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ACC DEAMINASE PRODUCING PGPR INVOKE CHANGES IN ANTIOXIDANT SYSTEMS TO MINIMIZE THE ADVERSE EFFECTS OF SALT IN SUNFLOWER

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ABSTRACT: In the present study, we demonstrated that the inoculation improved the growth of sunflower plants in upon salt stress with ACC deaminase producing plant growth promoting rhizobacteria (PGPR) isolates (*Pseudomonas otitidis* Rhizo SF 7 and *Acinetobacter calcoaceticus* Rhizo SF 9). Sunflower plants treated with both ACCd producing PGPR isolates exhibited significant improvement in plant height, shoot fresh and dry weights as well as the total chlorophyll content compared to control plants. The seed treatment with ACCd producing PGPR isolates enhanced the enzymatic (APX and SOD) and non-enzymatic (proline) antioxidants in sunflower upon stress induction compared to respective control. The MDA content decreased in ACCd producing PGPR treated sunflower plants. The antioxidant activities evaluated in the study indicated that the ACCD producing PGPR were able to mitigate the adverse effects of enhanced ethylene level, by utilizing the precursor of ethylene (ACC) produced by the plants and convert them to α-ketobutyrate and ammonia. The results confirmed that the ACCd producing PGPR possess a dual role in plant growth promotion and withstand high salt stress as observed by the increase in the levels of proline, APX, and SOD and decrease in the levels of MDA content.

KEYWORDS: Antioxidant enzymes, *Helianthus annuus* L., Lipid peroxidation, Rhizobacteria, Salinity.

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1.INTRODUCTION

Salinity is one of the severe and significant threats to agricultural land due to drastic changes in environmental conditions and human interference. It has been noted that about 50% of agricultural land throughout the world is expected to be affected by high salinity [1]. It has been observed that, the ethylene level of plants under salt stress increases which alters the physiological process of the plant resulting in the suppression of plant growth and may also lead to plant death [2]. Therefore, it is necessary to keep up the soil salinity for sustainable agriculture by utilizing the promising micro-organisms which can alleviate the salinity stress tolerance and improve plant growth and health [3,4]. Now-a-days, the use of beneficial microbes is gaining importance among researchers for their utilization in plants grown under salinity conditions [5,6,7]. Form the research findings of recent past it has been highlighted that, the plant growth promoting rhizobacteria (PGPR) with the efficacy to produce 1-amino-cyclopropane-1-carboxylic acid deaminase (ACCd) have the potential to improve plant growth and development under both biotic and abiotic conditions. These PGPR isolates possessing ACC deaminase (ACCd) enzyme can mitigate the adverse effects of enhanced ethylene level, by utilizing the precursor of ethylene (ACC as a sole nitrogen source) produced by the plants and convert them to α-ketobutyrate and ammonia [7,8]. There are many reports on the use of ACCd producing PGPR for the growth and development of plants in several crops [9,10,11]. ACCd producing bacteria treatment to sunflower has been reported which has resulted in enhanced plant growth and development under greenhouse conditions upon salt stress [12], but the mechanism involved in the promotion of plant growth is not yet studied. Hence, in this context, we have focused on the research work to explore the native sunflower ACCd producing PGPR to induce the salt stress tolerance in sunflower and to evaluate the physiological and biochemical changes occurring upon salt stress induction.

2. MATERIALS AND METHODS

2.1 ACCd producing PGPR

A total of ten ACCd producing PGPR which produced more than 50% of α- ketobutyrate compared to the isolates evaluated (unpublished data) were used throughout the study.

2.2 Evaluation of ACCd producing PGPR for salt stress tolerance

The ACC deaminase producing rhizobacteria were screened for their salt stress tolerance ability by microtiter plate method. About 300 µL of nutrient broth (NB) amended with the different concentrations of sodium chloride (NaCl) solution (0-15% w/v) was added to each well and inoculated with 10 µL of each ACCd producing bacterial suspension (1×10^8 CFU mL^{-1}). Each of the 96 well plates was incubated at 37° C in a rotary shaker for 24 h, and the absorbance was measured at 610 nm to check the bacterial growth with appropriate blank and controls. The concentration of NaCl where 50% suppression in bacterial growth was observed, and IC_{50} values for each were tabulated. The experiments were carried out in triplicates.

2.3 Evaluation of sensitivity of sunflower seeds to salt stress

The surface sterilized (4% NaOCl) sunflower (*Helianthus annuus* L.) seeds (cv. Mordan) were repeatedly washed with sterile distilled water and were germinated/ grown on Petri dishes containing three layers of sterile blotter discs saturated with Murashige and Skoog (MS) solution (½ strength). The Petri dishes were incubated at 28 ± 2 °C under standard long-day conditions (16/8 h light/dark photoperiod) for four days. After incubation, the germinated seedlings (with prominent plumule and primary root) were sown in an upright position (shoot) on MS media supplemented with different concentrations of sodium chloride (NaCl) of 0, 50, 100, 150, 200 and 250 mM. The plates containing germinated seedlings were incubated under standard long-day conditions as mentioned above. After ten days of incubation, the seedlings were evaluated for the growth of primary root length compared to control. The experiment was repeated thrice with ten seedlings per treatment. The percentage of inhibition in root growth was calculated by using the following formula.

$$\text{Inhibition in Root Growth (\%)} = \frac{\text{Root length on indicated salt concentration}}{\text{Root length on control}} \times 100$$

2.4 Seed bacterization

The fresh culture of ACCd producing PGPR grown in nutrient broth (NB) was centrifuged at 8,000 rpm for 10 min, and the pellet was re-suspended in sterile distilled water to adjust the bacterial count to 1×10^8 CFU mL^{-1}. The surface sterilized sunflower seeds were soaked for 12 h with ACCd producing PGPR suspension amended with CMC (0.2% w/v) on a rotary shaker at 150 rpm with appropriate control [13].

