INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 11, No. 1, p. 204-211, 2017

RESEARCH PAPER OPEN ACCESS

Productivity of maize through salt tolerant *Rhizobia* strains under salt stress conditions

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Key words: Inoculation, Maize, *Rhizobia* strains, Salinity

http://dx.doi.org/10.12692/ijb/11.1.204-211 Article published on July 27, 2017

Abstract

A study was conducted to investigate the potential of Rhizobia to improve the growth and productivity of maize under saline conditions. Maize seeds were inoculated with four pre-isolated Rhizobia strains (CRI-34, CRI-29, S-43 and LSI-25) along with un-inoculated control. Two salinity levels (6 dSm^{-1} and 12 dS^{-1}) along with original EC maintained in the pots using NaCl salt. Maize seeds were sown in the pots. Results indicated that Rhizobia has the potential to induce salt tolerance in plants under saline stress conditions. Significant increase in growth and yield of maize was recorded. Among the Rhizobia strains LSI-25 performed better in almost all the yield and growth parameters. Under LSI-25 inoculated seeds, 35% increase in growth and yield was recorded followed by S-43 with 24%, CRI-29 with 14% and CRI-34 with 11%. Rhizobia strains increased the crop growth and yield by maintaining the nutrient balance in maize plants.

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Introduction

Salinity is the major problem throughout the world limiting the agricultural productions especially in arid and semi-arid regions (Munns, 2002). Almost 800 million hectares of land is salt affected throughout the world. Soil salinity effects the plant growth through osmotic stress, production of ethylene, nutrient imbalance and disturbance with photosynthesis (Sairam and Tyagi, 2004). The ability of rhizobia is influenced by environmental factors which can be mainly categorized by physical factors, chemical factors and nutrient deficiencies (Giller, 2001). Soil salinity reduces plant growth, nutrient uptake especially phosphorus (P) uptake due to precipitations of phosphate ions with Ca ions in saline soils (Grattan and Grieve, 1999). Salinity causes toxic effects in plants like ionic effect and osmotic stress. Under saline soils, ionic effect including uptake in nitrogen (N) and intrusion in transportation of necessary ions within the plants and extreme lowering of photosynthesis activity in stressed plants while under osmotic stress, effects on reduction in cell wall extension and cellular expansion which leads to the stunted plant growth. The ability of plant to survive and retain their growth under saline conditions is identified as salt tolerance. Development of salt-tolerant crops is not an economic approach for sustainable agriculture whereas the yield and growth of maize can be improved by inoculation of seeds with efficient and competitive strains of rhizobia as it is the feasible way of production improvement (Ben Romdhane *et al.*, 2008). So, understanding the mechanism inducing salt tolerance in plants may be useful in developing new strategies to facilitate growth under saline conditions.

Rhizobia are considered as PGPR in non-legumes where they benefit plant growth. Plant growth promoting rhizobacteria stimulate the growth and colonize the plant roots (Arshad and Frankenberger, 2012). Plant growth promoting rhizobacteria able to produce various compounds like phytohormones, siderophores, organic acids and phosphorous solubilization and are also able to produce antibiotics that tackle the harmful bacteria.

Plant growth promoting rhizobacteria can affect the plant growth and development in a positive way by the synthesis of biologically active substances. The net effect of microbe-plant association on growth of plant could be positive, negative, or neutral. These bacteria are considered as efficient competitors with other microbes in root region (Kennedy, 2005; Nadeem *et al.*, 2006). They produce indole- acetic acid, cytokinins, gibberalins, and substances that inhibit the ethylene production. Plant growth can be facilitated by increasing the natural resistance of host against pathogens and by synthesizing the inhibitory substances (Cazorla *et al.*, 2007). Keeping in view the importance of maize and problems of salinity, present study was conducted to evaluate the potential of rhizobia for promoting growth and yield of maize under saline conditions in a pot trial.

Materials and methods

A pot trial was conducted on maize in the wire house of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad to evaluate the efficiency and potential of Rhizobium under salt stress conditions to improve the growth and yield of maize.

