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PERFORMANCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA CONTAINING ACC-DEAMINASE ACTIVITY FOR IMPROVING GROWTH OF MAIZE UNDER SALT-STRESSED CONDITIONS

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Bacteria carrying 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity lower stress induced ethylene levels and may be effective to improve plant growth under salt stress conditions. Twenty strains of rhizobacteria isolated from soil samples taken from different salt affected aras were screened for plant growth promotion and ACC- deaminase enzyme activity under axenic conditions at 6 dS m^{-1}. Three strains (S5, S15 and S20) that promoted growth to the greatest extent under axenic conditions were selected for further study in pot trial at 0, 5, 10 dS m^{-1}. Results of pot trial showed that the increase in salinity level decreased the growth of the maize seedlings. However, inoculation of maize seeds with these three rhizobacterial strains performed well at all salinity levels, and the strain S20 at 5 dS m^{-1} significantly increased root/shoot length, root fresh/dry weight and shoot fresh/dry weight up to 56/62, 51/71, 52/61%, respectively, over uninoculated control. At 10 dS m^{-1} increase was 120/63, 52/71, 59/118%, respectively, over uninoculated control. Similarly, increase in chlorophyll a, b and carotenoid contents of fresh leaves increased up to 86% at 5 dS m^{-1} and up to 84% at 10 dS m^{-1} by strain S20 over its respective control. Results revealed that it is highly likely that these rhizobacterial isolates deaminated endogenous ACC. Therefore, negative effects of stress induced ethylene could be partially eliminated through inoculation with ACC-deaminase containing rhizobacteria.
Key words: Salt stress, ACC-deaminase, maize growth, PGPR

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) could play a significant role in the development of sustainable agriculture. These are rhizobacteria that are beneficial to plants (Kloepper *et al.*, 1989) and affect plant growth directly or indirectly through various mechanisms of action (Glick *et al.*, 1998; Mantlelin and Touraine, 2004). Indirect promotion occurs when PGPR improved plant by preventing growth restricting conditions (Glick *et al.*, 1999). The direct promotion by PGPR entails either providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. They can fix atmospheric nitrogen, synthesize siderophores, phytochromes or some enzymes that modulate growth and development by influencing the levels of plant hormones (Glick *et al.*, 1996). Among the plant hormones whose concentrations are most likely to be altered by the PGPR include ethylene, auxin, gibberellins and cytokinin (Xie *et al.*, 1996; Zahir *et al.*, 2005).

The increase of ethylene in plants is directly related with the concentration of ACC in plant tissues (Machackov *et al.*, 1997). Recently, it was discovered that a number of PGPR promote plant growth by lowering endogenous ethylene synthesis in the roots through their ACC-deaminase activity (Glick *et al.*, 1998). When ACC deaminase containing PGPR are bound to the developing seedling, they may act as a sink for ACC ensuring that the ethylene level does not become elevated to the point where root growth is impaired (Grichko *et al.*, 2000). By reducing ethylene and facilitating the formation of longer roots, these bacteria may enhance the survival of some seedlings during the first few days after sowing (Glick *et al.*, 1998). Soil microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote plant growth by sequestering and cleaving plant-produced ACC, and thereby lowering the level of ethylene in the plant (Penrose *et al.*, 2001). A decreased ethylene level allows the plant to be more resistant to a wide variety of environmental stresses (Glick, 2005) such as salinity, drought and metal toxicity.

Salinity is one of the most critical constraints and hampers agriculture productions in many areas around the world, including Pakistan (Hasegawa *et al.*, 2000). Out of 22 million ha arable area of Pakistan, 6.67 million ha is salt affected (Khan, 1998). Moreover, about 40,000 ha land in Pakistan are lost annually to cultivation due to salinity. However, utilization of salt-affected soils for agriculture production is also indispensable to feed the burgeoning population of Pakistan. In addition to the use of traditional breeding of plants, a lot of measures such as physical, chemical

and recently the saline agriculture have been adopted. Because of the economic impact of stresses and the large amount of energy required to alter the environment to suit the plant, it is becoming increasingly important to utilize sustainable techniques for inducing salt tolerance in plants better adapted to stress. It is hypothesized that the use of plant growth promoting rhizobacteria carrying the trait of ACC-deaminase as inoculants can enhance plant growth by regulating the endogenous level of ACC and so ethylene in plant/crops under salt stress condition.