2.5 Seed treatment with ACCd producing PGPR in sunflower to salt stress

The ACCd producing bacteria treated and untreated control sunflower seeds were sown in poly cups (10 cm diameter) containing autoclaved potting medium [2: 1: 1 (red soil, coir peat, and FYM)] and arranged in a randomized complete block design (RBD). The seedlings were watered daily and maintained at 25 ± 2 °C with 80% relative humidity (RH). The water holding capacity of the potting medium was evaluated according to the gravimetric method of Ramegowda et al. [14] for the imposition of stress. The 21-day-old seedlings were watered with salt solution (150 mM NaCl, 170 mL) on alternate days for ten days. At the end of the treatment period, the plants were uprooted carefully without damaging the root system and used throughout the study. Each treatment consisted of 10 plants each with three replicates and the experiment was repeated thrice.

2.5.1 Evaluation of plant growth parameters

Ten plants from each treatment were selected randomly and evaluated for their vegetative growth parameters such as plant height (cm), shoot fresh and dry weights (g). The total chlorophyll content in leaves was evaluated following the method of Hiscox and Israelstam [15]. The experiments were carried out in quadruplicates with repetition.

2.5.2 Enzymatic analyses

2.5.2.1 Extraction of crude enzyme

About 1 g of fresh leaves (control and treatment) were frozen with liquid nitrogen and homogenized with 5 ml of potassium phosphate buffer (50 mM, pH 7.0) containing EDTA (1 mM), magnesium chloride (8 mM), β-mercaptoethanol (5 mM) and polyvinylpolypyrrolidone (1%, w/v) in pre-chilled mortar and pestle. The homogenate was centrifuged (at 6,000 rpm for 20 min at 4° C), and the resultant supernatant was used for the determination of protein content and enzyme activity. The total protein content was determined by the Bradford method [16] using bovine serum albumin (BSA) as a standard. The protein content was expressed as mg ml^{-1}. The experiment was carried out in triplicates and repeated thrice.

2.5.2.2 Ascorbate peroxidase (APX) assay

The APX activity was determined according to the method described by Nakano and Asada [17]. To 1 ml of reaction mixture [50 mM of potassium phosphate buffer (pH 7) contained 0.1 mM of H_2O_2, 0.5 mM of sodium ascorbate and 0.1 mM of EDTA]. About 100 µl of the enzyme extract was suspended into the reaction mixture to initiate the reaction. The APX activity was determined by measuring the decrease in absorbance at 290 nm due to the oxidation of ascorbate. The activity was calculated using molar extinction coefficient of 2.8 mM^{-1} cm^{-1} and 1 unit of enzyme activity were expressed as the amount of APX required to oxidize one µmol of ascorbate $min^{-1}$and the specific enzyme activity was expressed as U min^{-1} mg^{-1} of protein. The experiment was carried out in triplicates and repeated thrice.

2.5.2.3 Superoxide dismutase (SOD) assay

The SOD activity was measured according to Beauchamp and Fridovich [18]. To 3 ml reaction mixture [50 mM of potassium phosphate buffer (pH 7.8) contained 13 mM L-methionine, 100 µM EDTA, 75 µM NBT, 2 µM riboflavin], 50 µl of enzyme extract was added and exposed to white light for 15 min at room temperature to initiate the reaction. After exposure, the increase in absorbance due to the formation of blue formazan produced by the photoreduction of NBT was read at 560 nm. The SOD activity was determined using the SOD standard curve, and one unit of enzyme activity was expressed as the amount of SOD enzyme required to inhibit 50% of photochemical reduction of NBT. The specific activity was expressed as U min^{-1} mg^{-1} of protein. The experiment was carried out in triplicates and repeated thrice.

2.5.3 Non-enzymatic analyses

2.5.3.1 Estimation of Proline

The accumulation of proline in stress-induced and control plants were carried out following the method of Bates et al. [19] with modifications. In brief, about 0.5 g of fresh leaves (control and treatment) were frozen with liquid nitrogen, and to the powdered material, 10 ml of sulphosalicylic acid (3%) was added. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. To the

supernatant (2 ml) equal volume of acid-ninhydrin and acetic acid glacial was added and subjected for incubation at 100 °C for one h. After incubation, 4 ml of toluene was added and mixed well and allowed to settle for 20 min under dark conditions. Upper phase of the reaction mixture (toluene) to the sample was collected, and the absorbance was read at 520 nm. The proline content in each of the sample was determined using the proline standard curve and expressed as μg g^{-1} of FW. The experiment was carried out in triplicates and repeated thrice.

