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Mitigation of salinity in chickpea by Plant Growth Promoting Rhizobacteria and salicylic acid

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Abstract

For growth or development of pulses, biotic and abiotic environmental factors are more conspicuous under stress conditions. For the survival against abiotic stresses, salicylic acid (SA) is reported a universal remedy. At the Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, a pot study was conducted to monitor the role of Plant Growth Promoting Rhizobacteria (PGPR) and Salicylic acid in chickpea under salt stress. Eight treatments including control PGPR inoculation and Salicylic acid with their different combination were used. Results revealed that positive response of PGPR on productivity of chickpea but more enunciated response about grain yield was observed with the combined application of SA and PGPR compared to control. Growth parameters i.e root length, root mass, number of nodules and shoot mass were highly affected where SA was applied along with PGPR. From the study, it is proposed that under salt stress the combination of SA + PGPR can be a suitable practice for more production of chickpean Pakistan.

Keywords: Pulses, SA salt stress, microbes pulses.

Introduction

Salinity stress causes the major reduction in agricultural production of Pakistan by declining the crop yield through distressing the balance of water and micro and macronutrients of plants (Munns and Tester, 2008). There are a number of rhizospheric microorganisms which are known as plant growth promoting rhizobacteria (PGPR), when they develop strong relationship with a host plant improve the plant growth (Vessey, 2003). Nowadays, concentration of salts tremendously increasing which causes reduction in the agricultural output in major parts of the world (Hasanuzzaman et al., 2013).

Plant growth is suppressed under mainly by two ways; firstly by osmotic effects of salt stress and second by salt-specific effects (Sobhanian et al., 2010). However, salt tolerance can be enhanced in crops by various physiochemical pathways (Babu et al., 2012). To reduce the negative effects of salt stress on the growth and yield of plant a lot of research work has been conducted. Different techniques of biology like seed/plant treatments with plant growth promoting bacteria (PGPR) and exogenous application of growth hormones which make defense under stress conditions are previously studied (Hayat et al., 2010; Ullah et al., 2017; Qureshi et al., 2019). Among plant growth regulators salicylic acid is one of them which play a role in defensive system against different stresses either biotic or abiotic (Szalai et al., 2000). Among naturally existing phenolic compound, salicylic acid (SA) is one of them. A lot of evidences has been found that externally applied SA increased plant's tolerance to several abiotic stresses, including drought (Azooz and Youssef, 2010), osmotic stress (Al-Hakimi, 2006), heavy metal stress (Moussa and El-Gamel, 2010) and

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salinity (Gunes et al., 2007). Externally applied SA reduced transpiration and increased flower longevity, nitrate reductase activity as well as the yield of some plants which overall suggest that SA may enhance the multiple types of stress tolerance in plants through which interactive effects on several functional molecules or other signaling molecules participating in more complex stress response.

Biological Nitrogen Fixation is the biological mechanism where rhizobia symbiotically fix of atmospheric nitrogen into form which is available for plants in the presence of enzyme nitrogenase (Mohammadi and Sohrabi, 2012). The biological catalyst which is present in the bacteriod and mediates the reaction is a nitrogenase enzyme. BNF is cost effective and ecologically sound source of nitrogen and helps to decrease the dependence on external inputs

In pulses chickpea ranked at 3rd portion in overall world production. In many countries of the world chickpea (*Cicer arietinum* L.) is a key leguminous crop which is vital for its nutritional value. In arid areas chickpea is locally grown crop that has ability to adopt different biotic and abiotic environmental stress conditions (Rao et al., 2002). Through symbiotic nitrogen fixation chickpea fulfills its 70% of its N requirement (Siddique et al., 2005). So current study was planned to check the response of PGPR along with various concentrations of Plant growth regulators (Salicylic acid) under salinity stress.

Material and Methods

Isolation of PGPR

For the isolation of PGPR, rhizospheric soil of chickpea was collected from pulses research Institute, Faisalabad, Pakistan. Screening of microbes were carried out through auxin biosynthesis. Identification and characterization of selected isolate were done by using morphological, physiological, biochemical testing methods as described in Bergey's manual of Systematic Bacteriology.

