Pak. J. Bot., 48(1): 53-56, 2016.

APPLICATION OF ACC-DEAMINASE CONTAINING PGPR IMPROVES SUNFLOWER YIELD UNDER NATURAL SALINITY STRESS

MUHAMMAD ZAHID KIANI[1], TARIQ SULTAN[2], ARSHAD ALI[2] AND ZARRIN FATIMA RIZVI[3*]

[1]*PARC Institute of Advanced Studies in Agriculture, Park Road, Islamabad-45500, Pakistan*
[2]*Land Resources Research Institute, National Agricultural Research Center, Park Road, Islamabad-45500, Pakistan*
[3]*Department of Botany, GC Women University Sialkot, 51310, Pakistan*
[*]*Corresponding author's email: zarrinrizvi@yahoo.com; Cell# 03343182285*

Abstract

Salinity increase ethylene level in root rhizosphere. Plant growth promoting rhizobacteria having ACC deaminase have ability to mitigate the adverse effect of enhanced level of ethylene. Two field experiments were carried out at two different sites during 2013. The site1 had ECe 9.42 dS m^{-1} and SAR 20.75 $(mmol_c\ L^{-1})^{1/2}$, while site2 had ECe 7.51 dS m^{-1} and SAR 16.25 $(mmol_c\ L^{-1})^{1/2}$. Four PGPR strains (KS 44, KS 7, KS 41, KS 42) having ACC deaminase activity were selected for this study. Randomized completes block design (RCBD) was used and hybrid sunflower seeds (SMH-0917) were inoculated with these PGPR strains. The crop was harvested at maturity and data were recorded about plant height, shoot dry weight, head dry weight, number of seed $head^{-1}$, 1000 seed weight, seed weight $head^{-1}$ and seed yield kg ha^{-1}. The data were statistically analyzed by using soft ware statistic 8.1. The average increase in grain yield (site 1 + site 2) was 110.07, 108.89, 49.09, 65.77 and 69.70% over control due to inoculation with KS 44, KS 7, KS 41, KS 42 and KS mix treatments respectively. On the basis of statistical analysis, the bacterial strains KS 44 and KS 7, were declared as most promising strains on both sites. Hence these two strains (KS 44 and KS 7) could be used to mitigate negative impact of salinity stress on sunflower.

Key words: Salinity, Sunflower and PGPR.

Introduction

Sunflower (*Helianthus annus* L.) belongs to family Asteraceae and plant kingdom Plantae. It is very good source of edible oil all over the world because of low cholesterol. Its seed contain 47% oil which is ranked next to peanuts 48%. Pakistan imports edible oil 82% of its consumption and only 18% are being produced at country level. Sunflower contribution is 33% in local oil production. In 2012-13 US $ 1.595 billion (Rs 153.3 billion) were spent on import of edible oil and oil seed for extraction of oil (Anon., 2012-13). Pakistan is third largest importer of edible oil in the world and import bill is the largest drain on national exchequer (Siddiqui, 2010).

Better utilization of salt affected-soils and curtailing the spread of salinization and alkalizations is important for food security at national and international level. Irrigation system and arid to semi arid environment is main source of salinization (Szabolcs, 1994) because such environment received less rain fall and more water is transpired to air and ultimately net water movement is upward leaving salts on soil surface. Irrigated area produces one-third of total production, whereas productivity of rain-fed area is two time less than irrigated area. Salinity is a global issue, 7% of world land is salt affected, 15% of culture able land and 20% of irrigated land are affected by salinity (Szabolcs, 1994). High concentration of salts in root rhizosphere badly affects plant growth through a complex interaction of osmotic affect, ion toxicity, hormonal and minerals imbalance (Arbona *et al*., 2005). Ethylene is an important phytohormone, which is required for many physiological processes like seed germination, root elongation, ripening of fruit, organ senescence (Bleecker & Kende, 2000) but up to certain concentration. Salinity stress enhanced ethylene level in the root rhizosphere leading to physiological changes in leaf tissues (Zapata *et al*., 2004; Tank & Sarf, 2010). Ethylene acts as negative plant growth regulator as its concentration increases than required level (Holguin & Glick, 2001; Huang *et al*., 2003). 1-aminocyclopropane-1-carboxylate (ACC) is immediate precursor of ethylene. Salinity stress increased ACC level and as a result more ethylene (C_2H_4) production that ultimately increases plant damage (Botella *et al*., 2000). Etiolated pea seedlings demonstrate a characteristic classical ''triple'' response to ethylene (Arshad & Frankerberger, 1988). It involves reduction of stem elongation, swelling of hypocotyl, and change in the direction of growth.