2.5.3.2 Determination of malondialdehyde (MDA)

The lipid peroxidation generated upon membrane damage due to salt stress is expressed in terms of the amount of malondialdehyde as described by Davenport et al. [20]. In brief, about 1 g of fresh leaves (control and treatment) were frozen with liquid nitrogen and homogenized with 3 ml trichloroacetic acid (TCA) (10% w/v) in a pre-chilled mortar and pestle. The homogenate was centrifuged for at 15,000 rpm for 20 min at 4 °C. To 1 ml of supernatant an equal volume of TCA (10%) containing 0.5% thiobarbituric acid (w/v) along with 100 μl of 4% butylated hydroxytoluene (BHT, w/v) and subjected for incubation at 100 °C for 30 min. After incubation, the reaction mixture was centrifuged (15,000 rpm for 15 min at 4 °C) and the absorbance of collected supernatant was read at 532 and 600 nm, respectively. The MDA content was expressed as μmol g^{-1} of FW. The experiment was carried out in triplicates and repeated thrice.

2.6 Statistical Analysis

The experimental data from laboratory and greenhouse were statistically analyzed separately and subjected to arcsine transformation and analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) IBM Statistics, Version 23 (SPSS Inc., Chicago, IL). The significant differences between the treatment mean values were determined by Highest Significant Difference (HSD) obtained by Tukey's test at $p \leq 0.05$ level.

3. RESULTS AND DISCUSSION

3.1 Evaluation of ACCd producing PGPR for salt stress tolerance

Among the ten ACCd producing PGPR evaluated, it was observed that all the tested bacteria were able to grow at varied concentrations of NaCl thereby indicating their efficacy to tolerate stress (Table 1). The isolates namely *Pseudomonas otitidis* Rhizo SF 7, and *Acinetobacter calcoaceticus* Rhizo SF 9 were found to tolerate the maximum concentration of NaCl in broth with an IC_{50} value of 13.44 and 11.26, respectively. Hence, these two bacterial isolates (which offered IC_{50} value of >10) were selected and used for further studies.

Table 1: Salt tolerance ability of ACCd producing PGPR

Rhizobacterial Code	ACCd producing Rhizobacteria	Accession No.	*IC_{50}
Rhizo SF 4	*Enterobacter cancerogenus*	KC168093	4.46 ± 0.69^{bc}
Rhizo SF 7	*Pseudomonas otitidis*	KC168095	13.43 ± 2.34^{a}
Rhizo SF 9	*Acinetobacter calcoaceticus*	KC168096	11.26 ± 1.62^{ab}
Rhizo SF 23	*Bacillus thuringiensis*	KC168097	9.80 ± 1.96^{abc}
Rhizo SF 41	*Stenotrophomonas maltophilia*	KC168100	7.63 ± 1.04^{abc}
Rhizo SF 44	*Pseudomonas aeruginosa*	KF181207	8.03 ± 1.25^{abc}
Rhizo SF 48	*Bacillus subtilis*	KC168103	8.78 ± 1.59^{abc}
Rhizo SF 64	*Brevibacillus brevis*	MK424231	3.80 ± 0.78^{c}
Rhizo SF 90	*Bacillus subtilis*	MK424232	4.57 ± 1.05^{bc}
Rhizo SF 108	*Bacillus thuringiensis*	MK424233	7.35 ± 1.62^{abc}

Values are means of three independent replicates. ± indicate standard errors. Means followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD. *IC_{50} is expressed in a gram of NaCl L^{-1} of broth.

3.2 Evaluation of sensitivity of sunflower seeds to salt stress

The ten-day-old seedlings which were sown on MS medium supplemented with different concentrations of NaCl were evaluated for their sensitivity to salt. The results showed that the % of initial root length decreased with increase in the concentration of NaCl after incubation (Fig. 1). It was also observed that the seedlings grown upon MS media supplemented with 200 and 250 mM NaCl exhibited complete growth inhibition, and some were even killed at these levels.

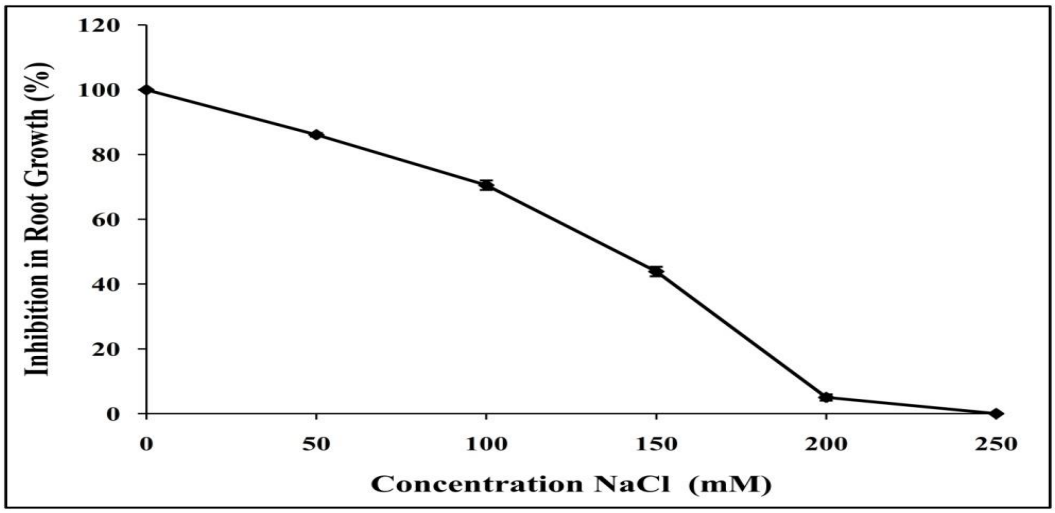

Figure 1: Dose responses of sunflower seedlings to different concentrations of NaCl. Values are means of three independent replicates, and express as the percentage inhibition in root growth after ten days. ± indicate standard errors.