For experiment, these PGPR isolates were grown on Luria Bertani (LB) media. A single loop was shifted to flasks of 250 mL which contains LB broth. Flasks are placed on a rotating shaker (95 rpm) for 24 h at 27ºC. The bacterial suspension concentration of 10^6 CFU ml^{-1} was maintained. 1 mL of log culture (110 cells) of each bacterial isolates was transferred as inoculum in the corresponding treatments. Auxin biosynthesis potential of screened isolates was determined in the terms of IAA equivalents (Sarwar et al., 1992).

Isolates	IAA Equivalents (µg mL^{-1})	Isolates	IAA Equivalents (µg mL^{-1})
Rhizobium sp. (ChickPea)		Azotobacter sp.	
CP-1		AZ-6	4.36
CP-2	5.89	AZ-7	5.56
CP-3	6.84	AZ-8	1.03
CP-4	4.36	AZ-9	5.04
CP-5	4.28	Azospirillum sp.	
		AS-1	2.08

Pot experiment

A glass house study in pots was carried out at the Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, to test the function of beneficial microorganisms (PGPR) along with Salicylic acid (SA) on chickpea crop in salinity stress conditions. Treatments were control (without inoculation), PGPR inoculation, Salicylic acid and their various combinations (T_1: Control, T_2: PGPR inoculation, T_3: Salicylic acid @ 10^{-3} M, T_4: Salicylic acid @ 10^{-4} M T_5: Salicylic acid @ 10^{-5} M, T_6: PGPR + Salicylic acid @ 10^{-3} M, T_7: PGPR + Salicylic acid @ 10^{-4} M, T_8: PGPR + Salicylic acid @ 10^{-5} M). Pots were filled with 10 kg soil having sandy clay loam texture, ECe 1.16 dSm^{-1}, pH 8.8 and saturation percentage age (33%). Used the chickpea (*Cicer arietinum* L.) seeds as plant materials. Chickpea seeds were sterilized in 1% $HgCl_2$ for 2 minutes, for surface sterilization and then, washed with sterilized distilled water at least 10 times to remove traces of toxic $HgCl_2$. After air drying Chickpea seeds were sown into sterilized pots (eight seeds per pot). All nitrogen and phosphorus doses were applied at time of sowing.According to treatment plan, seeds of chickpea were coated with peat based inoculum of selected strains of PGPR in a completely randomized design. Before pot filling salinity was developed by NaCl @ 5 dS m^{-1}. After 15days of seedling emergence foliar application of SA was carried out. All physiochemical parameters were recorded at the time of harvesting and after harvesting. N and P analysis was carried out by using method described by Jones (2001).

Chlorophyll and cartenoids concentration

To determine the chlorophyll and cartenoids, leaf sample of chickpea plant was taken in pre-weighed clean glass vials And add 10 mL of 80%. Bleached the leaf material and then decant off. Spectrophotometer (Spectronic Genesys-5, Milton Roy) was used to read the optical density at λ = 663, 646 and 470 nm using 80% acetone as a blank. Concentration of chlorophyll a, chlorophyll b and carotenoids (μg g^{-1}) was calculated according to Lichtenthaler and Wellburn (1983). Data was analyzed statistically by using statistics 8.1 (Steel et al., 1997).

Results

Physical parameters

Results revealed that positive response of microbes on growth and productivity of chickpea under salinity stress but distinctive effect was observed, in all growth characteristics of chickpea, where the p SA was applied along with co inoculation of PGPR. Maximum length of shoot was found in the treatment where PGPR and SA were applied. However, length of root and shoot was more in the treatment where only PGPR was applied as compare to un inoculated treatment but its effect was less as compare to combine treatment (PGPR and Salicylic acid) shown in Figure 1 and 2. Highest shoot and root length was recorded when PGPR and Salicylic acid 10^{-3} M was used. Maximum numbers of nodules were observed when combination of plant growth hormone (SA @ 10^{-5} M) and PGPR was applied (Table 1). Data regarding grain yield also showed that higher yield was recorded in treatment where PGPR + Salicylic acid 10^{-5} M was applied (Figure 3).

