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Application of ACC-deaminase Containing Rhizobacteria with Fertilizer Improves Maize Production under Drought and Salinity Stress

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Abstract

Maize (*Zea mays* L.) has a lot of dietary and industrial value around the globe and its yield potential is being extremely affected by abiotic stresses i.e., drought and soil salinity/sodicity etc. However, damaging effects of abiotic stresses can be ameliorated by the combined application of plant growth promoting rhizobacteria (PGPR) and mineral fertilizers. Therefore this field trial was designed to explore the effect of PGPR containing ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) alone and in combination with mineral fertilizers on yield and nutrient uptake of maize under drought in saline field. Two PGPR strains S_1 (*Pseudomonas syringae*) and S_2 (*Pseudomonas fluorescens*) were applied to the maize seeds in separate and along with full and half dose of recommended NPK fertilizers. Drought stress was imposed generally on whole experiment at tasseling by withholding irrigation up to ~50% field capacity (FC) level. The PGPR strains significantly improved the yield of maize when applied alone and further promising results were obtained when applied with mineral fertilizers. Maximum increase in number of cobs plant^{-1} (19.05%), cob length (68.02%) number of grain rows cob^{-1} (25.53%), number of grains cob^{-1} (28.29%) plant height (58.14%), number of grains per cob (28.29%), 1000-grain weight (35.92%) and grain yield (55.14%) was observed by the combined application of PGPR strains and full dose of recommended fertilizers over un-inoculated control and without mineral fertilizers application. Moreover combined application of PGPR strains and mineral fertilizers notably improved the nitrogen (N), phosphorus (P) and potassium (K) contents of grains and stalk of maize not only over control (un-inoculated seed and without mineral fertilizers application) but also over alone application of PGPR strains, and half and full dose of mineral fertilizers application as well. In conclusion, the PGPR strains containing ACC-deaminase activity along with full dose of artificial fertilizers improved the maize productivity due to notable expansion in yield related traits and nutrient uptake under dual stress conditions i.e., drought and soil salinity/sodicity.

Keywords: Salinity; Sodicity; Growth; Yield; Rhizobacteria

Introduction

Maize (*Zea mays* L.) is the 3rd important cereal crop (after rice and wheat) cultivated around the globe under a broader range of edaphic and climatic conditions. Maize is rightly said a versatile crop due to its multiple uses as food grain, component of animal and poultry feed, fodder and an industrial commodity. Its grain is used for the preparation of corn syrup, corn starch, corn flakes, corn oil, dextrose, lactic acid, gluten, grain cake and acetone which are used by different industries such as foundry, textile, fermentation and food etc. Maize grain has soaring dietary importance as it contains 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3% sugar and 1% ash (Chaudhary, 1983).

It is a high yielding cereal grown twice a year and can play important role in fulfilling the future food need of high populated countries like Pakistan. However, yield potential of maize is extremely affected by abiotic stresses like salinity, drought, extreme temperature, pollutants, flooding and poor or extreme irradiation etc. (Lawlor and Cornic, 2002; Akram *et al*., 2010). Among the abiotic stresses, drought is the most severe limitation to maize production (Sallah *et al*., 2002; Anjum *et al*., 2011). According to an estimate, only drought counts for 50% or more reduction in average yield universally (Wang *et al.*, 2003; Hussain *et al*., 2009). Similarly, salinity also causes numerous physiological and biochemical changes in plants which ultimately reduce the crop yield (Jensen and Mogensen, 1984; Hussain *et al*., 2013a).

Ethylene is a phytohormone taking part in evoking physiological responses in plants exposed to a variety of stresses including drought and salinity/sodicity etc. (Wang

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et al., 1990). It is thought that stress stimulates 1-aminocyclopropane-1-carboxylic acid (ACC) production which is an immediate ethylene precursor (Wang and Adams, 1982). Salinity and drought can increase the rate of ethylene biosynthesis via increased level of ACC (El-Beltagy *et al.*, 1997). Moreover, it is accepted around the globe that the ACC can be hydrolyzed into ammonia and α-ketobutyrate by bacterial enzyme ACC-deaminase (Glick *et al.,* 1998). The uptake and successive hydrolysis of ACC by the PGPR containing ACC deaminase; decrease the quantity of ACC in the plants, which consequently develop a better root system. The presence of ACC-deaminase has been observed in various plant growth promoting bacteria like *Enterobacter cloacae* (Penrose and Glick., 2001), *Pseudomonas, Variovorax*, *Alcaligenes* and *Bacillus* (Belimov *et al*., 2002). The PGPR containing ACC-deaminase has the potential to improve plants growth even in stress conditions (Nadeem *et al*., 2013).