3.3 Evaluation of seed treatment with ACCd producing PGPR in sunflower to salt stress

3.3.1 Evaluation of plant growth parameters

The sunflower seeds treated with ACCd producing PGPR (Rhizo SF 7 and 9) along with control were evaluated for their efficacy to tolerate salt stress (150 mM) under greenhouse conditions (Fig. 2). The results revealed that the sunflower seeds treated with ACCd producing PGPR isolates offered significant enhancement in the plant growth parameters evaluated compared to control (Fig. 3). Among the two isolates evaluated, Rhizo SF 7 offered maximum improvement in both stress-induced and non-stressed plants compared to Rhizo SF 9 treatment and control. A maximum of 14.52 cm, 2.18 g plant^{-1}, 0.30 g plant^{-1} and 1.25 mg g^{-1} FW of plant height, shoot fresh weight, dry weight and total chlorophyll, respectively were observed in Rhizo SF 7 treated seeds subjected to salt stress.

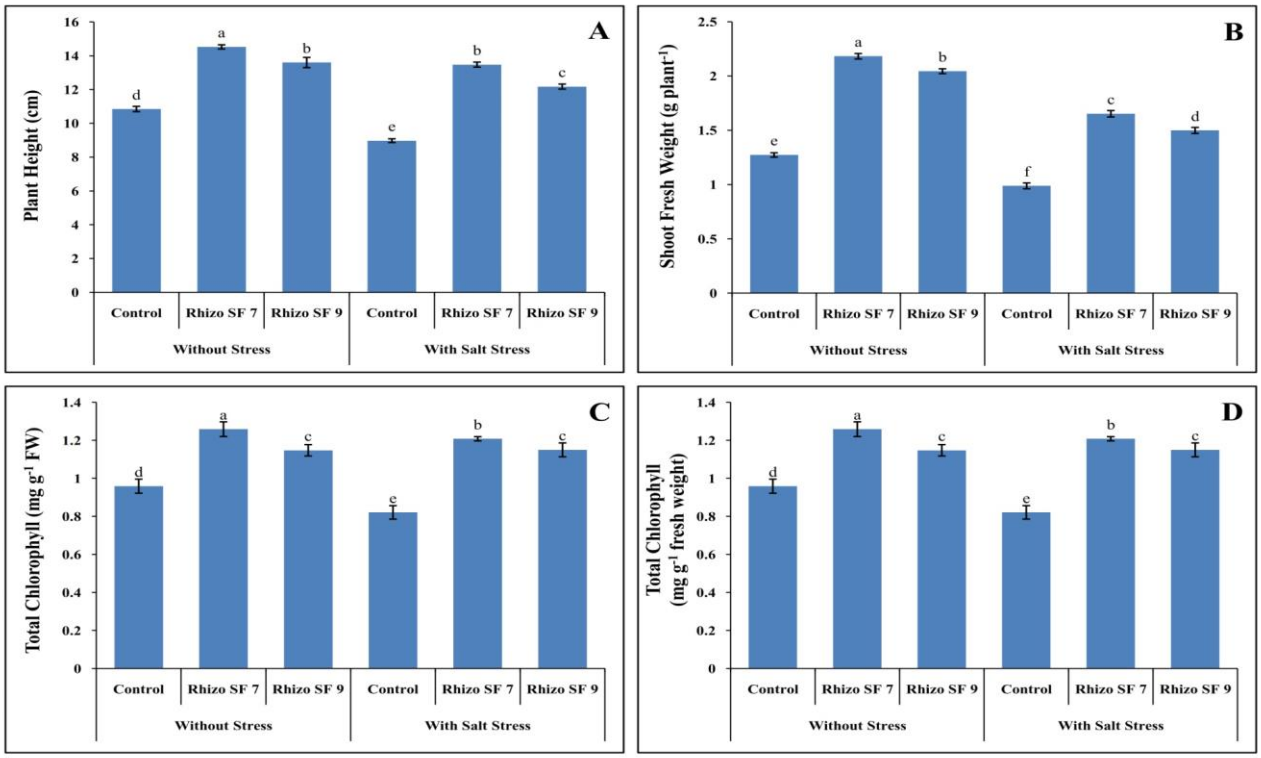

Figure 2: Effect on plant growth promoting properties in sunflower treated with ACCd producing PGPR upon induction of salt stress. Each value is the mean for four replicates (n = 4), and bars sharing the same letters are not different significantly at $p \leq 0.05$ according to Tukey's HSD. The vertical bar indicates the standard error.

Figure 3: Effect of seed treatment with ACCd producing *P. otitidis* Rhizo SF 7 upon induction of salt stress.

3.3.2 Enzymatic analyses

3.3.2.1 Ascorbate peroxidase (APX) assay

APX enzyme activity was estimated in with and without salt stress-induced seedlings raised from ACCd producing PGPR treated and untreated seeds. Significant differences in APX enzyme activity was observed in ACCd producing PGPR treated seedlings compared to control seedlings with or without salt stress induction (Fig. 4). The results revealed that irrespective of stress induction the proline content in sunflower plants increased in ACCd producing PGPR treated seeds compared to control. A maximum APX activity of 3.99 U min^{-1} mg^{-1} of protein was observed in Rhizo SF 7 treated salt stress-induced seedlings followed by Rhizo SF 9. The control seedlings showed APX activity of 1.81 and 0.76 U min^{-1} mg^{-1} of the protein upon stress and non-stress induction. A total of 0.5 and 0.9 fold increase in APX activity was observed in Rhizo SF 7 treated sunflower with or without induction of stress, respectively.