Table 1. Effect of treatments on yield parameters of chickpea

Treatments	Number of pods plant^{-1}	Pod yield (g pot^{-1})	Number of seed pod^{-1}	Branches plant^{-1}	Number of flowers plant^{-1}
T_1- Control	20.66 C	12.39 E	2	5 DE	26.00 BC
T_2- PGPR	24.66 C	14.63 D	2	6 CD	27.67 BC
T_3 - SA 10^{-3} M	20.66 C	15.40 D	2	3 E	22.67 C
T_4 - SA 10^{-4} M	21.33 C	16.01 CD	2	3 E	23.67BC
T_5 - SA 10^{-5} M	25.33 BC	17.36 BC	2	6 CD	27.33 BC
T_6 - PGPR + SA 10^{-3} M	30.33 AB	15.87 CD	2	7 BC	31.00 B
T_7 - PGPR + SA 10^{-4} M	31.00 A	18.61 B	2	9 AB	40.33 A
T_8 - PGPR + SA 10^{-5} M	25.66 ABC	21.33 A	2	10 A	47.67 A
LSD	5.519	1.702	N.S.	2.234	7.908

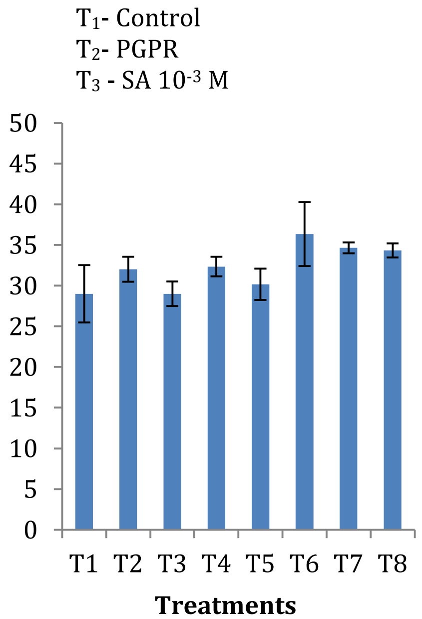

Figure 1. Effect of treatments on Plant Height (cm)

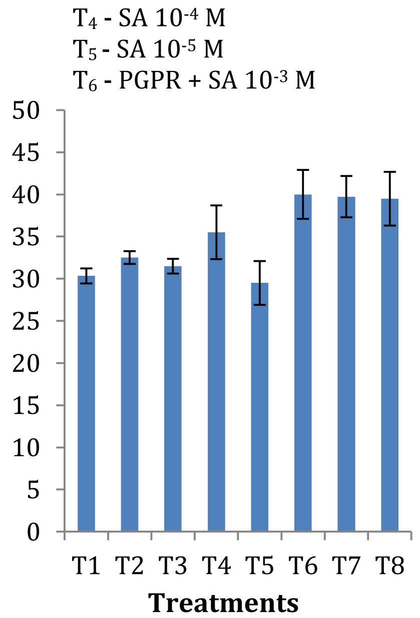

Figure 2. Effect of treatments on Root length (cm)

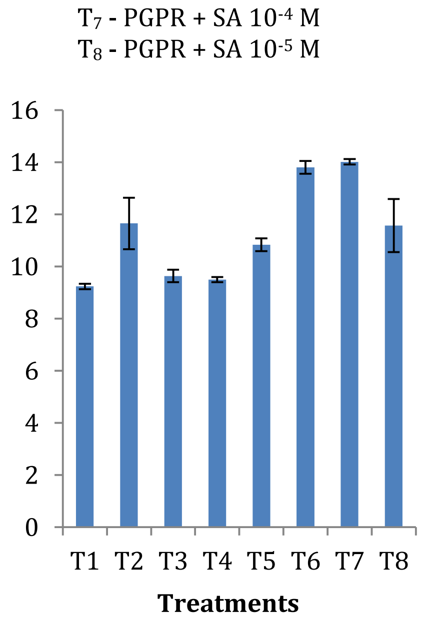

Figure 3. Effect of treatments on grain yield, g pot^{-1}

Data regarding chlorophyll and carotenoids, nodular mass, number of nodules revealed that maximum chlorophyll contents (a&b) were observed where SA@10^{-5} M along with PGPR present followed by other treatments as shown in Figure 4 and 5. But carotenoids were almost at par in all the treatments where SA and PGPR are combined as shown in Figure 6. It means efficiency of PGPR was enhanced in the presence of salicylic acid. In case of number of nodules maximum nodules were present in T_7 (SA@10^{-4}M+PGPR) followed by T_8 (SA@10^{-5} M+PGPR). Maximum nodular mass was observed in both the treatments T_7 & T_8 as compare to other treatments as shown in (Table 2).