Although the positive effects of PGPR containing ACC-deaminase as an inoculant on growth and productivity of crops under stress conditions are well reported but the effect of PGPR containing ACC-deaminase as an inoculant in combination with artificial fertilizers on the growth and productivity of maize under dual stress conditions i.e., drought and salt affected soil conditions is not reported. Therefore, this study was conducted with the hypothesis that PGPR containing ACC-deaminase as an inoculant along with chemical fertilizers can improve the maize productivity and nutrient uptake under dual stresses i.e., drought and salt affected soil conditions.

Materials and Methods

Site Depiction

This field trial was conducted in the experimental area of the Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan during spring 2011. The experimental soil was silty loam and saline sodic in nature (EC 4.78 dS m^{-1}, pH 9.2, SAR 17.73 (meq $L^{-1})^{1/2}$ and ESP 19.56).

Experimental Details

Two strains (S_1 and S_2) of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) enzyme were obtained from Soil Microbiology and Biochemistry Section, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Both the strains were applied alone and in combination with full dose (200, 150 and 100 kg ha^{-1} of NPK) and half dose of (100, 75 and 50 kg ha^{-1} of NPK) artificial fertilizers while full dose of fertilizers alone and nothing (no PGPR and fertilizers were applied) were taken as controls. For seed inoculation, the medium was prepared with the composition as follows; KH_2PO_4 = 4 g/L, Na_2HPO_4 = 6g/L, $MgSO_4.7H_2O$ = 0.25g/L, $FeSO_4.7H_2O$ = 1 mg/L, H_3BO_3 = 10 µg/L, $MnSO_4$ = 10 µg/L, $ZnSO_4$ = 70 ug/L, $CuSO_4$ = 50 µg/L, MoO_3 = 10 µg/L, Glucose = 10 g/L, Gluconoic acid = 2 g/L, Citric acid = 2 g/L, distilled water = 1L and ACC = 5 mM/0.658 g/L. The pH was adjusted using HCl/NaOH. The ACC was used as sole nitrogen source in the DF minimal salt medium (Dworkin and Foster, 1958). Each strain (S_1 and S_2) was inoculated and incubated at 28±1°C with shaking at 100 rpm for three days. The seeds were coated with the slurry, prepared by mixing sugar solution, broth bacterial culture and sterilized peat plus clay. Control was treated with sterilized peat plus clay containing sterilized broth and sugar solution. Inoculated seeds were placed overnight for drying before sowing. Maize hybrid "DK-6525" was used as test crop. The experiment was laid out in Randomized Complete Block Design with four replications. Drought stress was imposed on whole experiment at tasseling stage by withholding irrigation up to ~50% field capacity (FC) level; as Hussain *et al*. (2013b) reported that drought at tasseling up to ~50% FC caused substantial reduction in growth, yield and related traits of maize. This trial was conducted on saline field objectively to assess the role of *Pseudomonas* PGPR strains containing ACC- deaminase activity in improving maize production; as many authors reported their efficacy against salinity stress (Zahir *et al*., 2009; Nadeem *et al*., 2013).

Identification of the Strains S_1 and S_2

After 24 h growth on the plates of Biolog Agar, the bacterial strains S_1 and S_2 were identified using BIOLOG ® Identification Systems (Bochner, 1989). The strain S_1 proved to be *Pseudomonas syringae* while the S_2 found to be *Pseudomonas fluorescens*.

Crop Husbandry

A pre-sowing irrigation of 10 cm was given the field and when it attained the suitable moisture regime, seedbed was prepared by cultivating the field twice followed by planking. The crop was sown manually on 75 cm apart ridges in 2011 with plant to plant distance of 20 cm. Full and half doses of fertilizers were applied as urea, single super phosphate (SSP) and muriate of potash (MOP) according to the treatments. The whole dose of PK fertilizers was applied at the time of sowing as a basal dose while N was applied in two splits. Standard agronomic practices were followed to keep crop free from insects, weeds and diseases. Drought was imposed at tasseling stage by withholding irrigations in all treatments. Mature crop was harvested.