Cobalt ion (Co^{+2}) and aminoethoxyvinyle glycine (AVG) are chemical inhibitor to ethylene synthesis. These chemicals are not only expensive but also harmful for environment (Dodd & Belimov, 2009). There are some microbes (Bacteria and Fungi) that exhibit ACC deaminase activity. These microorganisms are successfully being used to mitigate the harmful effect of enhanced ethylene level due to salinity. Plant growth promoting rhizobacteria (PGPR) having ACC deaminase activity hydrolysis ACC into ammonia and a-ketobutyrate. Thus reduced the enhanced level of ethylene (Mayak *et al*., 2004) and promote the plant growth in a saline environment (Nadeem *et al*., 2010; Siddkee *et al*., 2010). PGPR facilitate the plant growth through a diverse mechanism like atmospheric nitrogen fixation, solubilization of inorganic phosphoric, siderophores production, Indile-3-acetic acid (IAA) production, anti-pathogen affect against diseases and mineralization of other nutrients (Nelson, 2004, Saharan & Nehra, 2011). The objective of this research work is to test the performance of rhizobacterial strains on sunflower, isolated from different salt affected field of Punjab (Pakistan). The more efficient strains selected can be used as inoculants to improve the yield of sunflower on sustainable bases in saline environment.

Materials and Methods

Four rhizobacterial strains (KS 44, KS 7, KS 41, and KS 42) were selected for two field trials. The following treatments were used. T_1= un-inoculated, T_2= KS 44, T_3= KS 7, T_4=KS 41, T_5= KS 42 and T_6= mixture of all. The selected bacterial strains were inoculated separately in 200 mL flask having Luria Bertani (LB) broth and incubated in shaker at 28±2°C for three to four days. These cultures were transferred to biofertilizer carrier (soil high in organic matter) at Soil Biology and biochemistry Laboratory NARC, Islamabad. Hybrid sunflower seed were surface sterilized with $HgCl_2$ (2%) before coating with slurry, which was made by adding 10% sugar solution in biofertilizer pocket.

Field study: Two sites were selected for these experiments[site1 was soil salinity research institute (SSRI) Pindi bhattian at ECe=9.43 dS m^{-1} and site 2 was farmer field at Zaidi Farm (Sheikherpura) where field salinity was 7.51 dS m^{-1}]. The selected PGPR strains were high in ACC-deaminase, IAA production and in phosphorus solubilization among all isolated strains (Table 2). Soil samples were taken from two depths (0-15 cm and 15-30 cm) to analyze physico-chemicals properties (Table 1). These experiments were laid down by using Randomized Complete Block design (RCBD). The Seeds of sunflower were inoculated with biofertilizer slurry and sowing was carried out on furrow with dibbling method. Plant to plant distance were maintained 30 cm and row to row 75 cm. Recommended dose of NPK fertilizers (100-60-50) were applied. All of phosphorous and potash were mixed in soil before sowing whereas nitrogenous fertilizer were applied in two split doses. The general protocol of experiment (plant protection measure, cultural practices and irrigation) were followed as and when required up to the crop maturity. After one hundred days of sowing, crop was harvested and data regarding selected parameters (plant height, shoot dry weight, number of seed $head^{-1}$, 1000 seed weight, and seed yield kg ha^{-1}) were recorded. Phosphorus, potassium, sodium and calcium contents in plant were measured by using method as explained by (Ryan *et al*., 2001).

Table 1. Physico-chemicals properties of two sites used for field experiment.