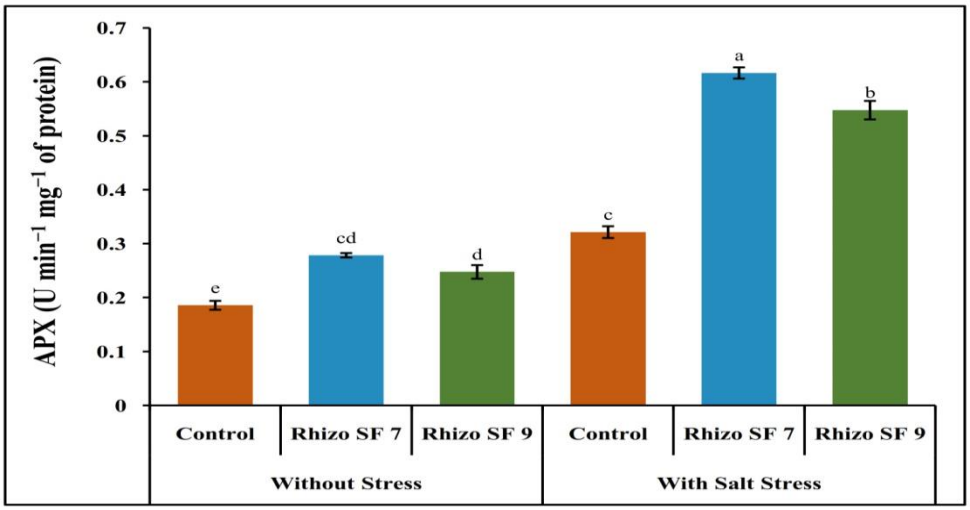

Figure 4: Effect on APX activity in sunflower treated with ACCd producing PGPR upon induction of salt stress. Each value is the mean for three replicates (n = 3), and bars sharing the same letters are not different significantly at $p \leq 0.05$ according to Tukey's HSD. The vertical bar indicates the standard error.

3.3.2.2 Superoxide dismutase (SOD) assay

The result of the SOD enzyme activity was in line with studies of APX assay. The results revealed that, the ACCd producing PGPR Rhizo SF 7 offered significant maximum enzyme activity of 27.27 and 19.87 U min^{-1} mg^{-1} of protein, while 23.84 and 18.94 U min^{-1} mg^{-1} of protein in plants treated with Rhizo SF 9 in plants subjected to stress and without stress, respectively (Fig. 5). There was an increase of about 0.3 and 0.2 fold in SOD enzyme activity in seedlings treated with Rhizo SF 7 and Rhizo SF 9, respectively compared to control. The control seedlings showed SOD activity of 16.23 and 21.51 U min^{-1} mg^{-1} of the protein upon stress and non-stress induction.

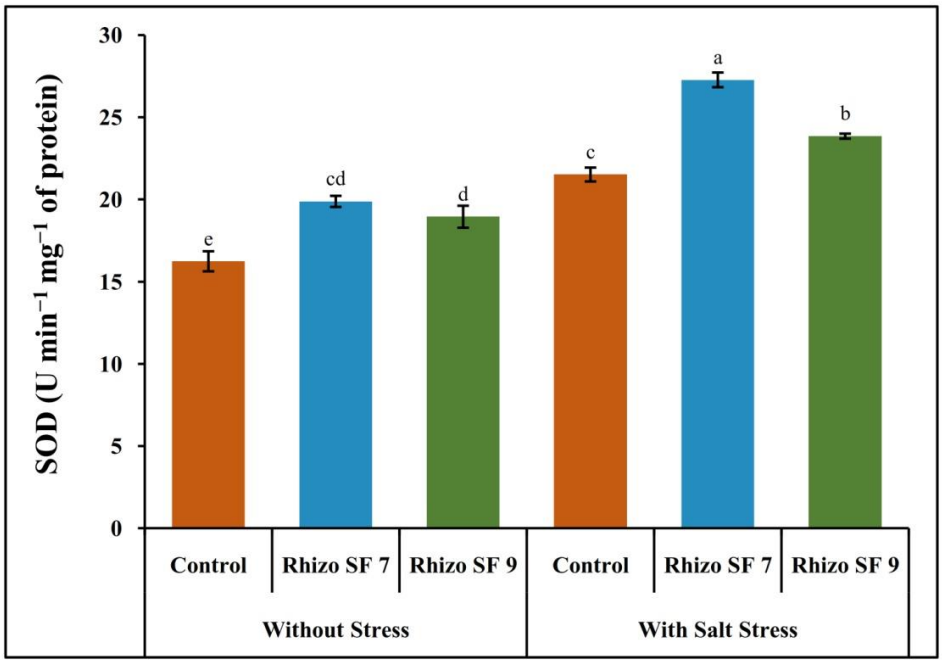

Figure 5: Effect on SOD activity in sunflower treated with ACCd producing PGPR upon induction of salt stress. Each value is the mean for three replicates (n = 3), and bars sharing the same letters are not different significantly at $p \leq 0.05$ according to Tukey's HSD. The vertical bar indicates the standard error.