The present study was conducted to evaluate the potential of microbial strains containing ACC-deaminase activity to promote growth of maize under salinity stress conditions.

MATERIALS AND METHODS

Isolation of rhizobacteria containing ACC-deaminase

Rhizobacteria were isolated from the rhizosphere of maize growing in salt affected soil by dilution plate technique using DF salt minimal medium (Dworkin and Foster, 1958) containing ACC as a sole source of nitrogen (enrichment technique). Bacterial colonies were isolated and purified by further streaking on fresh plates. These cultures were stored at 4±1°C on slants and maintained by transferring them on fresh slants weekly.

Preparation of inocula

A broth was prepared by using minimal salt medium containing ACC as a source of nitrogen. The medium was sterilized at 121°C for 20 min, while substrate ACC was sterilized separately by filtering through 0.2 µm membrane filter. Flasks containing 100 mL sterilized broth were inoculated with selected strains of rhizobacteria. Each flask was incubated at 30°C, under shaking at 100 rpm for 48 h. Culture broth was centrifuged for harvesting bacterial cells. Cell pellets were suspended in phosphate buffer saline. Population of each strain was maintained using the same buffer. Optical density of suspension was measured at 600 nm and a uniform cell density [OD_{600}: 0.5] was achieved.

Screening of rhizobacteria (Jar experiment)

Jar experiment was conducted on maize under controlled conditions to screen rhizobacteria containing ACC-deaminase for their growth promoting activity at different salinity levels. Sterilized glass jars were used for the experiment. Uniformly germinated seeds were inoculated with respective bacterial cell suspension and were sandwiched between two sterilized filter papers, which were rolled and placed in the glass jars. Hoagland nutrient solution (half strength) was applied for nutrition. Different salinity levels (0 and 6 dS m^{-1}) were developed by using NaCl salt. Jars were arranged using completely randomized design with four replications for each treatment. Plants were incubated in a growth chamber at 25 ± 2°C with light supplied for 12 h during the daytime. After two-week incubation, plants were harvested and data regarding root length shoot length and fresh weights of seedling (root+shoot) were recorded. Three promising isolates (S5, S15 and S20) showing maximum plant growth under axenic conditions were selected for further experimentation.

In vitro ACC-deaminase activity and ACC matabolism assay

ACC metabolism assay (qualitative) was carried out according to the method described by Jacobson et al. (1994). ACC-deaminase enzyme activity (quantitative) was determined according to Honma and Shimomura (1978).

ACC metabolism assay

Isolates were inoculated in 5mL ½ TSB (tryptic soy broth). Cultures were incubated for 48 hours at room temperature along with shaking at 150 rpm. These cultures were diluted ten times in autoclaved 0.1 M of $MgSO_4$. Diluted cultures were transferred to sterile petri dishes containing DF salts.

In 96-well plate, 150 µL DF salts was added in all wells. In lane 3, 6, 9 and 12, 15 µL 0.1 M $MgSO_4$ and in lane 2, 5, 8, and 11, 15µL 0.1 M $(NH_4)_2SO_4$ was added. The 3 mM ACC was filter sterilized with 0.2µL syringe filter and was stored at –20°C before the assay. This was allowed to thaw before use; 15µL of thawed ACC was filled in the lane 1, 4, 7 and 10. For inoculation of each well, 22µL bacterial culture was used. In uninoculated control wells, 22µL 0.1M $MgSO_4$ was used in place of bacteria. Optical density (OD) was measured after 0, 24, 48, 72 and 96 hours at 750 nm by Biolog ® identification system.

