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Research Paper

Bacteria in combination with fertilizers promote root and shoot growth of maize in saline-sodic soil

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Abstract

Salinity is the leading abiotic stress hampering maize (*Zea mays* L.) growth throughout the world, especially in Pakistan. During salinity stress, the endogenous ethylene level in plants increases, which retards proper root growth and consequent shoot growth of the plants. However, certain bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which converts 1-aminocyclopropane-1-carboxylic acid (an immediate precursor of ethylene biosynthesis in higher plants) into ammonia and α-ketobutyrate instead of ethylene. In the present study, two *Pseudomonas* bacterial strains containing ACC-deaminase were tested separately and in combinations with mineral fertilizers to determine their potential to minimize/undo the effects of salinity on maize plants grown under saline-sodic field conditions. The data recorded at 30, 50 and 70 days after sowing revealed that both the *Pseudomonas* bacterial strains improved root and shoot length, root and shoot fresh weight, and root and shoot dry weight up to 34, 43, 35, 71, 55 and 68%, respectively, when applied without chemical fertilizers: these parameter were enhanced up to 108, 95, 100, 131, 100 and 198%, respectively, when the strains were applied along with chemical fertilizers. It can be concluded that ACC-deaminase *Pseudomonas* bacterial strains applied alone and in conjunction with mineral fertilizers improved the root and shoot growth of maize seedlings grown in saline-sodic soil.

Key words: maize, ACC-deaminase, rhizobacteria, salinity.

Introduction

Novelty statement: Bacterial strains with ACC-deaminase activity improved the root and shoot growth of maize seedlings grown under saline-sodic field conditions at 30, 50 and 70 days after sowing when applied alone, and further improvement was achieved when these strains were applied with mineral fertilizers.

Maize (*Zea mays* L.) is an important cereal crop grown throughout the world (Araus *et al.*, 2002). It is a high yielding crop with significant commercial and industrial importance, as a large number of products are produced from its grains (Chaudhary *et al.*, 1997). Maize is a raw material for the preparation of corn starch, corn oil, dextrose, corn syrup, corn flakes, cosmetics, wax, alcohol and tanning material for the leather industry (Mujtaba, 2000). In developed countries, approximately 90% of maize is being used for making animal feed and other products as well (Rajoo, 1998).

In Pakistan, the average per hectare grain yield of maize is not only lower than that of other important maize growing countries but also less than the production potential of the cultivars being grown (Govt. of Pakistan, 2011). There are many reasons for the low yield but salinity stress is the most important (Rasheed *et al.*, 2003). Approximately, 7% of the world's land area, 20% of the world's cultivated land and nearly half of the irrigated land are significantly affected by salt contents (Zhu, 2001). Salinity-induced losses in plant growth are due to osmotic effects, ion-specific effects, imbalanced nutrition (particularly due to higher uptake of Na^+ at the expense of K^+) and oxidative stress (Pitman and Lauchli, 2002; Hussain *et al.*, 2012). This results in a reduction of the K^+ and Ca^{2+} contents and an increase in the levels of Na^+ and Cl^-. Salin-

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ity/sodicity stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids (Mittler, 2002).

