*Indian Journal of Ecology (2024) 51(3): 610-617*
*DOI: https://doi.org/10.55362/IJE/2024/4282*

**Manuscript Number: 4282**
**NAAS Rating: 5.38**

# Alleviation of Drought Stress by ACC (1-amino cyclopropane -1-carboxylate) Deaminase Producing Plant Growth Promoting Rhizobacteria Isolates in *Capsicum annum.* L

**Srinivasa Rao M. and Kishore N.***

*Department of Microbiology, Palamuru University Mahabubnagar, Telangana-509 001, India*
**E-mail: kishore_micro2003@yahoo.co.in*

***Abstract*:** Plant growth promoting rhizobacteria (PGPR) with multiple PGP traits and ACC deaminase activity have additional advantage to mitigate drought stress. Strains with these characters and resistant to drought conditions could be hypothesized to be efficient isolates to overcome drought stress. The present study shows the ability of two such isolates IS-7 (*Bacillus halotolerans*) and IS-74 (*Enterobacter hormaechei*) to help the capsicum plants sustain even severe drought conditions (12.5% WHC). Comparatively, both the isolates were able to make the plant sustain drought but with decreased shoot and root wet and dry weights, root volume, root shoot ratio, leaf number and chlorophyll content. However, significant difference was not observed between control plant without drought and inoculated + drought induced plants at 50% WHC. Control plants subjected to drought and without isolates could not survive 50% WHC drought severity. Plant responses to defend against reactive oxygen species (ROS) was more accelerated in the activity of enzymatic (increased -SOD, APOX, GPOX, MDA and decreased- GR and NR) and non-enzymatic (proline) antioxidants. Elevated morphological and biochemical status indicate the positive effect of the isolates compared to control without treatment.

***Keywords*:** ACC deaminase, PGPR, Drought stress, Antioxidants, *Capsicum annum*

Agricultural production has been a major challenge owing to the significant changes in climatic conditions like drought, salinity, modified monsoon and global warming (Chen et al 2014). Consequently, plants undergo abiotic and biotic stress, among which drought and salinity greatly effect on growth and development of plants in arid and semi-arid regions (Mohammadizad et al 2013). Among various environmental stresses drought stress stands first in reducing plant growth and productivity. Different studies indicate that the global population may increase by 9.2 billion by 2050 and food demand may further increase in the next decades (Silva 2018). To address the said need modern, sophisticated and sustainable agricultural strategies are required. For the last three decades, usage of commercial fertilizers and pesticides has been very intense causing adverse effects on human health and the environment (Hahn 2014).

During drought stress, plants are negatively affected, leading to changes in morphology and physiology due to the generation of reactive oxygen species (ROS) (Hossain and Dietz 2016, Chandra et al 2019) and an increased oxidative damage. To counteract this, plants developed defence using enzymatic - superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), nitrate reductase (NR), malondialdehyde (MDA) etc. and non-enzymatic protectants (proline, cysteine, glutathione etc.) as evident from recent studies, adverse effects of drought on plants is mitigated by the use of PGPR having ACC deaminase activity (Tiwari et al 2018, Singh et al 2019). Along with oxidative damage, an increased production of ethylene is observed in plants during stress leading to damage to plants. The negative effects of drought stress (ethylene production and oxidative damage) can be bypassed by the use of ACC deaminase-producing PGPR which can degrade the ACC to α-ketobutyrate and ammonia to reduce levels of ethylene and increase the non-enzymatic and enzymatic antioxidant machinery in the plant. Hypothetically, PGPR is the way to use as a biofertilizer and bio inoculant to alleviate drought stress and meet global food needs (Glick 2014). Recently, *Bacillus* and *Enterobacter* species are reported for their ability in tolerating extreme environments, acting on phytopathogens and as plant growth promoters (Tzipilevich et al 2021, Ajibade et al 2023). Chilli (*Capsicum annuum.* L) is a crop that grows in the tropical regions of America and later spread across the world. Chilli, the hot pepper is one of the most valuable commercial crops grown in India. Due to its long history of cultivation, out-crossing nature and popularity of the crop, large genetic diversity including local landraces has evolved in India. Chilli is mostly grown under rain-fed cultivation and the crops are often affected by low moisture stress leading to inconsistent yield and quality. Hence, the present study is mainly focused

on the effect of PGPR isolates *Enterobacter hormaechei*, and *Bacillus halotolerans* on the growth of chilli under various drought severities.