ACC-deaminase enzyme activity

Bacteria were grown at 30°C in nutrient broth to late lag phase, after which the cells were harvested by centrifugation and dissolved twice with 0.1 M Tris buffer, pH 7.5. A 200 mL aliquot of bacterial suspension was removed and 10 mL of toluene was added. The cells were vortexed vigorously to facilitate permeabilization, and then 5 mL of 500 mM ACC was added to 50 mL of bacterial lysate. ACC-deaminase assay was quantified by monitoring the amount of α-ketobutyrate that was produced by the deamination of ACC measured following the derivitization of α-

ketobutyrate with 2, 4-dinitrophenylhydrazine (Table 4) as described by Honma and Shimomura (1978).

Pot Trial

Isolates which could metabolize ACC more efficiently compared to $(NH_4)_2SO_4$ and $MgSO_4$ (N-free) (Figure 1 a, b, c) besides performing better under axenic conditions for improving root/shoot growth of maize seedlings were further evaluated in the pot trial at different salinity levels. The trial was conducted in the research area (wire house), Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad to evaluate the potential of effective strains of rhizobacteria containing ACC-deaminase activity to increase growth of maize under salt stress conditions. The inoculum for the pot trial was prepared by culturing the selected rhizobacterial isolates in DF minimal salt medium, and incubated at 28± 1°C for 72 hours. The

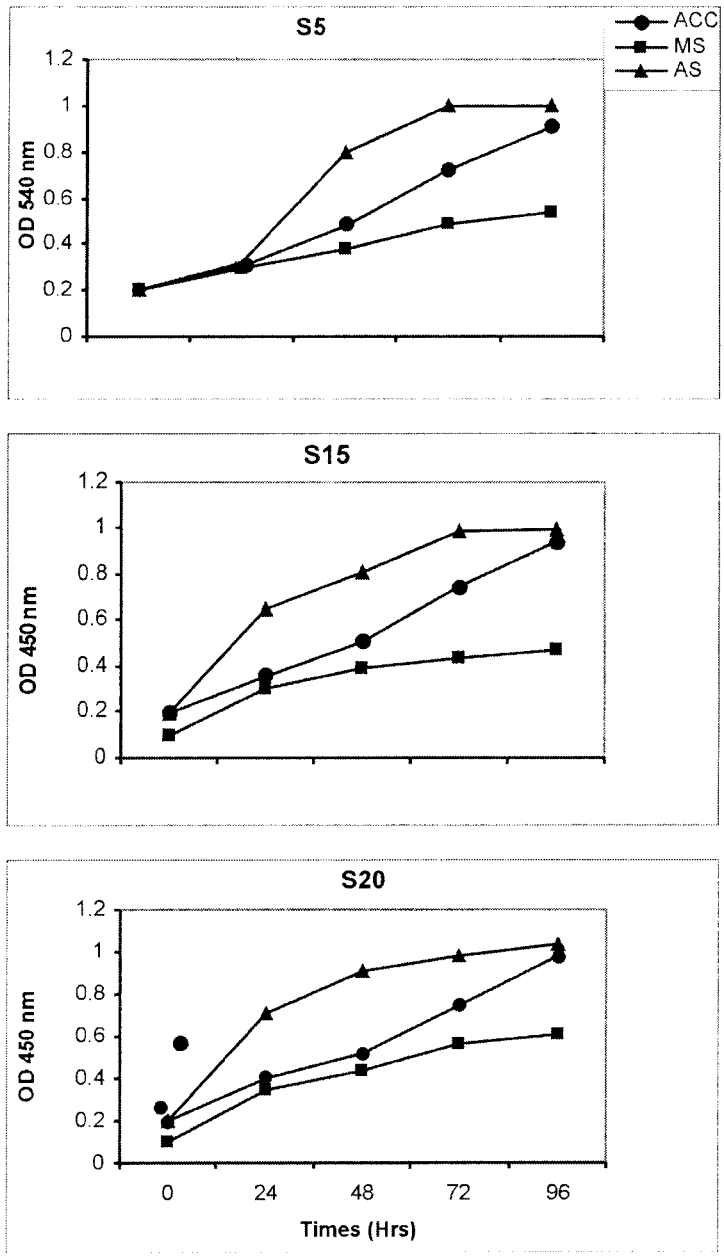

Figure 1. ACC utilizing potential of selected rhizobacterial strains.

