.86)**	(-33.62)**	(-24.66)**	(-68.18)**	(31.58)**
200mM NaCl + RA-5 + RRE-5	15.67	9.67	22.14	4.67	0.36	16.74	8.59	0.11	13.85
	(-37.20)**	(-26.53)**	(-28.23)**	(-56.07)**	(-54.20)**	(-31.21)**	(-29.30)**	(-73.17)**	(53.26)**

[Figures in brackets indicate percent change over control] * and ** indicates significant at 0.05 and 0.01 levels, respectively concentrations at 20 DAS. However, the control treatment showed the average number of leaves per plant for dual and single inoculated treatment to be 10.62 and 8.33, respectively at 20 DAS.

The average fresh weight per plant of dual inoculated treatment decreased from 0.53g to 0.36g and single inoculated treatment from 0.68g to 0.51g as the concentration of salt solution applied increased from 50mM to 200mM at 20 DAS. However, the control treatment showed the average fresh weight per plant of dual and single inoculated treatment to be 0.79g and 0.71g, respectively at the similar set of conditions. The average shoot length of dual inoculated saline treated plants at 20 DAS decreased from 21.33 cm to 16.74 cm when the concentration of salt solution applied was increased from 50mM to 200mM. The average shoot length of single inoculated saline treated plants under the same set of conditions decreased from 21.01 cm to 15.30 cm. However, the average shoot length of dual and single inoculated control plants was observed to be 24.33 cm and 23.03 cm, respectively at 20 DAS.

The average root length of dual inoculated saline treated plants at 20 DAS decreased from 10.00 cm to 8.59 cm when the concentration of salt solution applied was increased from 50mM to 200mM. The average root length of single inoculated saline treated plants at 20 DAS decreased from 9.67 cm to 8.04 cm when the concentration of salt solution applied was increased from 50mM to 200mM. However, the average root length of dual and single inoculated control plants (no salt) was observed to be 12.15 cm and 10.67 cm, respectively at 20 DAS. The average shoot and root dry weight of dual inoculated saline treated plants at 20 DAS decreased from 0.38 g to 0.11 g when the concentration of salt solution applied was increased from 50mM to 200mM. The average shoot and root dry weight of single inoculated saline treated plants at 20 DAS decreased from 0.19 g to 0.07 g at the same salt concentrations. However, the average shoot and root dry weight of dual and single inoculated control plants was observed to be 0.41 g and 0.22 g, respectively at 20 DAS.

The accumulation of sodium content in leaves revealed significant gain with an increase in concentration of the salt solution applied. Moreover, accumulation of sodium content in leaves for dual inoculated plants was significantly lower than the respective single inoculated plants, at different time intervals. The increase in accumulation of sodium content in leaves was observed to be 11.80 ppm to 14.07 ppm for single inoculated plants when the concentration of salt solution applied was increased from 50mM to 200mM at 20 days after sowing. Similarly, in dual inoculated plants the increase in accumulation of sodium content was observed to be 10.76 to 13.85 ppm under the similar set of conditions. The accumulation of sodium content in leaves for dual and single inoculated control plants was observed to be 9.04 g and 10.69 g, respectively at 20 DAS. Therefore, dual inoculated plants were observed to be more effective in alleviating salt stress than single inoculated ones. Similar trend as revealed by plant growth parameters at 20 DAS were also observed for 10 and 30 DAS (Tables 1, 2 and 3).

To summarise, all the plant growth parameters evaluated in present study except for accumulation of sodium content in leaves, were significantly higher in dual inoculated treatments as compared to respective single inoculated ones. However, accumulation of sodium content in leaves exhibited the reverse trend. Moreover, enhanced salt concentration of the solution applied imposes undesirable effects on the growth parameters. Best growth of plant was observed in treatment containing dual inoculation of cowpea *Bradyrhizobium* (RA-5) and PGPR (RRE-5) with no salt. Very poor growth was observed in treatments with single inoculation of cowpea *Bradyrhizobium* (RA-5) with 200 mM salt concentration.

Thus, treatments with single inoculation were less effective in mitigating the salinity effect as compared to that of dual inoculated ones. Han and Lee (2005) also conducted the similar studies in lettuce crop and reported that chlorophyll content of dual inoculated plants was significantly higher than the single inoculated plants. Ahmad *et al.* (2011) reported that salinity stress significantly reduced growth of mung bean plants and inoculation with PGPR enhanced the growth, thus reducing the inhibitory effect of salinity. Moreover, the combined application was reported to be more effective than single inoculation in mitigating the saline conditions. Egamberdieva *et al.* (2013)

Table 3. Effect of single (RA-5) and dual (RA-5 + RRE-5) inoculation on various growth parameters of pigeonpea under salt stress conditions at 30 DAS