## MATERIAL AND METHODS

**Screening of PGPR bacteria:** The rhizosphere soils of chilli were collected from five different locations in Jadcherla Taluka (16.769968" N, 78.148212" E), Mahabubnagar district of Telangana State, India. The soil samples were carefully taken into sterilized plastic covers, brought to the laboratory, and stored at 4°C. From these soils pure cultures were isolated on Cr-YEMA, Jensens's and Kings-B media and screened for PGPR traits, production of Indole acetic acid (IAA), Phosphate solubilisation, siderophore activity, HCN production, Gibberellic acid production, Nitrogen fixation and ACC deaminase activity (Khalid et al 2004, Kumar et al 2012).

**Screening of drought tolerant bacteria:** Isolates exhibiting a greater number of PGPR traits, including ACC-Deaminase activity, were evaluated for their capacity to grow in the presence of 40% PEG-6000. The log phase cultures were introduced into nutrient agar supplemented with varying concentrations of PEG-6000 (10, 20, 30 and 40%). The optical density at 600nm was subsequently measured after 24 hours of incubation.

**Molecular characterization:** The selected bacteria obtained in pure form were cultivated in nutrient broth under controlled conditions (30-32°C for 24 hours). Genomic DNA was extracted from the cultures using the Genomic DNA Extraction kit (Thermo-scientific), following the standard protocol. Subsequently, a 16S rRNA gene fragment of approximately 1500 base pairs was amplified through PCR using universal primers. The resulting sequences were then subjected to a BLAST search in the NCBI (https://www.ncbi.nlm.nih.gov/) database for phylogenetic identification.

**Experimental details:** The chilli seeds were surface sterilized using 75% ethyl alcohol (1 min), 1% sodium hypochlorite (30 secs) and washed in sterile water for 5-6 times. A bacterial broth culture of selected isolates ($5 \times 10^6$ CFU) was obtained and mixed with sterile seeds along with CMC as adhesive and was air dried. After seed germination in pots (15 cm) filled with 4 kg of sterile red loamy soil, pots were watered regularly until they reached four leaf stages. Later a greenhouse-based factorial experiment was conducted using a completely randomized block design, with each treatment replicated three times. The experimental treatments consisted of varying soil water holding capacities (WHC) at 75, 50, 25 and 12.5%. The soil's WHC had been previously determined following established procedures (Nejad et al 2023). Two control groups were established: Control 1, which involved no PGPR and drought stress, and control 2, which included no PGPR application and without drought stress.

**Measurement of morphological characters:** Following the harvest, an array of morphological characters, including shoot length, root length, leaf count, leaf area, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, and root volume, were carefully measured. To determine the dry weights, plant samples were subjected to drying in a hot air oven at a temperature of 70°C for a duration ranging from 24 to 48 hours, ensuring complete desiccation.

**Estimation of chlorophyll:** One gram of fresh leaf sample was cut into small discs and placed in a test tube containing 5 mL of dimethyl sulfoxide (DMSO). After incubation, the absorbance was measured using a spectrophotometer at wavelengths 645 nm and 663 nm, with DMSO serving as the blank (Arnon 1949).The calculations for chlorophyll content were performed as follows

- Chlorophyll a (mg/g) = (12.7 × A663) - (2.59 × A645)
- Chlorophyll b (mg/g) = (22.9 × A645) - (4.7 × A663)
- Total chlorophyll (mg/g) = (8.2 × A663) + (20.2 × A645)