3.3.3 Non-enzymatic analyses

3.3.3.1 Estimation of Proline

The sunflower seeds treated with ACCd producing PGPR isolates were evaluated for their efficacy to accumulate proline in plants under stress and non-stress conditions. The results revealed that the Rhizo SF 7 and Rhizo SF 9 treated seedlings offered greater amount in the accumulation of proline compared to control plants irrespective of their exposure to salt stress (Fig. 6). Maximum accumulation of 0.97 and 3.48 µg g^{-1} of FW was observed in Rhizo SF 7 treated non-stress and stress-induced plants, respectively followed by Rhizo SF 9. A significant increase in proline accumulation was observed in plants treated with ACCd producing PGPR compared to control plants irrespective of stress exposure. It was noticed that an increase of 0.7 to 1.2 fold in Rhizo SF 7 treated plants compared to control.

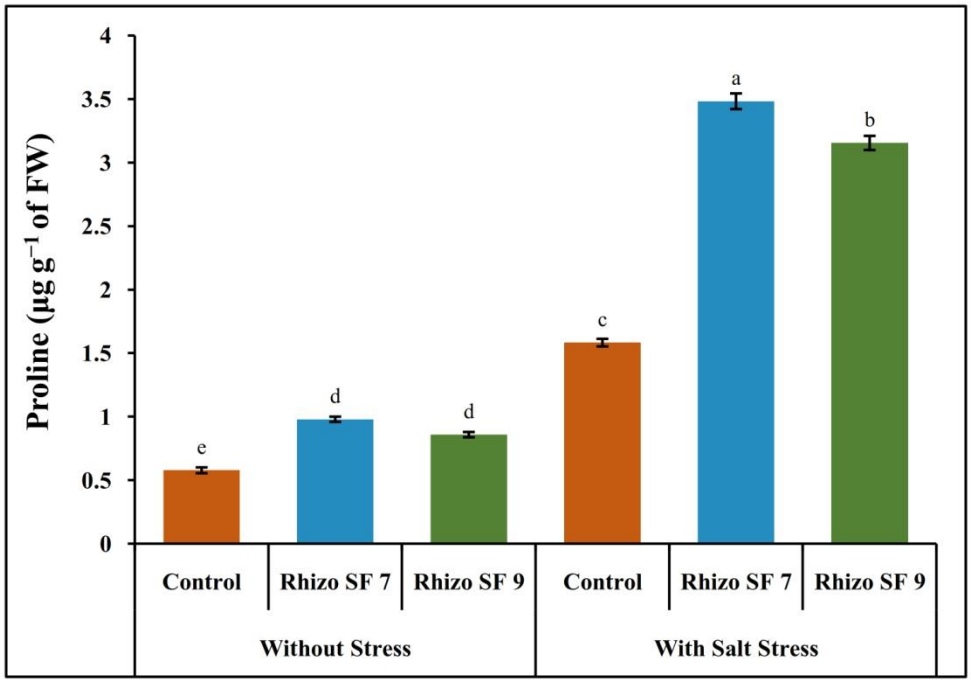

Figure 6: Effect on proline accumulation in sunflower treated with ACCd producing PGPR upon induction of salt stress. Each value is the mean for three replicates (n = 3), and bars sharing the same letters are not different significantly at $p \leq 0.05$ according to Tukey's HSD. The vertical bar indicates the standard error.

3.3.3.2 Determination of malondialdehyde (MDA)

Lipid peroxidation occurring due to membrane damage plays an important role in plants exposed to oxidative stress which is measured in terms of accumulation of MDA. It was observed that, the plants which were subjected to salt stress accumulated a more significant amount of MDA compared non-stressed plants irrespective of the treatment with or without ACCd producing PGPR. The results demonstrated that there was a decrease in the accumulation of MDA in Rhizo SF 7 and Rhizo SF 9 treated seedlings compared to control which were subjected to salt stress induction (Fig. 7). It was observed that MDA content decreased up to 0.2 and 0.4 fold in Rhizo SF 7 treated plants exposed to salt tolerance compared to control salt exposed plants.