Treatment	Plant height (cm)	Chlorophyll content	No. of nodules per plant	No. of leaves per plant	Fresh weight per plant (g)	Shoot length (cm)	Root length (cm)	Shoot and root dry weight (g)	Na content
No Salt + RA-5 (control)	24.5	13.15	32.5	8.67	1.06	23.67	11.5	0.52	11.14
No salt + RA-5 + RRE-5 (control)	27.11	13.96	33.48	11.59	1.14	27.33	12.67	0.76	10.48
50mM NaCl + RA-5	22.33 (-8.84)**	12.29 (-6.54)**	23.62 (-27.31)**	6.33 (-26.92)**	0.52 (-50.79)**	21.67 (-8.45)**	10.47 (-8.99)**	0.48 (-7.10)**	13.15 (18.01)**
50mM NaCl + RA-5 + RRE-5	25.75 (-5.03)**	12.33 (-11.65)**	31 (-7.40)**	9.48 (-18.20)**	0.75 (-34.02)**	22.33 (-18.32)**	11.17 (-11.84)**	0.57 (-24.56)**	12.67 (20.90)**
100mM NaCl + RA-5	19.67 (-19.73)**	10.98 (-16.48)**	23.37 (-28.10)**	4 (-53.85)**	0.39 (-62.78)**	20.86 (-11.87)**	9.04 (-21.39)**	0.29 (-44.52)**	13.16 (18.10)**
100mM NaCl + RA-5 + RRE-5	22.56 (-16.77)**	11.93 (-14.57)**	27.59 (-17.58)**	8 (-30.99)**	0.77 (-31.96)**	21.55 (-21.15)**	10.93 (-13.74)**	0.5 (-33.77)**	11.95 (14.03)**
200mM NaCl + RA-5	18.59 (-24.11)**	9.73 (-25.98)**	19.8 (-39.07)**	2.67 (-69.23)**	0.55 (-47.63)**	18.33 (-22.54)**	9 (-21.74)**	0.23 (-55.48)**	13.18 (18.31)**
200mM NaCl + RA-5 + RRE-5	21 (-22.54)**	10.85 (-22.25)**	21.17 (-36.75)**	6.33 (-45.37)**	0.61 (-46.33)**	19.67 (-28.05)**	9.67 (-23.68)**	0.47 (-38.60)**	11.88 (13.43)**

[Figures in brackets indicate percent change over control] * and ** indicates significant at 0.05 and 0.01 levels, respectively.

reported that co-inoculation treatment in legume crops was able to alleviate salt stress more than the single inoculation with *Bradyrhizobium*. Younesi *et al.* (2013) reported that under saline conditions, co-inoculated alfalfa (*Medicago sativa*) plants with *Rhizobium* and *Pseudomonas* have markedly longer plant height, number of nodules, shoot length, root length, shoot and root dry weight than the plants inoculated with *Rhizobium* alone. Thus, co-inoculation was reported to be more effective in alleviating the effect of salt as compared to the single inoculation.

REFERENCES

1. Ahmad, M., Zahir, Z.A., Asghar, H.N. and Asghar, M., Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. *Can J Microbiol*, 2011; **57**(7): 578-589.
2. Cicek, N. and Cakirlar, H., The effect of salinity on some physiological parameters in two maize cultivars. *Bulg J Plant Physiol*, 2002; **281**: 66-74.
3. Dixon. R.K., Garg, V.K. and Rao, M., Inoculation of Leucaena and Prosopis seedlings with Glomus and Rhizobium species in saline soil: rhizosphere relations and seedlings growth. *Arid Soil Res Rehabil*, 1993; 7: 133-144.
4. Egamberdieva, D., Berg, G., Lindstrom, K. and Rasanen, L.A., Alleviation of salt stress of symbiotic *Galega officinalis* L. (Goat's Rue) by co-inoculation of *rhizobium* with root colonising *Pseudomonas. Plant and Soil*, 2013; **369**(1): 453-465.
5. Han, H.S. and Lee, K.D., Plant Growth Promoting Rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Res J Agri Biol Sci*, 2005; **1**(3): 210-215.
6. Mayak, S., Tirosh, T. and Glick, B.R., Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol Biochem*, 2004; **42:** 565-572.
7. Parida, A.K. and Das, A.B., Salt tolerance and salinity effects on plants: A Review. *Ecotox Eviron Safety*, 2005; **60**: 324-349.
8. Tank, N.D. and Saraf, M.S., Salinity resistant PGPR ameliorates NaCl stress on tomato plants. *J Plant Interact*, 2010; **5**: 51-58.
9. Yildirim, E. and Taylor, A.G., Effect of biological treatments on growth of bean plants under salt stress. *Ann Rep Bean Improv Coop*, 2005; **48**: 176-177.
10. Younesi, O., Baghbani, A. and Namdari, A., The effects of *Pseudomonas fluorescence* and *Rhizobium meliloti* co-inoculation on nodulation and mineral nutrient contents in alfalfa (*Medicago sativa*) under salinity stress. *Intl J Agri Crop Sci*, 2013; **5**(14): 1500-1507.