Different types of stresses (*i.e.*, temperature extremes, chemicals, ultraviolet light, water stress, pathogen attack, salinity/sodicity and other trauma-causing agents) increase the ethylene level in plants. A high ethylene level may inhibit plant growth (Glick *et al.*, 1999). If the ethylene level is high during germination, root elongation is inhibited. A substantial declines in root and shoot elongation and in root and shoot fresh and dry weights under saline conditions have been well documented in the published literature (Farhoudi *et al.*, 2012; Hussain *et al.*, 2012, 2013a). In higher plants, 1-aminocyclopropane-1-carboxylic acid (ACC) is an immediate precursor of ethylene synthesis. Thus a decrease in the ACC level in plants results in a decline in the ethylene level. There are certain bacteria/rhizobacteria that possess an enzyme known as 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase). The presence of ACC deaminase has previously been reported in Gram negative bacteria (Belimov *et al.*, 2001; Wang *et al.*, 2001; Babalola *et al.*, 2003), Gram positive bacteria (Belimov *et al.*, 2001) and fungi (Jia *et al.*, 1999) as well. ACC-deaminase activity has also been observed in *Achromobacter*, *Azospirillum*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Enterobacter*, *Pseudomonas and Ralstonia* (Blaha *et al.*, 2006). Ectophytic rhizobacteria which are found in the rhizosphere of all higher plants, regulate ethylene production in emerging seedlings by hydrolyzing ACC into ammonia and α-ketobutyrate via the action of ACC deaminase. In this way the negative effects of increased ethylene may be minimized/eliminated using ACC deaminase-containing bacteria (Hall *et al.*, 1996). Penrose *et al.* (2001) performed an experiment to study the effect of bacteria containing an ACC-deaminase enzyme that reduced ethylene production and increased the root length of canola. These authors concluded that the canola roots were elongated and the ACC level was reduced. Similar findings have been reported by Zahir *et al.* (2011) and Zafar-ul-Hye *et al.* (2013) in other crops.

The findings of some in vitro studies have elucidated the efficacy of ACC-deaminase-containing bacteria in promoting the root and shoot growth of maize plants under saline conditions (Nadeem *et al.*, 2006, 2007); however, their ability to improve maize root and shoot growth under saline-sodic field conditions has yet to be explored. Therefore, this field trial was conducted based on the hypothesis that application of ACC-deaminase-containing bacteria alone and in combination with mineral fertilizers have the potential to improve the root and shoot growth of maize seedlings in saline-sodic soil.

Materials and Methods

Maize variety "DK 6525" was sown in saline-sodic soil (EC 4.78 dSm^{-1}, pH 9.2, SAR 17.73 $(meq\ L^{-1})^{1/2}$ and ESP 19.56) in the experimental field of the Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan. The bacterial strains were obtained from the Microbiology and Biochemistry Section, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. These strains were isolated from the rhizosphere of maize using a dilution plate technique with DF salt minimal medium (Dworkin and Foster, 1958) and ACC as the only nitrogen source. The trial was conducted in 2011 using a randomized complete block design with eight treatments: Control (without fertilizer or bacterial strains), recommended dose of NPK fertilizers, bacterial strain 1, bacterial strain 2, half of the recommended dose of NPK fertilizers + bacterial strain 1, half of the recommended dose of NPK fertilizers + bacterial strain 2, full recommended dose of NPK fertlizers + bacterial strain 1 and full recommended dose of NPK fertilizers + bacterial strain 2. Four replications were performed. The efficacy of both these bacterial strains in improving the root and shoot growth of maize plants under saline conditions was previously tested in *in vitro* trials (Nadeem *et al.*, 2006, 2007). Therefore, in this study both these strains alone and in combination with mineral fertilizers were directly used under saline-sodic field conditions.

Seed inoculation

The DF salt minimal medium was prepared as follows: -KH_2PO_4 = 4 g/L, Na_2HPO_4 = 6 g/L, $MgSO_4.7H_2O$ = 0.25 g/L, $FeSO_4.7H_2O$ = 1 mg/L, H_3BO_3 = 10 ug/L, $MnSO_4$ = 10 ug/L, $ZnSO_4$ = 70 ug/L, $CuSO_4$ = 50 ug/L, MoO_3 = 10 ug/L, glucose = 10 g/L, gluconic acid = 2 g/L, citric acid = 2 g/L, distilled water = 1 L and ACC = 5 mM/0.66 g/L (Dworkin and Foster, 1958). The only nitrogen source was ACC. The procedure by Sharma *et al.* (2003) was used for seed inoculation with a few modifications. Broth cultures of both the strains were incubated at 28 ± 1 °C with shaking at 100 rpm for 72 h. A slurry was prepared by mixing a sugar solution with the respective broth culture and adding sterilized peat and clay; this slurry was used to coat the seeds.