salinity was produced by using sodium chloride. For inoculation, seed dressing was done with inoculated peat mixed with 25 ml, 10% sugar solution. In the case of uninoculated control, seeds were coated with the sterilized (autoclaved) peat treated with broth and 25 ml, 10% sugar solution. The coated seeds of maize were sown in pots having 12 kg pot $^{-1}$ sandy clay loam soil. Recommended doses of NPK at 120-100-50 kg ha^{-1} were applied to treatments. The whole dose of PK fertilizers was applied at the time of sowing as a basal dose while N was applied in two split doses.

Maize seeds were inoculated with three strains (S5, S15 and S20) at three salinity levels (0, 5 and 10 dSm^{-1}) in pot trial. There were three replications and pots were arranged in wire house under ambient light and temperature according to completely randomized design. Canal water was used for irrigation. Data regarding shoot length was recorded before harvesting and root length at harvesting. Fresh root and shoot weight was immediately recorded after harvesting. The harvested plants were oven dried at 65°C to get oven dry weight of roots and shoots. The collected data were statistically analyzed (Steel and Torrie, 1984) using completely randomized design and means were compared by Duncan's multiple range test (Duncan, 1955).

Chlorophyll contents

The chlorophyll a, b and carotenoid contents were determined according to the method of Arnon (1949). The fresh leaves weighing 0.2 g were cut and extracted overnight with 80% acetone at 0-4 °C. The extracts were centrifuged at 100,000 xg for 5 minutes. The absorbance of the supernatant was read at 663, 645 and 480 nm using a spectrophotometer.

RESULTS

Rhizobacterial strains containing ACC-deaminase were isolated and screened through ACC metabolism assay.

Table 1. Effect of inoculation with rhizobacteria containing ACC- deaminase on shoot fresh & dry weight, root fresh & dry weight, root length and shoot length at salinity levels 6 dS m^{-1} (Average of 4 replicates).

Strains	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	19.43	0.51	0.02	20.30	0.79	0.06
S1	21.13	0.47	0.03	22.97	0.84	0.11
S2	19.67	0.60	0.02	21.00	0.96	0.08
S3	17.03	0.46	0.03	21.87	1.01	0.08
S4	18.07	0.49	0.04	21.77	1.00	0.09
S5	25.47	0.69	0.08	24.43	1.12	0.17
S6	19.47	0.51	0.06	21.70	1.03	0.11
S7	22.47	0.52	0.04	22.07	1.00	0.13
S8	22.07	0.58	0.03	21.50	0.97	0.13
S9	24.00	0.45	0.03	20.57	0.83	0.09
S10	23.83	0.63	0.04	22.57	1.02	0.07
S12	21.33	0.53	0.03	22.93	0.92	0.16
S13	22.63	0.52	0.03	23.10	1.04	0.12
S14	24.43	0.67	0.04	23.77	0.73	0.11
S15	24.57	0.68	0.06	25.27	1.35	0.22
S16	19.43	0.36	0.02	15.77	0.49	0.04
S17	23.57	0.56	0.05	21.33	0.97	0.07
S18	21.87	0.64	0.04	21.47	1.06	0.13
S19	24.87	0.43	0.03	19.73	0.79	0.07
S20	26.10	0.91	0.10	24.50	1.19	0.19
LSD value	1.45	0.05	0.05	0.26	0.21	0.05

Table 2. Effect of inoculation with rhizobacteria containing ACC- deaminase on root characteristics at different salinity levels (Average of 4 replicates).