Parameter	Units	Site 1	Site 2
ECe	dS m^{-1}	9.43	7.51
Na	meq L^{-1}	88	65
K	meq L^{-1}	3.65	4.78
Ca^{+2}+ Mg^{+2}	meq L^{-1}	18	16
SAR	$(mmol_c\ L^{-1})^{1/2}$	20.75	16.25
Organic matter	%	0.68	0.79
Saturation percentage	%	33	32
Textural class		Sandy clay loam	Sandy clay loam

Table 2 Quantification of plant growth promoting activities of bacterial strains.

Sr. No.	Isolate	IAA mg L^{-1}	ACC deaminase µmol $kg^{-1}hr^{-1}$	PSB (SI)
1.	KS 44	25.54	450 ± 12	2.28
2.	KS 7	23.44	430 ± 10	2.70
3.	KS 41	17.04	400 ± 8	3.35
4.	KS 42	19.2	410 ± 11	2.20

Results and Discussion

The data collected from both sites on plant height indicated significant increase over control. The strain KS 44 showed maximum increase of 56 and 36% in plant height on site1 and site 2, followed by KS7 with an increase of 55 and 31% respectively as compared to control (Fig. 1). While non-significant difference was noted between isolates at site 1, whereas isolate KS 44 significantly differs from others at site 2. The PGPR inoculated treatments showed significant increase in shoot dry weight over control. The bacterial strains KS 44 indicated highest increases (182 and 42%) at site 1 and site 2 in shoot dry weight followed by KS7 (150 and 27%) over control on both sites respectively. These strains (KS 44 and KS 7) statistically differ from all other strains and also from each other at two sites. The isolate KS 41 showed minimum increase (21%) at site 1 followed by mixture treatment (17%) at site 2 over control (Fig. 2). Like other parameters PGPR inoculation also significantly enhanced number of seed per head on both sites over control. At site 1, maximum improvement (103 and 97%) was noted with strains KS 44 and KS 7 respectively as compared to control. While at site 2, isolates KS 44 and KS 7 gave an increase of 54 and 47% as compared to un-inoculated. Whereas strains KS 44 and KS 7 were at par statistically at site 2, but significantly differ at sit 1 from each other and from other strains on both sides. Among all strains KS 41 and KS mixture showed minimum increase (40 and 17%) in number of seed per head at site 1 and site 2 respectively(Fig. 3). However in case of 1000 grain weight, strain KS 44 demonstrated highest increase (41%) followed by KS 7 (37%) over control at site 1, while this increase was 36 and 35% respectively over control at site 2(Fig. 4). The data collected on seed yield (Kg ha^{-1}) revealed that all strains produced significantly higher yield as compared to un-inoculated. The bacterial strains KS 44 and KS 7 produced maximum seed yield (163 and 160% higher) respectively over control at site 1. While an increase of 57.05 and 57% respectively was recorded over un-inoculated treatments at site 2. The strain KS 42 produced 101 and 30% more seed at site1 and at site 2 respectively over control. The lowest increase in seed yield over control was recorded due to KS 41 at site 1 and KS

mix at site 2 (55 and 23%) respectively. Among all tested PGPR strains the KS 44 and KS 7 consistently gave better performance in term of growth and yield parameters of sunflower under saline conditions, hence ranked as first and second respectively. While KS 41, KS 42 and KS mix showed variable performance for growth and yield parameters (Fig. 5).