Table 2. Effect of treatments on growth parameters of chickpea

Treatments	Fresh shoot wt. (g pot^{-1})	Dry shoot wt. (g pot^{-1})	Fresh root wt. (g pot^{-1})	Dry root wt. (g pot^{-1})	Number of Nodules Plant^{-1}	Nodular Mass (g)
T_1- Control	40.70 F	8.06 E	32.71 C	3.06 E	35.00D	0.08 BCD
T_2- PGPR	42.50 EF	8.43 DE	41.00 B	5.23 BC	37.00 CD	0.10 ABCD
T_3 - SA 10^{-3} M	41.50 EF	8.40 DE	43.22 B	3.96 DE	39.66 C	0.07 CD
T_4 - SA 10^{-4} M	43.50 DE	8.83 CD	43.67 B	3.88 DE	47.00 A	0.06 D
T_5 - SA 10^{-5} M	45.16 CD	9.23 BC	48.50 A	5.50 BC	39.00 C	0.10 ABCD
T_6 - PGPR + SA 10^{-3} M	47.30 BC	9.40 ABC	48.93 A	6.27 B	47.66 A	0.11 ABC
T_7 - PGPR + SA 10^{-4} M	48.83 AB	9.83 AB	50.51 A	7.86 A	49.00 A	0.12 AB
T_8 - PGPR + SA 10^{-5} M	51.00 A	10.10 A	43.71 B	4.72 CD	43.66 B	0.12 A
LSD	2.298	0.728	3.483	1.174	3.238	0.037