Treatments	Length (cm)			Fresh weight (g)			Dry weight (g)		
	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}
Control	26.00±0.47	18.00±0.85	10.00±0.71	26.80±0.62	18.50±1.08	10.50±0.47	18.33±0.54	12.00±0.62	7.20±0.36
S5	35.00±1.31	24.00±1.63	18.00±0.94	37.83±1.74	26.00±1.41	18.33±0.83	20.00±0.94	17.00±0.47	10.00±0.71
S15	28.00±0.82	26.00±0.94	14.00±0.63	34.50±1.43	22.00±0.62	16.00±1.25	22.33±0.72	19.00±0.85	12.00±0.82
S20	32.67±0.98	28.00±0.47	22.00±1.32	32.50±1.03	28.00±0.82	21.00±1.08	26.00±0.82	20.47±1.18	16.00±0.47

* Average ± Standard error of four replications.

Table 3. Effect of inoculation with rhizobacteria containing ACC- deaminase on shoot characteristics at different salinity levels (Average of 4 replicates)

Treatments	Length (cm)			Fresh weight (g)			Dry weight (g)		
	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}
Control	65.50±3.63	55.50±2.01	29.50±2.72	68.00±1.65	46.00±1.84	28.00±0.95	27.50±0.85	18.00±0.62	11.00±0.94
S5	85.50±2.10	76.00±2.45	48.00±0.94	80.00±0.94	53.50±3.08	42.50±1.85	38.00±0.41	27.00±0.82	20.00±1.78
S15	105.00±3.30	83.50±1.87	45.00±2.94	95.00±2.16	60.00±1.41	36.50±1.55	33.50±0.62	25.00±0.94	16.50±0.41
S20	90.00±2.49	90.00±3.27	53.00±3.68	90.00±3.27	73.00±2.45	49.50±2.66	32.00±1.25	29.00±0.41	24.00±0.62

* Average ± Standard error of four replications.

Table 4. Effect of inoculation with rhizobacteria containing ACC- deaminase on chlorophyll a, chlorophyll b and carotenoid contents at different salinity levels (Average of 4 replicates)

Treatments	Chlorophyll a			Chlorophyll b			Carotenoid			ACC-deaminase α-ketobutyrate nmol g^{-1} biomass
	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	0 dSm^{-1}	5 dSm^{-1}	10 dSm^{-1}	
Control	0.52±0.04	0.39±0.03	0.25±0.04	0.70±0.04	0.48±0.03	0.30±0.02	0.27±0.01	0.17±0.03	0.10±0.01	
S5	0.65±0.04	0.45±0.04	0.27±0.03	0.80±0.04	0.56±0.02	0.37±0.04	0.25±0.02	0.19±0.02	0.11±0.02	440
S15	0.78±0.05	0.54±0.03	0.32±0.04	1.15±0.04	0.62±0.02	0.41±0.03	0.37±0.02	0.23±0.02	0.15±0.01	405
S20	0.75±0.06	0.61±0.02	0.46±0.03	0.98±0.01	0.72±0.03	0.48±0.01	0.31±0.011	0.28±0.03	0.18±0.02	442

* Average ± Standard error of four replications.

Results of ACC metabolism assay revealed that all the strains metabolized ACC but with different degree of efficacy (data not shown). On the basis of results we categorized all the strains into three groups. Strains S5, S8, S9, S10, S14, S15, S17 and S20 have high ACC utilization rate, strains S1, S4, S7, S12, S13, S16, S18 and S19 falls in the medium ranges while strains S2, S3 and S6 have lower ACC utilization rate.

Screening under axenic conditions

Twenty rhizobacterial strains (S1-S20) containing ACC-deaminase were evaluated for their growth promoting activity in maize at salinity level (6 dSm^{-1}) under gnotobiotic conditions. Results are summarized below.