Data Recorded

Data of plant height and number of cobs per plant were recorded by random selection of ten plants at maturity from each treatment. Likewise data of cob length, number of grain rows per cob and number of grains per cob were taken from random selected ten cobs from each treatment. Three random samples of 1000 grains from each treatment were taken; weighed on an electric balance and averaged to note 1000-grain weight. At maturity, crop was harvested and cobs were separated, threshed manually and grains were separated to compute grain yield by using an electric balance. Grain yield was adjusted to 10% moisture contents by taking random samples and converted into kg ha^{-1} following unitary method.

Plant Analysis

The dried and ground material (0.1 g) of grains and stalk was digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982). The volume of extracts was made up to 50 mL with distilled water, filtered and used for determination of mineral elements. Potash was determined with flame photometer (Jenway PFP-7). The values of K from flame photometer were compared with standard curve and total quantities were computed (Richards *et al*., 1954). The extracted material (5 mL) was mixed in 10 mL of barton reagents and total volume was made as 50 mL. The samples were kept for half an hour and phosphorus contents were determined by spectrophotometer using standard curve (Richards *et al*., 1954). The barton reagent was prepared as described by Ashraf *et al*. (1992). Total nitrogen was determined by Kjeldhal method described by Jackson (1962).

Statistical Analysis

The data collected were subjected to analysis of variance technique (Steel *et al*., 1997) and Duncan's multiple rang test (DMR) was applied at 5% probability to compare treatment means (Duncan, 1955).

Results

The data revealed that under drought and salt affected soil conditions, ACC-deaminase containing PGPR strains improved maize yield and related traits significantly and when the strains were applied in combination with mineral fertilizers, further significant improvement was noted (Tables 1 and 2). As far as plant height, number of cobs per plant, cob length and number of grain rows per cob were concerned, maximum and significant increase was observed as a result of combined application of bacterial strains and full dose of NPK fertilizers (Table 1). As far as, alone recommended NPK fertilizers doses are concerned, significant increase in plant height and cob length and non-significant increase in number of cobs per plant and number of grain rows per cob were observed as compared to the control. Both the strains S_1 and S_2 produced statistically different plant height over control, while similar results over control were observed in case of cob length, number of cobs per plant and number of grain rows per cob. The combination of both the strains with half of the recommended NPK doses, caused significant improvement in plant height and cob length and non-significant increase in cobs per plant and number of grain rows per cob as compared to the control. Increase in plant height (11%), cob length (15), number of cobs per plant (8) and number of grain rows per cob (11) was noted as a result of combined application of PGPR strains and recommended NPK dose as compared to application of recommended NPK doses without PGPR strains. Maximum increase in plant height (58.14%), number of cobs per plant (19.05%), number of grain rows cob^{-1} (25.53%) and number of grains cob^{-1} (28.29%) as compared to the control was obtained, where the strain S_2 was applied in combination with full dose of NPK fertilizers. However, maximum increase in cob length (68.02%) as compared to the control was observed, when the strain S_1 was used along with full of the recommended doses of NPK fertilizers (Table 1).

Similarly, both the bacterial strains significantly improved the number of grains cob^{-1}, 1000-grain weight, grain yield and stalk yield in separate application as well as in combination with NPK fertilizers compared with the control and even the only NPK fertilizers application without any bacterial strain performed better compared with control (Table 2). When only recommended NPK dose was applied, significant improvement over control was noticed in number of grains per cob, 1000-grain weight and grain and stalk yield. Similarly, only PGPR strains without fertilizers also significantly improved these parameters as compared to the control. Effect of PGPR strains inoculation in combination with half of the recommended NPK doses were found to be similar to the full of recommended NPK doses without PGPR strains in case of number of grains per cob, 1000-grain weight, grain yield and stalk yield. Improvement in number of grains per cob, 1000-grain weight, grain yield and stalk yield remained up to 15, 5, 12 and 13%, respectively as a result of PGPR strains inoculation in conjunction with full of the recommended NPK doses as compared to recommended NPK dose without PGPR strains. Maximum increase in number of grains cob^{-1} (28.29%), 1000-grain weight (35.92%), grain yield (55.14%) and stalk yield (46.36%) was observed with the combined application of strain S_2 and full dose of NPK fertilizers as compared to the control (Table 2).