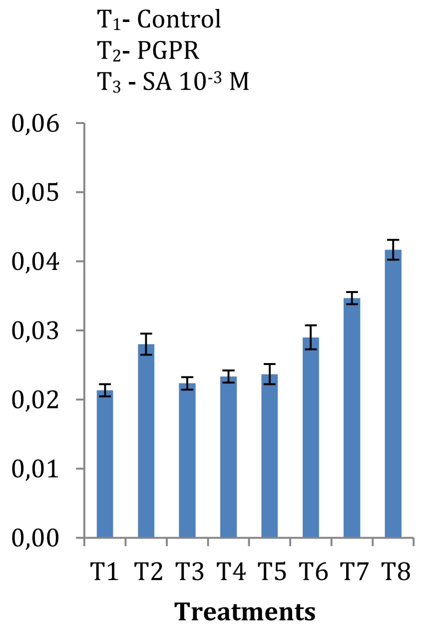

Figure 4. Effect of treatments on Chlorophyll a (μg g^{-1}) concentration

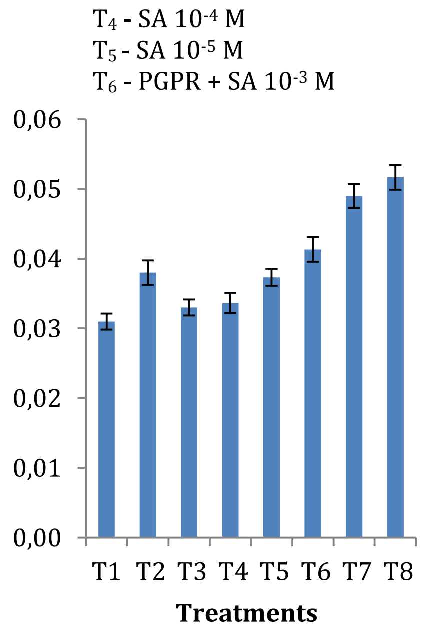

Figure 5. Effect of treatments on Chlorophyll b (μg g^{-1}) concentration

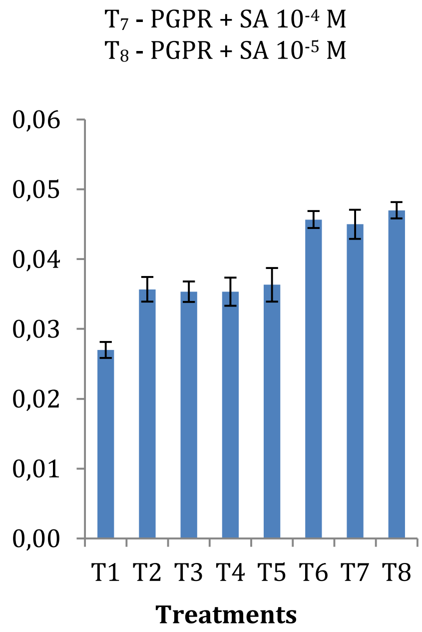

Figure 6. Effect of treatments on Cartenoids (μg g^{-1}) concentration

Agronomic parameters

Data regarding grain yield revealed that maximum grain yield (14.08 g pot^{-1}) was observed in T_7 treatment where PGPR was applied along with salicylic acid @ 10^{-4} M, following by T6 treatment the exogenous application of plant growth hormone@10^{-3} in combination with PGPR (13.81g) as compared to control (9.23g). Similarly maximum plant height (34.67cm), number of nodules pot^{-1} (49.0) was observed in T_7 treatment. Results regarding different agronomic parameters revealed that maximum pod yield (21.33 g pot^{-1}), number of flowers (47.67 plant^{-1}), fresh shoot mass (51.0g), number of branches (9.67 plant^{-1}), root length (40.0 cm) were obtained in T_6. Maximum root mass (51.51g pot^{-1}), dry root mass (7.86 g) was observed in T_7 as compared to control (32.72, 3.06 g pot^{-1}) respectively.

Chemical Parameters

In case of chemical analysis of chickpea grain for nitrogen and phosphorus the same trend was observed like the other parameters. The concentration of nitrogen in chickpea grain increased in those treatments where plant growth promoting rhizobacteria were applied as compare to other treatments. When PGPR combined with salicylic acid the nitrogen contents enhanced as compared to that treatments where only PGPR or SA applied (Figure 7). Similar trend was observed in case of % P contents in chickpea grain (Figure 8).