Results of axenic trial (Table 1) showed that out of twenty strains, one strain showed non-significant effect on root length and four strains were statistically at par to control while maximum increase in root length (84% over uninoculated control) was observed in case of inoculation by S20. Similarly, in case of shoot length, two strains affected non-significantly and the strain S15 showed maximum increase in shoot weight i.e. 61% over control. Regarding root fresh weight, three strains showed non-significant response, seven strains showed statistically similar results compared to control and strain S20 increased maximum root fresh weight (149%) over control. In case of root dry weight, one strain showed non-significant effect, fourteen strains were statistically similar to control and strain S15 increased root dry weight by 433% over uninoculated control. Regarding shoot fresh weight, two strains were below and six strains were statistically at par with control while maximum increase (176% over control) was recorded in S20. In shoot dry weight, eight strains showed statistically similar results and S15 responded significantly i.e. 450% increase over uninoculated control.

Pot Trial

Result of pot trial showed that on an overall average basis the increase in salinity level decreased the growth of the maize seedlings. However, inoculation of maize seeds with PGPR containing ACC-deaminase activity significantly affected the growth of maize under salinity stress.

Results showed that inoculation with strain S20 significantly increased the root length at EC 10 and 5 dS m^{-1} (120, 56% over respective control) followed by strains S5 and S15 (Table 2). At original EC, rhizobacterial strains S5 and S20 increased root elongation significantly that was 35 and 26% higher than control, respectively.

Inoculation with isolate S20 significantly increased root fresh weight (up to 100%) at EC 10 dS m^{-1} while 52% increase was recorded at EC 5 dS m^{-1}over respective control, followed by S5 and S15 (Table 2). At original EC, all rhizobacterial strains significantly increased root fresh weight (21 to 40%) compared to control.

In case of original EC, inoculation with PGPR increased root dry weight that was 10-42% higher as compared to control (Table 2). At EC 10 and 5 dS m^{-1} strain S20 was found more effective and increased root dry weight i.e. 122 and 71%, respectively, higher than control, while S15 increased 67 and 58 % and S5 showed (39 and 42%) at same salinity levels.

Data regarding the effect of inoculation on shoot length of maize revealed that strain S20 caused maximum increase in the shoot length i.e. 80 and 63% over control at 10 and 5 dS m^{-1} (Table 3). At original EC, all strains significantly increased the shoot length that was 31, 60 and 37%, higher than control.

The increase in shoots fresh weight by inoculation with rhizobacterial strains at different salinity levels ranged from 18 to 77% over control (Table 3). At original EC, strain S15 significantly increased shoot fresh weight that was 40% higher as compared to uninoculated control. At EC of 10 and 5 dS m^{-1} S20 was found more effective and increased weight significantly that was 77 and 59% higher than control.

The data regarding shoot dry weight indicated that PGPR containing ACC-deaminase significantly affected the shoot dry weight at different salinity levels (Table 3). At EC 0 and 5 dS m^{-1}, strain S5 increased shoot dry weight by 38 and 50%, respectively over respective control. At EC 10 dS m^{-1} S20 caused maximum increase (118%) in shoot dry weight over uinoculated control followed by S5 and S15.

Chlorophyll Contents

Result of pot study showed that on an overall average basis the increase in salinity level decreased the chlorophyll contents (chlorophyll a, b and carotenoid) of the maize seedlings. However, inoculation with PGPR containing ACC-deaminase activity significantly affected the pigments under salinity stress.

Data regarding the effect of inoculation on chlorophyll 'a' contents in maize plant revealed that the strain S20 caused maximum increased in chlorophyll 'a' contents (up to 84 and 56% over respective control) (Table 4) at EC 10 and 5 dS m^{-1} followed by S15 and S5. At original EC, strains S5, S15 and S20 caused significant increase in chlorophyll 'a' contents, which was 25, 50 and 44% higher over control, respectively.

Data in Table 4 depicted the significant effect of strains inoculation on chlorophyll 'b' contents in maize plant. At original EC, S15 and S20 caused significant increase in chlorophyll 'b' contents that were

64 and 40% higher than control, respectively. At EC of 5 dS m^{-1}, strains S20 and S15 enhanced chlorophyll 'b' contents by 50 and 29 % compared to control, respectively. At EC 10 dS m^{-1}, all the three strains were effective and enhanced chlorophyll 'b' contents (60, 37 and 23%, respectively compared to control).