Salinity is a global issue in arid and semi arid region of world. Plants at germination stage are more sensitive to salts than later developmental stages (Ashraf & Foolad, 2005). Higher concentrations of salts not only negatively affect plant growth and yield parameters but also disturb hormonal and nutritional balance in plants (Sidari *et al*., 2008). Ethylene is important phytohormones required for many physiological processes up to certain concentration levels. Higher concentration inhibits root proliferation and retard plant growth (Holguin & Glick, 2001; Zapata *et al*., 2004; Arbona *et al*., 2005). Reduction in the enhanced levels of ethylene is necessary for successful growth in saline environment (Ashraf *et al*., 2004; Mayak *et al*., 2004; Saleem *et al*., 2007). In the above mentioned study four rhizobacterial strains were used as inoculum. Before testing in natural field condition these strains were also tested in pot experiment under different salinity levels. The strains KS 44 and KS7, like field trial results also ranked as first and second position respectively on the basis of performance in pot experiment(pot expt. data is not presented here). This is because of high ACC- deaminase activity in these strains as compared to others that hydrolysis ACC into ammonia and α-ketobutyrate and thus reduces enhanced level of ethylene in root rhizosphere and promotes plant growth. During Bio-chemical characterization of isolated strains these two strains were found high in ACC deaminase and IAA production (Table 2). Husen *et al*., 2009 reported that strains high in IAA production only exhibit ACC activity. Other researcher have also reported that inoculation with PGPR strains enhanced plant growth under salt-affected condition (Zahir *et al*., 2009; Tank & Sarf 2010; Nadeem *et al*., 2013). Nadeem *et al*. (2010) also mentioned similar results, when wheat seed were treated with PGPR strains under different salinity levels.

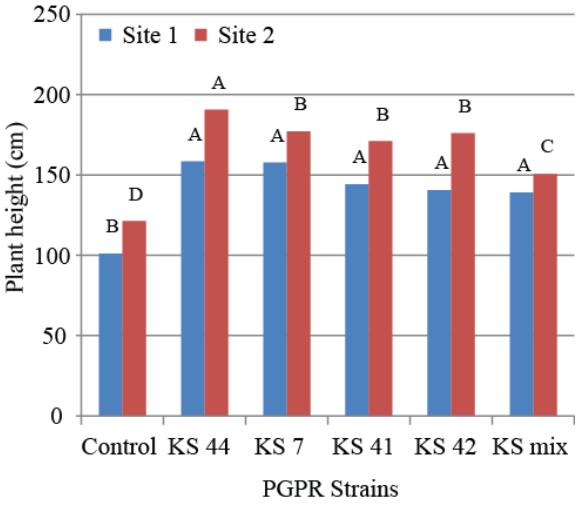

Fig. 1. Effect of PGPR strains on plant height.

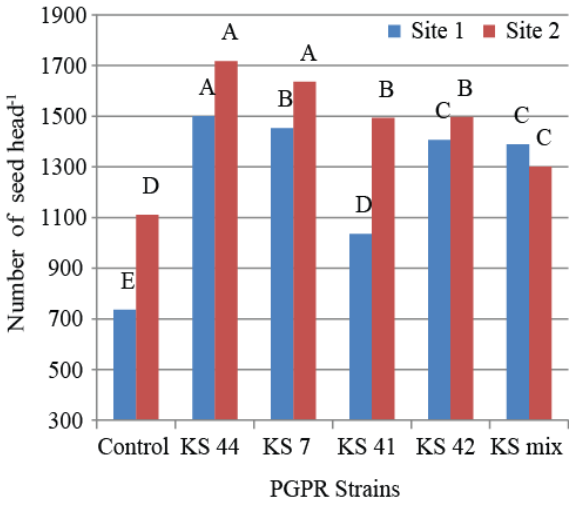

Fig. 3. Effect of PGPR strains on number of seed head^{-1}.

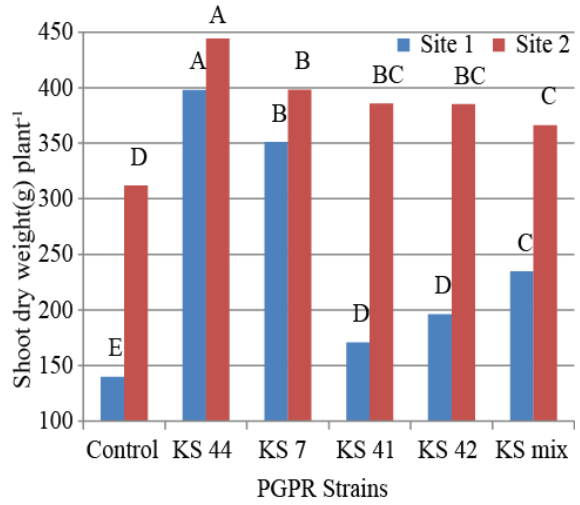

Fig. 2. Effect of PGPR strains on shoot dry weight.