**Soluble carbohydrates:** Homogenate one gram of fresh leaves was made using a mixture of ethanol, chloroform, and water in a ratio of 60:25:15 (v/v). The resulting mixture was incubated at 60°C for duration of 2 hours. Subsequently, the samples were subjected to centrifugation at 10,000 rpm for 30 minutes to separate the components. A 0.2 mL portion of the supernatant was then adjusted to a total volume of 1 mL with distilled water and combined with 1 mL of a 5% phenol solution and 5 mL of 96% $H_2SO_4$. Soluble carbohydrates were determined against glucose standard curve at 490 nm (Dubois et al 1951).

**Relative water content (RWC) :** In a clean Petri plate take one gram of finely cut fresh leaves (circular discs) were immersed in 25 mL of distilled water for a 6-hour period and then the leaf samples were gently dried by blotting and weighed. Subsequently, the leaves were subjected to oven drying at 70°C for 24 hours, and their final weight was documented (González and González 2001).

RWC = (Fresh Weight - Dry Weight) / (Saturated Weight - Dry Weight) × 100.

**Ammonia production:** In the tubes containing freshly grown bacterial cultures (24hrs), 0.5 mL of Nessler's reagent was added. The presence of a light-yellow colour (+) and a deep yellow to brown colour (++) indicate the production of ammonia (Dye 1962).

**Protease production:** Protease production was assessed through a spot inoculation method, where log-phase bacterial cultures were individually placed on Skim Milk Agar

(SMA) medium. This medium comprised 5.0 grams of casamino acids, 2.5 grams of yeast extract, 1.0 gram of glucose, 100 millilitres of skim milk solution, and 15 grams of agar-agar, all adjusted to a pH of 7.0. The inoculated plates were then incubated for a period of 4-5 days at a temperature of 30°C. Identification of bacterial isolates capable of producing protease was achieved by observing the formation of a halo zone surrounding the bacterial colony (Maurhofer et al 1994).

**β – 1, 3 – Glucanase assay:** The β-glucanase activity of specific bacterial isolates was assessed through the utilization of β-glucan agar, following the plate method with Congo red staining. The presence of a distinct halo zone surrounding the bacterial colony was an indication of β-glucanase production (Teather and Wood 1982).

**Chitinase production:** For the chitinase enzyme assay, a reaction mixture that consists of 0.25 ml of the supernatant (enzyme source), 0.3 ml of sodium acetate buffer at pH 5.3, and 0.5 ml of 0.1% colloidal chitin was made. After incubation of this reaction mixture in a water bath at 50°C for 4 hours, 1 ml of a DNS reagent was added. The mixture was boiled and 1 ml of a 40% Rochelle salt (sodium potassium tartrate) solution was added and cooled in running tap water. The development of a purple colour in the reaction mixture signifies the presence of reducing sugars and, consequently, the presence of chitinase enzyme (Legrand et al 1987).

**Total proline:** The homogenate was made with one gram of fresh leaves, 10 ml of 3% sulfosalicylic acid and centrifuged at 10000 rpm for 10 min at 4°C. To 1ml supernatant 2 ml of each ninhydrin, glacial acetic acid was added and incubated at $100^0$ C for 1 hr. The contents were cooled immediately and 4 ml of toluene was added and incubated in dark (20 min). Optical density was read at 520nm and proline content was estimated using a proline standard curve (Bates et al 1973)

**Lipid peroxidation or malondialdehyde (MDA):** One gram of fresh leaves was ground and then mixed with 10 ml of a solution containing 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The resulting mixture was subjected to incubation in a water bath at 95°C for 30 minutes and was then immediately transferred to an ice bath to cool. After cooling, the mixture was centrifuged at 10,000 rpm for 15 minutes at 25°C to separate the solution. The optical density of this solution was measured at two wavelengths, specifically at 532 nm and 600 nm (Davenport et al 2009).

$$\text{MDA (µ moles/g of FW)} = \frac{\text{Abs 532} - \text{Abs 600)}}{\text{Extinction coefficient}} \times 1000$$

**Evaluation of Antioxidant enzyme activities:** To assess superoxide dismutase (SOD), the method established by Beauchamp and Fridovich (1971) was employed. As for ascorbate peroxidase (APX), the protocol developed by Nakano and Asada (1981) was used. Guaiacol peroxidase was quantified by the methodology described by Castillo et al (1984).