Data revealed that inoculation with rhizobacterial strain S20 significantly increased carotenoid contents (Table 4). At original EC, S15 showed significant effect and increased carotenoid contents up to 37% as compared to control. At 10 and 5 dS m^{-1}, strain S20 increased the carotenoid contents of maize plant by 80 and 65% higher than control, respectively, followed by strains S15 and S5.

DISCUSSION

In this study, twenty strains of PGPR containing ACC-deaminase activity were screened for their growth promoting activity under axenic conditions at salinity levels (0, 6 dS m^{-1}) by conducting jar experiment on maize. Three promising ACC-deaminase-rich strains of PGPR (S5, S15 and S20) were further evaluated at different salinity levels (0, 5 and 10) in a pot trial. It was observed that inoculation with these rhizobacterial strains containing ACC-deaminase activity significantly promoted root, shoot and other growth contributing parameters of maize at all levels of salinity under axenic and pot conditions. Inoculation was effective even in the presence of higher salinity levels (5 and 10 dS m^{-1}). Ethylene is recognized as stress hormone i.e. produced at higher concentration under any kind of stress including salinity. The higher concentration of ethylene is inhibitory to plant growth. It is very likely that the PGPR strain S20 promoted root growth under axenic conditions by lowering the endogenous inhibitory levels of ethylene in roots because of its high ACC-metabolising ability (Figure 1 c). This may imply that the inoculation with rhizobacteria containing ACC-deaminase could result in the development of much better root system, which subsequently affects shoot growth positively. Very recently, Shaharoona *et al.* (2006) reported a significantly positive correlation between ACC-deaminase activity and root elongation in maize due to inoculation with rhizobacteria containing ACC-deaminase activity under axenic conditions. Other researchers have also reported that under gnotobiotic conditions, seed and/or root inoculation with rhizobacteria promotes root growth through ACC-deaminase activity (Glick *et al.*, 1998; Wang *et al.*, 2000; Belimove *et al.*, 2002).

The three most effective strains selected on the basis of inducing prolific root systems under gnotobiotic conditions also promoted plant growth in pot trial. The data revealed that inoculation with rhizobacterial strain S20 proved to be the most effective at all salinity levels which increased root growth and other parameters even at high salt stress i.e. EC 10 dS m^{-1} followed by S5. This growth promotion might be attributed to the decreased ethylene levels due to inoculation with these isolates containing ACC-deaminase activity. Because production of ethylene is accelerated during seed germination that becomes more pronounced (intense) when plants are exposed to salinity stress, this may have inhibitory effects on seed germination and root growth (Glick *et al.*, 1998; Mayak *et al.*, 2004). So it is highly likely that inoculation with these rhizobacterial isolates might have decreased endogenous inhibitory levels of ethylene in developing seeds and roots and thus resulted in formation of longer roots because of their high ACC-deaminase activity (Table 4) that subsequently promote shoot growth positively.

It was also observed that inoculation with strains containing ACC-deaminase increased the chlorophyll pigments (a, b and carotenoid contents) of maize, and these results confirm the previous finding of Glick *et al.* (1997). Similar results were also reported by Han and Lee (2005) that PGPR inoculation increased the chlorophyll content in lettuce.

Data showed that isolate S20 increased the chlorophyll pigments (a, b and carotenoid contents) up to 84, 60 and 80% at EC 10 dS m^{-1}. This may be the result of increased photosynthetic leaf area of plant even at high salt stress by PGPR inoculation compared to control where leaf area reduced due to stress (Marcelis and Van Hooijdonk 1999).

It is concluded that inoculation with ACC-deaminase PGPR could be effective to improve the growth of maize even in the presence of high salinity stress. However, further work is needed to explore the effectiveness of such strains under field conditions.

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