Details of experiment are given below:

Acquisition of Rhizobia isolates

Pre-isolated Rhizobium strains were attained from the Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. These strains included the following:

CRI-34 (*Mesorhizobium ciceri*)

CRI-29 (*Mesorhizobium ciceri*)

LSI-25 (*Rhizobium leguminosorum*)

S-43 (*Rhizobium phaseoli*)

Preparation of inoculums

Fresh inoculums of each strain were developed using sterilized yeast extract mannitol broth. After three days of incubation of inoculated broth (at 28^{o}C and 100rpm) with respective isolate (CRI-34, CRI-29, LSI-25 and S-43), optical density was measured and dilutions were made if needed to have uniform population.

Seed inoculation

Maize seeds were inoculated with respective bacterial strains. For this purpose, slurry was used which was prepared with peat, broth culture and sugar solution 10% in the ratio 5:4:1. In case of un-inoculated control, peat treated with sterilized broth was used for seed coating.

Pot trial

Soil was obtained from the research area of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Salt (Sodium Chloride) was added to soil. The recommended level of chemical fertilizers (N: P: K @ 20:60:60kg ha^{-1}) was applied at the time of sowing. The soil was analyzed for physicochemical characteristics before filling the pot at 10kg pot^{-1}.

Three salinity levels of 1.41 dS m^{-1} (Control original soil), 6 dS m^{-1}, and 12 dS m^{-1} were developed by using calculated amount of NaCl in each pot. Seeds of maize were sown at the rate of five inoculated seeds pot-1. Each treatment was replicated thrice. Pots were arranged in completely randomized design at ambient light and temperature. Good quality irrigation water which meets the irrigation quality criteria for crop was used to irrigate the pots. After germination, uniform plant population of two plants pot-1 was maintained by thinning. Plant parameters were studied including plant height, root length, grain yield, 1000 grain weight, cob fresh weight, cob dry weight, N P K contents in straw and grain. Soil textural class was determined by using international soil classification system. After 90 days, at maturity, plants were harvested and data about growth and yield parameters were recorded.

Statistical Analysis

Analysis of variance (ANOVA) technique was used from Steel *et al*., (1997) and Duncan's Multiple Range (DMR) test was applied to see the significance of difference among the treatment mean (Duncan, 1955).

Results

Inoculation of LSI 25 was prominent for improving root length of the crop with an increase of 35% followed by S-43 with 24%, CRI-29 with 14% and CRI-29 with 11% improvement. Plant height of maize is extensively affected by salinity where 12 dS m-1 maximally reduced the plant height followed by 6 dS m-1. Plant height was reduced up to 25% at 12 dS m-1 and 6 dS m-1 decreased it up to 15% compared to control treatment. Mainly inoculation of LSI-25 remained important for improving plant height of the crop with an increase of 28% followed by CRI-34 with 10% improvement, while other inoculated isolates (CRI-29 and S-43) improved the plant height but remained statistically at par with un-inoculated control. Strain LSI-25 significantly improved the root length up to 35%, plant height up to 28%. Strain S-43 significantly improved the cob length up to 25%. 12 dS m-1 maximally reduced the 1000 grain weight compared to control treatment.

Mainly inoculation of CRI-34 remained prominent for improving 1000 grain weight of the crop with an increase of 27% followed by LSI-25 with 24%, S-43 with 26% and CRI-29 with 29% improvement. Mainly inoculation of LSI-25 remained prominent for improving cob yield dry of the crop with an increase of 45% as compared to control. Inoculation of CRI-34 remained prominent for improving cob yield fresh of the crop with an increase of 31% than control. All the inoculated isolates improved the grain yield but remained statistically at par with un-inoculated control. All the inoculated isolates significantly improved the % N in grain and % in straw as compared to control treatment. CRI-34 remained prominent for improving % P in grain of the crop with an increase of 25% and S-43 remained prominent for improving % P in straw of the crop with an increase of 30% as compared to control.

Under normal i.e. original salinity inoculation remained beneficial for improving the % K in grain and straw of the crop but most of the isolates remained statistically similar to un-inoculated control but LSI-25 was significantly different from inoculated as well as respective un-inoculated control. Overall, LSI-25 and CRI-34 remained most prominent strains for improving growth and productivity of maize under saline conditions.

Table 1. Physico-chemical properties of soil.