Likewise data regarding NPK concentration in maize grains and stalks highlighted that again both the strains along with full dose of fertilizers remained significantly better as compared to other treatments (Table 3). It is evident from the data that recommended NPK fertilizers without PGPR strains significantly improved NPK contents of grains and stalk as compared to the control. Similarly, without NPK fertilizers both the strains, S_1 and S_2 increased NPK contents over control in grains and stalk over control, except N and P concentration in grains, which remained to be similar to control as a result of S_1 application without NPK fertilizers. Both the strains along with half of the recommended NPK doses significantly improved NPK contents in grains and stalk over control. The increase in N in grains and stalk, P in grains and stalk

Table 1: Effect of PGPR strains applied alone and in combination with mineral fertilizers on plant height and yield related traits of maize under drought and salinity stress

Treatments	Plant height (cm)	Cob length (cm)	Number of cobs per plant	Number of grain rows per cob
Control	115.52 g	13.57 f	1.05 c	11.75 c
200-150-100 kg NPK ha^{-1}	164.02 cd	19.82 bc	1.15 abc	13.25 abc
PGPR S_1 (*Pseudomonas syringae*)	137.80 f	14.45 ef	1.05 c	12.00 bc
PGPR S_2 (*Pseudomonas fluorescens*)	148.19 e	15.30 e	1.10 bc	12.50 bc
100-75-50 kg NPK ha^{-1} + S_1	156.64 de	18.42 cd	1.15 abc	12.75 bc
100-75-50 kg NPK ha^{-1} + S_2	167.80 bc	17.35 d	1.15 abc	13.25 abc
200-150-100 kg NPK ha^{-1} + S_1	175.23 ab	20.85 b	1.20 ab	13.50 ab
200-150-100 kg NPK ha^{-1} + S_2	182.69 a	22.80 a	1.25 a	14.75 a
LSD Value at 5%	9.60	1.67	0.14	1.66

Table 2: Effect of PGPR strains applied alone and in combination with mineral fertilizers on yield and yield related traits of maize under drought and salinity stress

Treatments	Number of grain per cob	1000-grain weight (g)	Grain yield (kg ha^{-1})	Stalk yield (kg ha^{-1})
Control	312.75 e	132.86 e	2395.6 f	7139 e
200-150-100 kg NPK ha^{-1}	349.50 bc	172.22 abc	3316.9 bcd	9281 c
PGPR S_1 (*Pseudomonas syringae*)	320.25 de	163.35 cd	2904.1 e	7892 d
PGPR S_2 (*Pseudomonas fluorescens*)	337.50 cd	158.05 d	3050.3 de	8192 d
100-75-50 kg NPK ha^{-1} + S_1	360.50 b	165.80 bcd	3206.4 cde	9884 ab
100-75-50 kg NPK ha^{-1} + S_2	357.50 bc	173.08 ab	3381.8 bc	9544 bc
200-150-100 kg NPK ha^{-1} + S_1	371.50 b	177.34 a	3521.7 ab	9757 bc
200-150-100 kg NPK ha^{-1} + S_2	401.75 a	180.58 a	3716.4 a	10449 a
LSD Value at 5%	22.44	9.61	311.53	569.78

Table 3: Effect of PGPR strains applied alone and in combination with mineral fertilizers on NPK contents of maize grain and stalk under drought and salinity stress

Treatments	Grain N contents (%)	Stalk N contents (%)	Grain P contents (%)	Stalk P contents (%)	Grain K contents (%)	Stalk K contents (%)
Control	0.98 f	0.37 e	0.31 f	0.41 e	0.42 e	1.24 f
200-150-100 kg NPK ha^{-1}	1.67 cd	0.68 ab	0.56 bc	0.62 b	0.71 c	1.99 cd
PGPR S_1 (*Pseudomonas syringae*)	1.17 ef	0.43 de	0.39 ef	0.45 de	0.58 d	1.60 e
PGPR S_2 (*Pseudomonas fluorescens*)	1.24 e	0.47 d	0.43 de	0.51 cd	0.53 d	1.49 e
100-75-50 kg NPK ha^{-1} + S_1	1.59 d	0.58 c	0.52 cd	0.56 c	0.73 bc	1.85 d
100-75-50 kg NPK ha^{-1} + S_2	1.85 bc	0.62 bc	0.64 ab	0.65 b	0.76 bc	2.12 c
200-150-100 kg NPK ha^{-1} + S_1	2.01 ab	0.72 a	0.66 ab	0.71 a	0.89 a	2.66 a
200-150-100 kg NPK ha^{-1} + S_2	2.08 a	0.68 ab	0.71 a	0.77 a	0.83 ab	2.47 b
LSD Value at 5%	0.20	0.08	0.11	0.06	0.10	0.17