Identification of bacterial strains

The bacterial strains were grown for 24 h on Biolog Agar plates and then identified using BIOLOG® Identification Systems (Bochner, 1989). Strain 1 was found to be *Pseudomonas syringae* while strain 2 proved to be *Pseudomonas fluorescens*.

Crop husbandry

To establish a suitable moisture level, a pre-soaking irrigation (*i.e.*, 10) cm was applied to the field. The field was plowed twice followed by planking to prepare the seed bed. The ridges were kept 75 cm apart with a 20 cm plant-to-plant distance while sowing the crop (Hussain *et al.*, 2013b). The full recommended dose of NPK fertilizer (200-150-100 kg ha^{-1}, respectively) and half of the recommended dose of NPK fertilizer (100-75-50 kg ha^{-1}, respectively) were applied using urea, single super phosphate (SSP) and muriate of potash (MOP) in accordance with the treatments. Standard agronomic practices were followed.

Observations

The root and shoot length, fresh root and shoot weight and dry root and shoot weights of 30-, 50- and 70-day-old plants were recorded. The root length of three plants selected randomly from each plot were measured. Plants were uprooted with extensive care to avoid root damage, washed with water and air-dried. The root length was measured using measuring tape and the average root length was calculated. The length of fresh shoots obtained from the uprooted plants mentioned above was measured in centimeters using a measuring tape and the average shoot length was calculated.

Subsequently, the fresh weight of the aforementioned air-dried roots and shoots of three plants was measured in grams using an electronic balance, and the average was calculated. Plants roots were placed in a Kraft paper bag and dried in an electric oven at 65 ± 5 °C for 72 h. After drying, the dry root weight per plant was recorded in grams using an electronic balance. The shoots were also dried as described above, and their weight was recorded in grams.

Statistical analysis

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

Results

After 30 days of sowing, the data revealed that the ACC-deaminase-containing rhizobacterial strains significantly improved the root and shoot growth of maize plants grown on saline-sodic soil. Moreover, a further significant improvement in root and shoot growth was achieved from the data, when the same bacterial strains were applied in combination with the full recommended dose of NPK fertilizer (Table 1). Without chemical fertilizers, the bacterial strains increased the root length, shoot length, fresh root weight, fresh shoot weight, dry root weight and dry shoot weight up to 26, 16, 35, 71, 35 and 68%, respectively, compared to the control. The maximum increases over the control in root length (85%), fresh shoot weight (131%) and dry shoot weight (198%) were observed as a result of the application of strain 1 coupled with the full recommended dose of NPK fertilizer. The maximum increases compared to the control in shoot length (48%), fresh root weight (100%) and dry root weight (100%) were noted due to the strain 2 application along with the full recommended dose of NPK fertilizer. However, the two strains had statistically similar effects on root length, shoot length and fresh shoot weight, when applied in conjunction with the full recommended dose of NPK fertilizer.

The data recorded after 50 days of sowing showed the same trend. The bacterial strains promoted root and shoot growth significantly, when applied separately, while further improvement in growth was achieved by applying the bacteria in combination with NPK fertilizer (Table 1). In the absence of NPK fertilizer, the increases in root length, shoot length, fresh shoot weight, fresh root weight, dry root weight and dry shoot weight were 26, 38, 33, 39, 55 and 38%, respectively when compared with the control. The data also indicated that after 50 days, bacterial strain 2 with the full recommended dose of NPK fertilizer yielded maximum increases in root length (86%), shoot length (80%), fresh root weight (97%), fresh shoot weight (100%), dry root weight (98%) and dry shoot weight (98%) compared to the control.