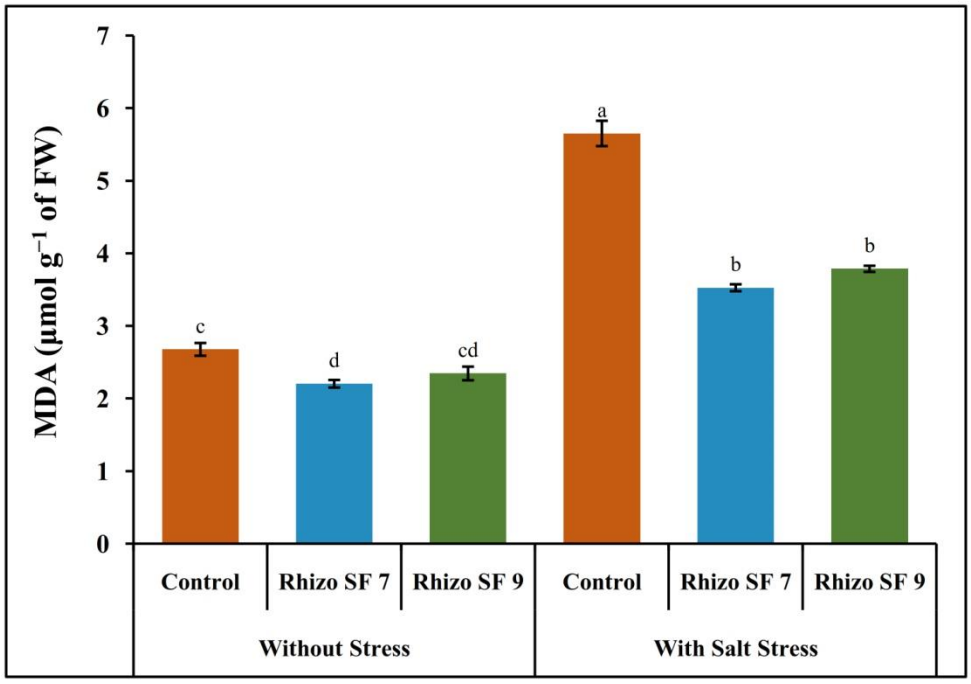

Figure 7: Effect on MDA content in sunflower treated with ACCd producing PGPR upon induction of salt stress. Each value is the mean for three replicates (n = 3), and bars sharing the same letters are not different significantly at $p \leq 0.05$ according to Tukey's HSD. The vertical bar indicates the standard error.

DISCUSSION

The study of bacteria residing in the rhizosphere of the plants are interesting due to its numerous beneficial aspects as they are in close association with the roots of the plants where key host-microbe interactions take place (adverse to favorable conditions). In the present study, a total of ten ACCd producing PGPR were evaluated for their ability to tolerate salt stress. Among assessed isolates, all were able to grow in NB medium supplemented with different concentration of NaCl (up to 15%). The isolates namely *P. otitidis* Rhizo SF 7 and *A. calcoaceticus* Rhizo SF 9 offered a significant IC_{50} value >10 and were used for further studies. The method employed here is highly accredited for the evaluation of stress tolerance ability of micro-organisms [21]. Likewise, Tiwari et al. [7] have evaluated the efficacy of ACCd producing rhizobacteria for salt and drought tolerance by microtiter plate method and reported about thirteen rhizobacterial isolates were able to grow on NA media amended with NaCl. The evaluation of sunflower seeds for their sensitivity to salt stress on MS media supplemented with different concentrations of NaCl revealed that the seed was able to tolerate salt stress up to 150 mM concentration of NaCl and above to this concentration the plants showed a significant reduction in the growth and some were also killed at 250 mM NaCl concentration. Similar experiments are also carried out by many researchers to evaluate the sensitivity of seeds/ seedlings of *Arabidopsis* on MS media supplemented with different concentrations of NaCl [22]. The sunflower seeds treated with ACCd producing PGPR and subjected to salt stress showed

significant enhancement in plant growth parameters compared to control. The bacterial isolate Rhizo SF 7 offered maximum improvement in plant growth parameters followed by Rhizo SF 9. The results are in agreement with the findings of Kiani et al. [12], wherein they evaluated two PGPR isolates in sunflower for their ability to tolerate salt stress under greenhouse conditions and found both the PGPR were able to tolerate and enhanced plant growth parameters to varying degrees. Also, Saravanakumar and Samiyappan [5] reported that groundnut seed treatment with ACC deaminase producing *P. fluorescens* strain TDK rhizobacteria enhanced plant growth parameters subjected to salt stress compared to control. Gamalero et al. [6] reported that the ACC deaminase-producing *P. putida* UM4 improved the salt tolerance ability in cucumber plants upon treatment. The antioxidant enzyme activity of sunflower seeds with or without salt stress induction increased in the present work. The results are by the studies of Prochazkova et al. [23], wherein they have reported enhanced antioxidant enzyme activity upon stress induction. It has been notified that SOD catalyzes the dismutation of O_2 and H_2O_2, while APX scavenges H_2O_2 with ascorbate resulting in the production of MDA which in turn helps in the detoxification of H_2O_2 to water thereby protecting cells against oxidative damage [24, 25]. The results revealed that an increase in antioxidant enzymes (APX and SOD) was observed irrespective of stress induction. The results also showed that the antioxidant enzyme level increased in plants treated with ACCd producing PGPR compared control salt stress-induced plants. The above results are in corroboration with the findings of Gurumani et al. [26], wherein treatment of ACCd producing PGPR to *Solanum tuberosum* increased the levels of antioxidant enzymes both under salt and control conditions, thereby conferring better tolerance to plants upon induction of salt stress. In accordance, Karthikeyan et al. [27] have noted increased levels of antioxidant enzymes in plants treated with ACCd producing bacteria. Proline accumulation due to salt stress is related to the protection of cell membrane and scavenges the free hydroxyl radicals [28], while the increase in MDA content is correlated to the increase in reactive oxygen species in plants caused by cell membrane damage [29]. The present study revealed that the plants treated with ACCd producing PGPR increased the accumulation of proline and decreased the amount of MDA irrespective of salt stress induction. The results are in line with the findings of Tiwari et al. [7], wherein they have reported higher accumulation in proline content in *Panicum maximum* plants treated with ACCd producing rhizobacteria irrespective of stress exposure. Likewise, Singh and Jha [30] indicated a decrease in MDA content in wheat plants treated with ACCd aminase producing *Stenotrophomonas maltophilia*. They also stated that the reduction in MDA content in plants exposed to salinity stress is attributed to the ACCd activity due to a reduction in the level of ethylene synthesis.