Means not sharing the same letter within a column differ significantly from each other at 5% level of probability

and K in grains and stalk remained up to 25, 6, 27, 24 25 and 34%, respectively as compared to the NPK fertilizers application without PGPR strains. Comparing with the control, maximum increase in N concentration in grains (112.24%) and P concentration in grains (129.03%) and stalk (87.80%) was recorded when the strain S_2 was applied with full dose of NPK fertilizers. While maximum increase in N concentration as compared to the control in stalk (94.59%) and K concentration in grains (111.90%) and stalk (114.52%) was noted when the strain S_1 was used in combination with full of the recommended doses of NPK fertilizers (Table 3).

Another important aspect of the results is that both the strains performed significantly better when applied in combination with full dose of fertilizers as compared to the half of the recommended dose of NPK fertilizers, full dose of fertilizers alone and bacterial strains alone (Tables 1-3).

Discussion

Stress conditions are known to suppress the plant growth (Cuartero and Fernandez-Munoz, 1999) but greater reduction in productivity was observed in control. However, where the seeds were treated with PGPR containing ACC-deaminase, the extent of growth suppression was decreased and the bacterially treated plants showed better results as compared to the control (Tables 1 and 2). It has been suggested that bacteria containing ACC-deaminase activity might have reduced the level of stress ethylene and thus granted resistance to plants against both the stresses (Glick *et al.*, 1998). Moreover, elevated nutrient uptake by the application of bacterial strains with full dose of fertilizers might be responsible for the improved yield components of maize (Tables 1-3). Notable improvement in yield related traits of maize with the application of bacterial strains along with full dose of fertilizers improved the maize yield under dual stress conditions i.e., drought and salinity compared with control (Table 1, 2). A number of researchers earlier reported significant increase in plant growth and development due to inoculation with ACC-deaminase containing PGPR (Zahir *et al*., 2011; Zafar-ul-Hye *et al*., 2013).

The PGPR strains alone and in combination with mineral fertilizers improved yield parameters and gave promising results as compared to the control. The reason behind might be the reduction of ethylene with ACC-deaminase containing rhizobacteria which converted ACC into ammonia (NH_3) and α-ketobutyrate instead of ethylene. The PGPR strains might have improved the use of mineral fertilizers by helping in the availability of mineral nutrients. It was interesting to note that PGPR strains were highly beneficial in terms of improving maize yield under aforementioned dual stress conditions when applied with full dose of mineral fertilizers compared with their alone application and with half dose of mineral fertilizers (Table 2). It might be due to the fact that these strains overcome the stress effects by declining the ethylene production but could not fulfil the nutrient demands without fertilizer application. The arguments are strongly supported by the work of several researchers (Belimov *et al*., 2002; Zahir *et al*., 2009). The results of the present study, regarding N, P and K contents in grains and stalk are in line with those of several researchers (Pal *et al.,* 2000; Zahir *et al.*, 2009).

Under field conditions, there is a complex system and various biotic and abiotic factors may cause modification in the behavior of particular PGPR strains. As we observed that out of two selected strains, inoculation with PGPR strain S_2 was highly effective in improving growth and yield of maize alone and along with 100% of recommended dose of mineral fertilizers. The PGPR strain S_2 might also have other mechanisms of action, which might have helped in better nutrient mobilization, availability and thus uptake by the plants (Belimov *et al*., 2002).

In conclusion, the PGPR *Pseudomonas* strains containing ACC-deaminase activity had the potential to improve productivity when applied with full dose of mineral fertilizers due to notable expansion in yield related traits and nutrient uptake under dual stress conditions i.e., drought and soil salinity/sodicity. Moreover it is interesting to note that alone application of PGPR strains or full dose of mineral fertilizers application was not sufficient to elevate maize production under dual stress of drought and salinity.

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