A similar trend was observed when the data were recorded after 70 days of sowing maize plants on saline-sodic soil. Again, the ACC-deaminase-containing rhizobacteria improved the root and shoot the growth of maize plants. The addition of NPK fertilizer amplified the beneficial effects of application of both the bacterial strains (Table 1). Increases in root length, shoot length, fresh root weight, fresh shoot weight, dry root weight and dry shoot weight of 34, 43, 34, 28, 34 and 32% over the control were recoded, respectively, when the bacterial strains were applied without chemical fertilizers. In the presence of the full recommended dose of NPK fertilizer, strain 2 caused maximum increases in root length (108%), shoot length (95%), fresh shoot weight (88%), dry root weight (98%) and dry shoot weight (88%) compared to the control, while the maximum increase in fresh root weight (97%) was obtained with strain 1 application along with the full recommended dose of NPK fertilizer. Both the strains remained statistically similar with respect to fresh and dry root and shoot weights, when applied with the full recommended dose of NPK fertilizer.

Discussion

Stress conditions are known to suppress the plant growth (Cuartero and Fernandez-Munoz, 1999). Increasing stress has been reported to reduce plant growth. However, when plants are treated with PGPR containing ACC-deaminase, the extent of growth suppression was decreased and the plants treated with bateria showed increased root and shoot growth as well as greater root and shoot fresh and dry weights compared with untreated plants. It is likely that bacteria with ACC-deaminase activity might have reduced

Table 1 - Combined effect of ACC-deaminase producing bacteria and mineral fertilizers on root and shoot growth of 30-, 50- and 70-day-old maize plants in saline-sodic soil.

Treatment	Root length (cm)	Shoot length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)
30 days after sowing (DAS)						
Control	13.42 f	26.47 f	0.51 e	3.51 e	0.17 f	0.90 e
NPK fertilizer (200-150-100 kg ha^{-1} respectively)	21.10 bc	35.87 a-c	0.84 bc	6.65 bc	0.26 cd	1.50 cd
Strain 1=*Pseudomonas syringae*	15.55 ef	28.70 ef	0.66 d	4.56 de	0.23 de	1.16 de
Strain 2 =*Pseudomonas fluorescens*	16.85 de	30.65 de	0.69 d	5.99 cd	0.21 e	1.51 cd
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 1	19. 40 cd	33.00 cd	0.80 c	4.68 de	0.24 d	1.43 d
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 2	20.00 bcd	35.17 bc	0.88 b	7.55 bc	0.28 bc	1.90 bc
NPK fertilizer (200-150-100 kg ha^{-1} respectively) + Strain 1	24.82 a	38.50 ab	0.85 bc	9.44 a	0.29 b	2.68 a
NPK fertilizer (200-150-100 kg ha^{-1} respectively) + Strain 2	22.70 ab	39.25 a	1.02 a	8.10 ab	0.34 a	2.02 b
LSD Value	3.20	3.75	0.07	1.57	0.03	0.41
50 DAS						
Control	18.67 e	44.25 f	4.26 f	18.01 d	1.56 f	5.34 d
NPK fertilizer (200-150-100 kg ha^{-1} respectively)	29.50 bc	64.15 cd	6.60 cd	30.77 a-c	2.46 c	8.79 a-c
Strain 1=*Pseudomonas syringae*	23.52 d	51.70 e	5.66 de	25.09 b-d	1.92 ef	7.38 cd
Strain 2 =*Pseudomonas fluorescens*	21.60 de	60.90 d	5.23 ef	23.45 cd	2.42 cd	6.89 cd
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 1	28.05 c	66.50 c	6.68 c	27.87 bc	2.08 de	8.22 bc
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 2	27.92 c	69.25 bc	7.04 bc	28.98 a-c	2.58 bc	8.03 bc
NPK fertilizer (200-150-100 kg ha^{-1} respectively) + Strain 1	31.70 ab	73.57 b	7.77 ab	32.85 ab	2.85 ab	9.66 ab
NPK fertilizer (200-150-100 kg ha^{-1} respectively) + Strain 2	34.67 a	79.82 a	8.39 a	36.05 a	3.09 a	10.60 a
LSD Value	3.02	5.50	0.99	7.92	0.36	2.05
70 DAS						
Control	27.35 e	66.36 f	13.97 f	76.89 e	3.75 f	22.09 e
NPK fertilizer (200-150-100 kg ha^{-1} respectively)	44.27 bc	104.88 c	21.97 c	109.36 b-d	5.91 c	31.43 b-d
Strain 1=*Pseudomonas syringae*	32.20 de	79.07 e	18.67 de	92.02 de	5.02 de	26.45 de
Strain 2 =*Pseudomonas fluorescens*	36.52 d	94.95 d	17.22 ef	98.43 de	4.62 ef	29.22 c-e
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 1	42.75 c	100.89 cd	21.73 cd	101.65 c-e	5.84 cd	28.29 de
NPK fertilizer (100-75-50 kg ha^{-1} respectively) + Strain 2	44.60 bc	105.66 c	23.17 bc	124.13 a-c	6.23 bc	35.67 a-c
NPK fertilizer (200-150-100 kg ha^{-1} respectively) + Strain 1	48.65 b	117.99 b	27.59 a	133.23 ab	6.88 ab	38.29 ab
NPK fertilizer (200-150-100 kg ha^{-1} respectively)+ Strain 2	56.92 a	129.16 a	25.58 ab	144.58 a	7.42 a	41.55 a
LSD Value	4.70	9.27	3.26	24.86	0.87	7.14