4. CONCLUSION

In conclusion, from the results it is confirmed that, the ACCd producing PGPR possess dual role in plant growth promotion and withstand high salt stress as observed by the increase in the levels of proline, APX, and SOD and decrease in the levels of MDA content which also are in confirmation with the studies of Sergeeva et al. [31]. The observed results authenticate the significant plant growth promoting properties of ACCd producing PGPR in sunflower and thus allowing them to withstand the salt stress.

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CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology. 2005; 16(2):123–132.
2. Nagar S, Arora A, Singh VP, Ramakrishnan S, Umesh DK, Kumar S, Saini RP. Effect of cytokinin analogues on cytokinin metabolism and stress responsive genes under osmotic stress in wheat. The Bioscan. 2015; 10(1):67–72.
3. Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World Journal of Microbiology and Biotechnology. 2011; 27(5):1231–1240.
4. Singh JS, Pandey VC, Singh, D. P. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems & Environment. 2011; 140(3-4):339–353.
5. Saravanakumar D, Samiyappan R. ACC deaminase from Pseudomonas fluorescens mediated saline resistance in groundnut (*Arachis hypogea*) plants. Journal of Applied Microbiology. 2007; 102(5):1283–1292.
6. Gamalero E, Berta G, Massa N, Glick BR, Lingua G. Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt stress conditions. Journal of Applied Microbiology. 2010; 108(1):236–245.
7. Tiwari G, Duraivadivel P, Sharma S, Hariprasad P. 1-Aminocyclopropane-1-carboxylic acid deaminase producing beneficial rhizobacteria ameliorate the biomass characters of *Panicum maximum* Jacq. by mitigating drought and salt stress. Scientific Reports. 2018;8.
8. Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Research. 2014; 169(1):30–39.

9. Yadav J, Verma JP, Jaiswal DK, Kumar A. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). Ecological Engineering. 2014; 62:123–128.
10. Meena RK, Singh RK, Singh NP, Meena SK, Meena VS. Isolation of low temperature surviving plant growth–promoting rhizobacteria (PGPR) from pea (*Pisum sativum* L.) and documentation of their plant growth promoting traits. Biocatalysis and Agricultural Biotechnology. 2015; 4(4):806–811.
11. Egamberdieva D, Jabborova D, Berg G. Synergistic interactions between *Bradyrhizobium japonicum* and the endophyte *Stenotrophomonas rhizophila* and their effects on growth, and nodulation of soybean under salt stress. Plant and Soil. 2016; 405(1–2):35–45.
12. Kiani MZ, Ali A, Sultan T, Ahmad R, Hydar SI. Plant growth promoting rhizobacteria having 1-aminocyclopropane-1-carboxylic acid deaminase to induce salt tolerance in sunflower (*Helianthus annus* L.). Natural Resources. 2015; 6(06):391.
13. Silva HS, Romeiro RD, Mounteer A. Development of a root colonization bioassay for rapid screening of rhizobacteria for potential biocontrol agents. Journal of Phytopathology. 2003; 151(1):42–46.
14. Ramegowda V, Senthil-Kumar M, Ishiga Y, Kaundal A, Udayakumar M, Mysore KS. Drought stress acclimation imparts tolerance to *Sclerotinia sclerotiorum* and *Pseudomonas syringae* in *Nicotiana benthamiana*. International Journal of Molecular Sciences. 2013; 14(5):9497–9513.
15. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany. 1979; 57(12):1332–1334.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72(1–2):248–254.
17. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 1981; 22(5):867–880.
18. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971; 44(1):276–287.
19. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973; 39(1):205–207.
20. Davenport SB, Gallego SM, Benavides MP, Tomaro ML. Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. Plant Growth Regulation. 2003; 40(1):81–88.
21. Muscolo A, Junker A, Klukas C, Weigelt-Fischer K, Riewe D, Altmann T. Phenotypic and metabolic responses to drought and salinity of four contrasting lentil accessions. Journal of Experimental Botany. 2015; 66(18):5467–5480.

22. Liu JX, Srivastava R, Che P, Howell SH. Salt stress responses in *Arabidopsis* utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. The Plant Journal. 2007; 51(5):897–909.
23. Prochazkova D, Sairam RK, Srivastava GC, Singh DV. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Science. 2001; 161(4):765–771.
24. Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010; 48(12): 909–930.
25. Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. International Journal of Molecular Sciences. 2015; 16(6):13561–13578.
26. Gururani MA, Upadhyaya CP, Baskar V, Venkatesh J, Nookaraju A, Park SW. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in Solanum tuberosum through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. Journal of Plant Growth Regulation. 2013;32(2):245-258.
27. Karthikeyan B, Jaleel CA, Lakshmanan GA, Deiveekasundaram M, Studies on rhizosphere microbial diversity of some commercially important medicinal plants. Colloids and Surfaces B: Biointerfaces. 2008; 62:143–145.
28. Claussen W. Proline as a measure of stress in tomato plants. Plant Science. 2005; 168(1): 241–248.
29. Koca H, Ozdemir F, Turkan I. Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii.* Biologia Plantarum. 2006; 50(4): 745–748.
30. Singh RP, Jha PN. The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. Frontiers in Microbiology. 2017; 8:1945.
31. Sergeeva E, Shah S, Glick BR. Growth of transgenic canola (*Brassica napus* cv. Westar) expressing a bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene on high concentrations of salt. World Journal of Microbiology and Biotechnology. 2006; 22(3):277–282.