Characteristics	Value
Particle size analysis	
Sand	29.2%
Silt	57.80%
Clay	13%
Texture	
Chemical analysis	
Organic matter	0.46%
Saturation percentage	34.98%
pH	9.93
EC	4.48 dS m^{-1}
Organic Matter	0.73%
Available phosphorous	6.3 ppm
Extractable potash	500 ppm
Na^+	46.48meqL^{-1}
Ca + Mg	2.73meqL^{-1}
K^+	500 ppm
SAR	37%
ESP	18

Table 2. Effect of rhizobium strains and salinity on maize yield parameters at different salinity levels.

Salinity level	Rhizobia species	1000 grain wt (g)	Grain yield (g)	Cob fresh yield (g)	Cob dry yield (g)
Control	Control	121hi	42cdef	36de	62def
6 dSm-1		107j	35f	26f	50g
12 dSm-1		138ge	53ab	45abc	75abc
Control	S 43	128fg	49abcd	37de	72bcd
6 dSm-1		125gh	41def	34c	66bcd
12 dSm-1		149b	58a	52a	81ab
Control	LSI 25	145bc	55ab	46abc	73abc
6 dSm-1		136de	52abc	37de	61ef
12 dSm-1		166a	53ab	47ab	83a
Control	CRI 34	141cd	47bcde	43bcd	76abc
6 dSm-1		132ef	41def	37de	63def
12 dSm-1		141cd	54ab	38cde	71cde
Control		123ghi	47bcde	35e	63def
6 dSm-1		119i	41cdef	31ef	61def
12 dSm-1		118i	39ef	3ef	56fg

Table 3. Effect of rhizobium strains on N, P, K in grain and straw at different salinity levels.

Salinity Level control	Rhizobium species	% N in grain	% N in straw	% P in grain	% P in grain	% K in grain	% K in grain
Control		1.54e	1.05b	0.46b	0.26cde	1.48cd	0.22bc
6 dSm-1		1.38f	0.73d	0.26d	0.22ef	1.38efg	0.20bc
12 dSm-1		0.88i	0.35e	0.13e	0.14g	1.51i	0.18c
Control	S 43	1.69ab	1.26a	0.55a	0.31ab	1.61ab	0.26ab
6 dSm-1		1.59d	1.01b	0.33c	0.26cde	1.49cd	0.23abc
12 dSm-1		1.23h	0.73c	0.18e	0.24def	1.28gh	0.18c
Control	LSI 25	1.72a	1.27a	0.48b	0.27bcd	1.69a	0.29a
6 dSm-1		1.63c	1.08b	0.31cd	0.24def	1.45cde	0.21bc
12 dSm-1		1.22h	0.90c	0.16e	0.21f	1.22hi	0.20bc
Control	CRI 34	1.69ab	1.25a	0.56a	0.29abc	1.60ab	0.25ab
6 dSm-1		1.66bc	1.04b	0.33c	0.26cd	1.55bc	0.24abc
12 dSm-1		1.33g	0.81cd	0.17e	0.23def	1.39def	0.23abc
Control	CRI 29	1.69ab	1.21a	0.48b	0.31a	1.52bc	0.25ab
6 dSm-1		1.57de	0.82cd	0.31cd	0.25cde	1.41def	0.22abc
12 dSm-1		1.25h	0.36e		0.21f	1.39fgh	0.21bc

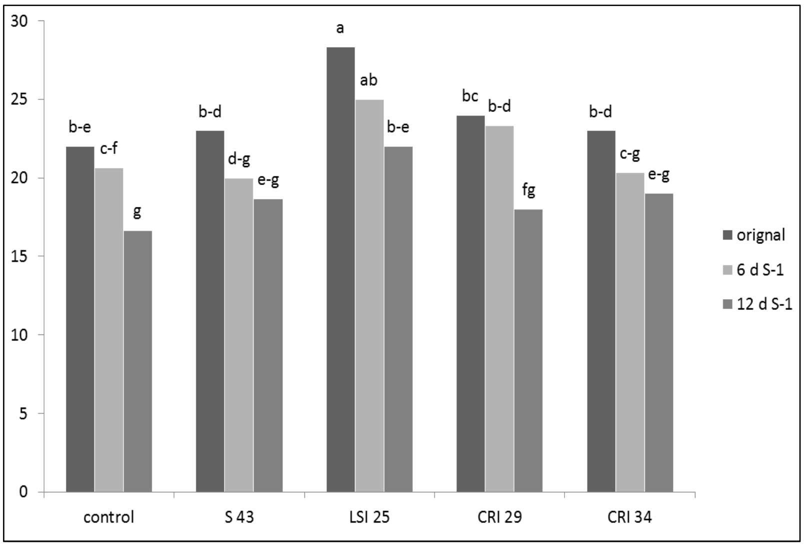

Fig. 1. Effect of *Rhizobia* strains on root dry weight of maize at different salinity levels.