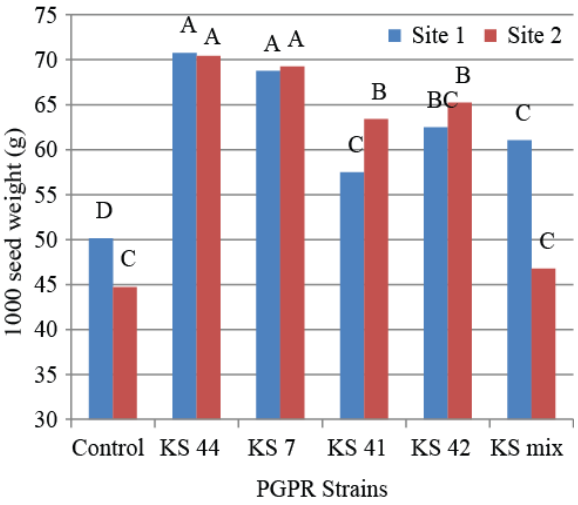

Fig. 4. Effect of PGPR strains on 1000 seed weight (g).

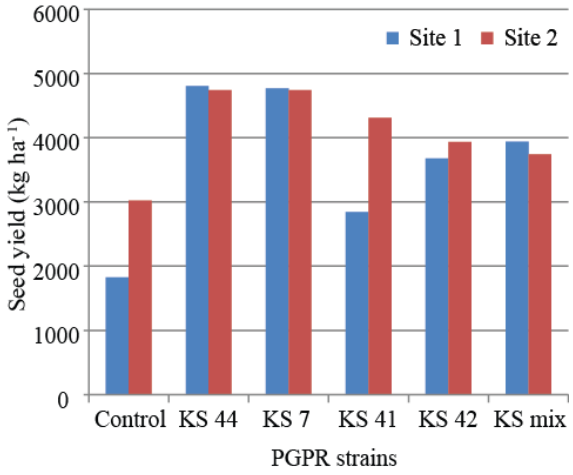

Fig. 5. PGPR strains vs seed yield (kg ha^{-1}).

Conclusion

We studied four strains tested separately in two different fields having different salinity levels. The increase in growth and yield due to mixture treatment was less than separate inoculation. This might be due to non-compatible combination of these strains. While bacterial isolates KS 44 and KS 7 demonstrated better performance on both sites. These results were encouraging for productive use of salt-affected soils for safer environment and also for national food security. These two strains could be used as inoculants in biofertilizer.

Effect of PGPR on plant growth and yield parameters to induce salt tolerance in sunflower; F1: plant height; F2: shoot dry weight; F3: number of seed head^{-1}; F4: 1000 seed weight; F5: seed yield (kg ha^{-1}); the values are average of three, statistical analysis were carried out by using statistic 8.1. Different letters mentioned above the bars are different at 5% level of significance.

References

Anonymous. 2012-13. Pakistan Bureau of Statistics, Economic Affairs Division, Central Statistical Office, Islamabad.

Arbona, V., A.J. Macro, D.J. Iglessias, M.F. Lopez-Climent, M. Talon and A. Gomez-Cadnas. 2005. Carbohydrate depletion in roots and leaves of salt- affected potted *Citrus clementina* L. *Plant Growth Regul*., 46: 153-160.

Arshad, M. and W.T. Frankenberger. 1988. Influence of ethylene produced by soil microorganisms on etiolated pea seedlings. *Appl. Environ. Microbiol*., 54: 2728-2732.

Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment-A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron*., 88: 223-271.

Ashraf, M., S. Hasnain, O. Berge and T. Mahmood. 2004. Inoculating wheat seedling with exopolysacchride-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fertile Soils*, 40: 157-162.

Bleecker, A.B. and H. Kende. 2000. Ethylene: a gaseous signal molecule in plants. *Ann. Rev. Cell Dev. Biol*., 16(1):1-18.