Means sharing the same letter(s) within a column did not differ significantly with respect to each other at 5% probability level.

the level of stress ethylene and thus caused the plants to become stress-resistant (Glick *et al.*, 1998).

In the present study, it was observerd that under saline-sodic field conditions, root parameters such as root length, fresh root weight and dry root weight were improved in plants inoculated with ACC-deaminase-containing rhizobacterial strains compared with control plants (Table 1). The underlying reason might be the reduction of the stress ethylene level with ACC-deaminase-containing rhizobacteria, which convert ACC (an immediate precursor of ethylene biosynthesis) into ammonia (NH_3) and α-ketobutyrate instead of ethylene. Similar results were reported by several other researchers (Belimov *et al.*, 2002; Zahir *et al.*, 2009; Naz *et al.*, 2013; Zafar-ul-Hye *et al.*, 2014a; Zafar-ul-Hye *et al.*, 2014b).

Similarly, shoot length and fresh and dry shoot weights were found to be increased by PGPR strains. This result might be due to improved root growth, which consequently promoted shoot growth. Similar findings were reported by Kausar and Shahzad (2006), who demonstrated that inoculation of maize with PGPR strains caused a significant increase in shoot dry matter. Nadeem *et al.* (2006) also reported similar findings.

The use of PGPR strains in combination with chemical fertilizers further improved root and shoot growth compared to the control. PGPR might have improved the solubilization, mobilization, availability and uptake of N, P and K by the plants, which stimulated the performance of crop due to the production of plant growth regulators (Zahir *et al.*, 2004). The results obtained in the present study, with respect to N, P and K are in agreement with those of several other researchers (Pal *et al.*, 2000; Zahir *et al.*, 2009).

Field conditions are complex, and various biotic and abiotic factors may modify the behavior of particular PGPR strains. For example, we observed that both strains behaved differently when applied alone and when used with mineral fertilizers. The strains might exhibit differences in their mechanisms of action, including ACC-deaminase activity, IAA production, siderophore production, phosphate solubilization and others. These differeneces might have resulted in differences in their effectiveness in root and shoot growth promotion in maize grown under saline-sodic field conditions (Belimov *et al.*, 2002; Zahir *et al.*, 2004).

It is suggested that PGPR strains with ACC-deaminase activity have the potential to reduce the stress ethylene level in plants and may be used for improving crop growth under stressful conditions. It is further proposed that the effectiveness of the bacteria under saline-sodic field conditions could be enhanced when they are applied in combination with mineral fertilizers.

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