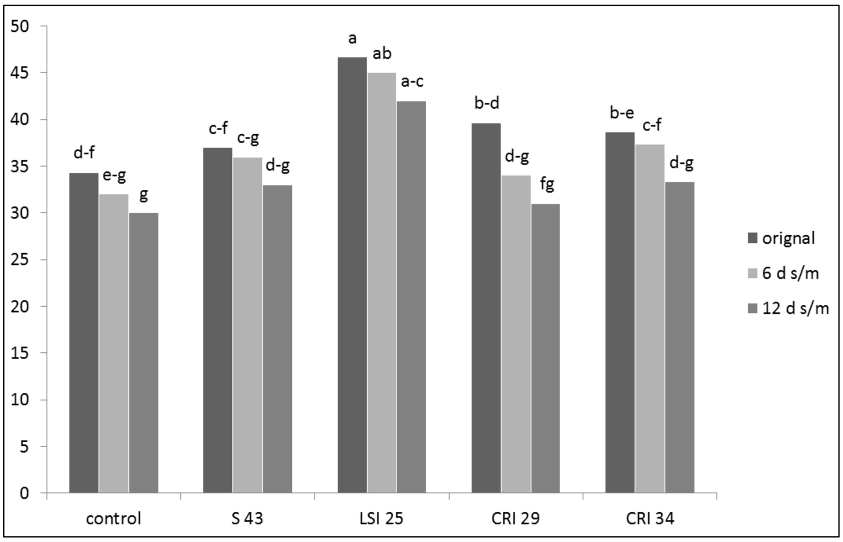

Fig. 2. Effect of *Rhizobia* strains on root fresh weight of maize at different salinity levels.

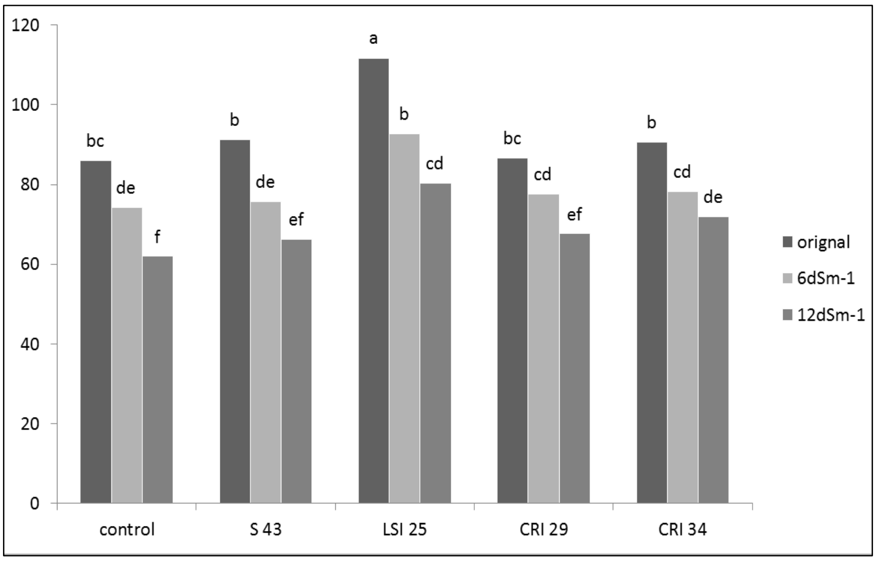

Fig. 3. Effect of *Rhizobia* strains on plant height of maize at different salinity levels.