Botella, M.A., D. Amor, F.A. Amoros, Serrano, M.V. Martinez and A. Cerda. 2000. Polyamine ethylene and other physiochemical parameters in tomato (*Lycopersicon esculentum*) fruits as affected by salinity. *Plant Physiol*., 109(4): 428-434.

Dodd, I.C., A.A. Belimov, W.Y. Sobeih, V.I. Safronova, D. Grierson and W.J. Davies. 2009. Will modifying plant ethylene status improve plant productivity in water-limited environments? In: *Proceedings for the 4th International Crop Science Congress, Brisbane, Australia*, 26, Sept. – 1 Oct. 2004.

Holguin, G. and B.R. Glick. 2001. Expression of the ACC-deaminase gene from *Enterobacter cloacae* UW4 in *Azospirillum brasilense*. *Microbiol. Ecol*., 41: 281-288.

Huang, Y., L.H. Hutchison, C.E. Laskey and J.J. Kieber. 2003. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. *Plant J*., 33(2): 221-233.

Husen, E., A.T. Wahyudi, A. Suwanto and R. Saraswati. 2009. Soybean Seedling root growth promotion by 1-aminocyclopropane-1-Carboxylate deaminase-producing pseudomonads. *Ind. J. Agri. Sci*., 10(1): 19-25.

Mayak, S., T. Tirosh and B.R. Glick. 2004. Plant growth promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem*., 42: 565-572.

Nadeem, S.M., Z.A. Zahir, M. Naveed and S. Nawas. 2013. Mitigation of salinity- induced negative impact on the growth and yield of wheat by plant growth promoting rhizobacteria in naturally saline conditions. *Annals of Microbiology*, 33(1): 225-232.

Nadeem, S.M., Z.A. Zahir, M. Naveed, H.N. Asghar and M. Arshad. 2010. Rhizobacteria capable of producing ACC deaminase may mitigate salt stress in wheat. *Soil Sci. Soc. Am. J*., 74: 533-542.

Nelson, L.M. 2004. Plant growth promoting rhizobacteria (PGPR): Prospects for New Inoculants. On line crop manag. doi: 10.1094/ CM-2004-0301-05-RV.

Ryan, J., G. Estenfan and A. Rashid. 2001. Soil and plant analysis laboartary manual. International Center for Agriculture Research in the dry Areas (ICARDA), Aleppo, Syria 172pp.

Saharan, B.S. and V. Nehra. 2011. Plant growth promoting bacteria: a critical review. *Life Sci. Med. Res*., 2011: LSMR-21

Saleem, M., M. Arshad, S. Hussain and A.S. Bhatti. 2007. Prospectus of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biotechnol*., 34: 635-648.

Sidari, M., C. Mallamaci and A. Muscolo. 2008. Drought, salinity and heat differently effect seed germination of *Pinus pinea*. *J. for Res*., 13: 326-330.

Siddikee, M.A., P.S. Chauhan, R. Anandham, G. Han and S. Tongmin. 2010. Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. *J. Microbiol. Biotechnol*., 20(11): 1577-1584 .

Siddiqui. 2010. *Nutrient management for sunflower production*. PhD diss., Tendo Jam univ. Sindh, (Pakistan).

Szabolcs, I. 1994. Soils and salinisation. In *Handbook of Plant and Crop Stress*. (Ed.): Pessarakali, M. pp. 3-11. Marcel Dekker, New York.

Tank, N. and M. Sarf. 2010. Salinity resistant plant growth promoting rhizobacteria ameliorates sodium choleride strss on tomoto plants. *J. Plant Interact*., 5: 51-58.

Zahir, Z.A., U. Ghani, M. Naveed, S.M. Nadeem and H.N. Asghar. 2009. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt stress condition. *Arch. Microbiol*., 191: 415-424.

Zapata, P.J., M. Serrano, M.T. Pretel, A. Amoros and M.A. Botella. 2004. Polyamines and ethylene changes during germination of different plant species under salinity. *Plant Sci*., 167: 781-788.

(Received for publication 11 January 2015)