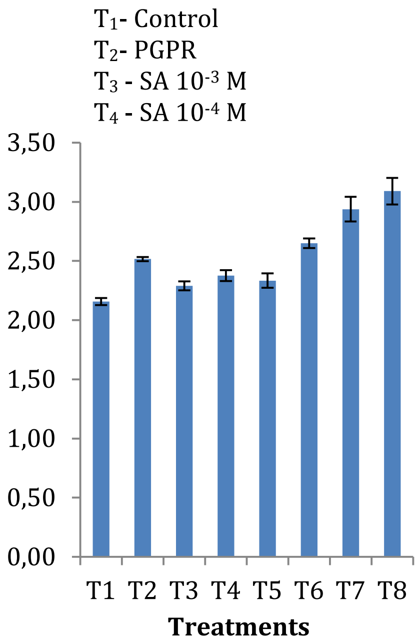

Figure 7. Effect of treatments on N (%) in chickpea grain

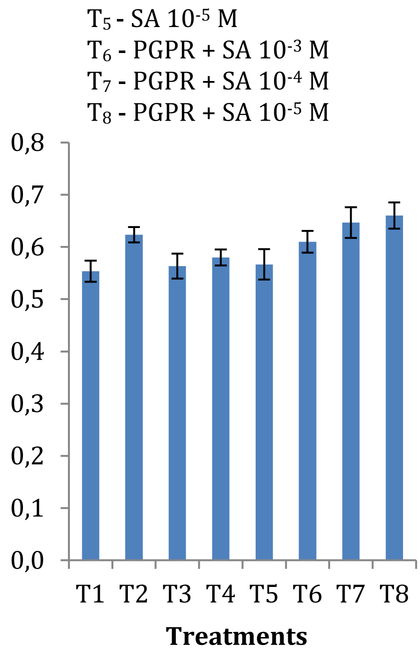

Figure 8. Effect of treatments on P (%) in Chickpea grain

Discussion

According to our results under stress conditions salicylic acid improves the growth and yield of chickpea crop. But when it combines with PGPR it not only improves the growth but also increased the grain yield. All growth and yield parameters improve by the addition of salicylic acid and PGPR. Exogenous application of salicylic acid in combination with PGPR under salt stress conditions increased growth of chickpea. Our findings were similar to Baniaghil et al. (2013) which describes that in canola plant various species of microorganisms such as Azospirillum sp. and Pseudomonas sp. enhanced growth and biomass by altering the oxidative stress enzymes and essential nutrient under saline conditions. Internal ion flux of plants severely affected under salinity stress conditions. According to the results under salinity, the control plants had less nutrient uptake as compared to PGPR and SA application, our findings are similar to the results of (Rojas-Tapias et al., 2012). During mutualistic relationship production of SA improves the growth of plants by initiating induced systemic resistance under all type of stresses whether they are biotic or abiotic (Pozo and Azcón-Aguilar, 2007). There was significant increase in number of nodules and nodular mass was observed by seed treatments with PGPR. The positive response of Rhizobium specie on nodular dry mass and number per plant in chickpea is studied by many researchers (Eusuf Zai et al 1999; Bhuiyaan et al, 2008). Rhizobium inoculation resulted in excellent nodulation in contrast to poor nodulation in control (Khattak et al, 2006). Suryawanshi et al. (2007) observed positive effect of inoculation on nodule number and nodule dry weight. Inoculation studies have showed increase nodule number and nodule dry and fresh weight per plant in chickpea (Verma et al, 2010; Sahai and Chandra, 2010). Similarly, Singh et al. (2014) observed higher number (27.6%) and dry weight (22.2%) of nodules per plant as compared to un-inoculated control in chickpea. Leghaemoglobin, red iron-containing protein, occurs in the root nodules of leguminous plants where it facilitates the diffusion of oxygen to the symbiotic bacteroids in order to promote nitrogen fixation. Moreover, Leghaemoglobin is synthesized by the symbiotic partners viz. the Rhizobia and the host plant. Rhizobium synthesizes the “haem” portion and plant synthesizes the “globine” portion. Lakshmanarao and Singh (1983) evaluated positive relationship between leghaemoglobin content and nitrogen fixation. Tagore et al. (2013) observed higher leghaemoglobin content in the nodular tissue of chickpea inoculated treatment. Further, there was a positive correlation between nitrogenase activity and both number and dry weight of nodules (Miller et al., 1986). Seed inoculation showed significant increase in the nitrogenase activity in contrast to uninoculated control (Dutta and Bandhyopadhyay, 2009). Malik and Sindhu (2011) also reported similar findings in chickpea. Such positive benefits have also been reported by Das et al (2013) which may be attributed to presence of low or useless population of indigenous rhizobia nodulating chickpea in the soil allowing the inoculant strain to form greater portion of effective nodules on plant roots (Parul and Chandra, 2009). There is positive effect observed in all yield parameters by the application of PGPR. Many researchers also reported positive effect of rhizobium inoculation and various other yield parameters viz number of pods per plant, seeds per pod, thousand seed weight. Numbers of pods per plant were reported to be 21% higher with rhizobium over uninoculated control (Sharar et al., 2000). Further Ali et al., (2004) revealed that thousand seed weight was significantly better with inoculation. The increase in yield components through seed inoculation may be due to higher nodulation and more nutrient availability resulting in increase in plant growth and yield (Namvar et al., 2013). The present investigation suggests positive effect of salicylic acid on plant biomass and grain yield. This may be due to tolerance of plants against stressful environment. Several reports were published in the last decade that represents salicylic acid may play a vital role in signaling for plant resistance to environmental stresses (Hayat et al., 2008).

Increase in nitrogen and phosphorus uptake was observed by the application of PGPR and salicylic acid. These findings are online with those of Tagore et al. (2013) in chickpea. Kumar et al. (2014) were also found significant increase in nutrient contents by the application of rhizobium as comparison to control. İn this study exogenous application of salicylic acid and PGPR increased the chlorophyll contents and carotenoids. Our results are in agreement with the observation of Hayat et al. (2008; 2012). That describe when plants were subjected to environmental stresses chlorophyll a,b and carotenoid concentration was significantly reduced that were overcome under salicylic acid application that enhanced the activity of Rubisco and PEP carboxylase under stress.

Physiological and agronomic characteristics of plants to salinity are controlled by different types of genes, whose expressions are affected by different factors of environment (Foolad, 2004). In exogenous use of SA in the presence of salinity cause higher fresh and dry weight of plants as compared to those without SA treatment. Foliar use of SA on mustard plant results in increasing the tolerance of salinity stress (Ghoulam et al., 2002). In spite of this SA has potential to induce systemic resistance against pathogenic attack in plants.

Conclusion

From the study, it is concluded that exogenous application of salicylic acid along plant growth promoting rhizobacteria has a potential to improve the growth and yield of chickpea under salinity stress. So it is recommended that during salinity plant growth regulator (SA) along with PGPR can be an appropriate approach for better chickpea production.

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