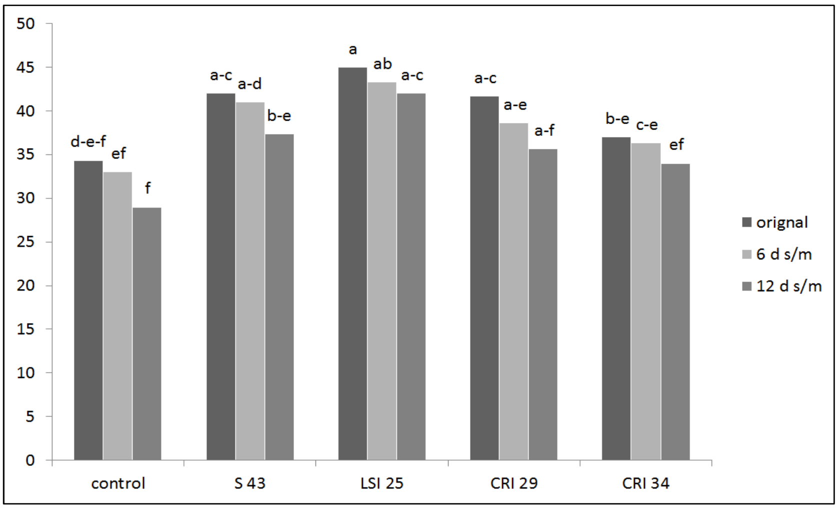

Fig. 4. Effect of *Rhizobia* strains on root length of maize at different salinity levels.

Discussion

The result of this study showed that rhizobium strains promoted the growth of maize with different efficiency. Thousand grain weight, cob length, cob weight, total biomass, and straw yield increased significantly over un-inoculated control but the root length and cob yield showed non-significant results under inoculation. On the other hand, inoculation was more effective under high salinity. Some strains were more effective and some were less efficient at different salinity levels as shown in the results. Momentary adjustment in ionic balance may likely be a mechanism of action in salt conditions and Rhizobium changes the metabolism of cytoplasmic low molecular weight compounds in response of salinity (Yap and Lim, 1983). Several species of bacteria become accustomed under saline conditions by the intracellular accumulation of low molecular weight organic solutes called osmolytes (Csonka and Hanson, 1991). This accumulation of osmolytes neutralizes the dehydration effect of low water movement in medium but it does not interfere with the structure or function of macro molecules (Smith *et al*., 1994). Plants under stressed conditions showed significant improvement in growth and yield compared to un-inoculated control. The results from our trial also uncovered that the effect of the rhizobial isolates on growth and yield of maize varied from pot to pot. This variability in the effect of rhizobial inoculants on crop with respect to pot could be explained on the basis of changing soil-plant and micro floral components in any experimental pot.

Hoflich (2000) conducted trials on sandy loam and loamy sand fields and reported considerable improvement in maize growth equal to 10% with R39 on loamy sand field whilst no considerable results regarding maize growth were obtained on the sandy loam field. Due to presence of microorganisms in rhizosphere of maize some macronutrients like N and P concentrations were positively increased. Likewise, Han and Lee (2005) reported that uptake of N and P were significantly improved in soyabeen under salt stressed conditions when inoculated with Rhizobium when compared with un-inoculated control under salinity. Inoculation by the Rhizobium could be responsible for the observed higher N uptake of inoculated plants under both non-saline and saline conditions (Kantar *et al*., 2003; Oğutcu *et al*., 2008). From the work of different scientists it was evident that increase in Na+ contents, caused an increase in Na+ in general and resulted in high Na+/K+ ratio. As Na+ in the saline environment is considerably greater than K+, therefore it competes with the K+ uptake effectively through common transport systems (Maathuis *et al*., 1996; Rains and Epstein, 1967). In the study the improvement in growth under salinity is due to the maintaining the nutrition balance in plant. Decrease in Na+ concentration may be due to the production of exopolysaccharides (EPSs) by rhizobia. Rhizobial strains are capable of producing exopolysaccharides which have the ability to bind cations including Na+ (Geddie and Sutherland, 1993). These EPS-producing rhizobia under salt stress conditions have been found to restrict Na+ uptake by roots (Ashraf *et al*., 2004).

Protein concentration can be increased and the inhibitory effect of salinity on growth can be minimized by the inoculation with selective strains. Hamdia *et al*., (2004) also reported significant increase in concentration of protein synthesis in maize crop under salt stressed conditions because of inoculation with *Azospirillum brasilense*.

Conclusion

Rhizobia inoculation could increase nutrient availability, increase resistance against salt stress and improve crop growth under saline conditions. Therefore, rhizobium inoculation is a good tool in promotion of growth and yield of crops in saline conditions.

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