**Catalase activity:** Enzyme extract (0.1 ml) was mixed with 100 mM phosphate buffer (pH 7.0) and 0.5 ml of a 75 mM hydrogen peroxide ($H_2O_2$) solution. The reaction mixture was completed by adding 950 µl of distilled water. Subsequently, the optical density (OD) at 240 nm was measured (Aebi 1974).

**Nitrate reductase activity:** Plant leaf material (0.5 grams) was ground in 1 ml of a 50 mM potassium phosphate buffer (pH 8.0) containing 1 mM EDTA, 25 mM cysteine, and 3% (w/v) BSA. Enzyme sample was obtained from the supernatant after centrifugation (12,000 rpm, 20 minutes, 40°C). The reaction mixture was made with 200 µl of 50 mM potassium nitrate, 200 µl of 0.5 mM NADH, 400 µl of the enzyme extract, and 1,200 µl of 50 mM potassium phosphate buffer (pH 7.0) and incubated for 15 minutes at 25°C. Absorbance of the pink coloured complex formed was measured at 540nm (Hageman and Hucklesby 1971)

**Glutathione reductase activity:** Mixture of 10 mM potassium phosphate buffer, 0.5 mM 5, 5 dithiobis-2-nitrobenzoic acids (DTNB), 2.0 mM NADPH, 0.33 mM EDTA, 0.1 ml of enzyme extract, and 20mM GSSG (oxidised glutathione) was made. Double distilled water was added to make up the solution to 3 ml. Optical density was read at 412 nm after development of red colour in tubes (Gutteridge and Halliwell, 2000).

**Statistical analysis:** The data were subjected to statistical analysis using Fischer's one-way with the help of SPSS software version 24.

## RESULTS AND DISCUSSION

The rhizosphere soils of capsicum collected from five different locations from which fifteen isolates were obtained in pure culture and were evaluated for PGPR traits (Table 1). The presence of multiple traits are being recently studied for selecting efficient isolates (Singh et al 2010, Anuradha et al 2022). Among the fifteen isolates, IS-7, IS-74 expressed more number of PGP traits than other strains. In the present study, presence of more number of PGPR traits was considered as criterion for selecting efficient PGPR isolate.

The selected isolates with PGPR traits were further evaluated for drought tolerance by growing in a media amended with PEG-6000 at 10, 20, 30 and 40%. Isolates able to grow in the presence of different concentrations of amended PEG 6000 are considered to be resistant to drought stress (Michel and Kaufmann 1973). All the isolates

could grow at 40% PEG except IS-8 and IS-91 (Table 2). Isolates IS-7, IS-93, IS-27 and IS-74 were showing more tolerance and higher OD. Even though, IS-93 and 27 were tolerant than IS-74, owing to the number of PGPR traits as innate character, IS-7 and IS-74 were selected for further study and were found to be efficient pant growth promoters in chilli plant experiment .

The selected isolates, IS-7 and IS-74 were further evaluated for their ability to express PGPR traits even in presence of 40% PEG 6000 amended media. Under stressed conditions isolates may lose the ability to show the required PGPR trait (Ahmad et al 2022). Both the isolates could express the traits at 40% PEG. With the increase in PEG concentration, there is a decrease in quantitative production of IAA, 'P' solubilisation and ACC deaminase activity. Compared to IS-74, IS-7 was more efficient at 40% PEG (Table 3). IS-7 could retain the ability of siderophore and glucanase production even at 40% PEG but lost the ability to produce HCN whereas, IS-74 couldn't retain the ability to show siderophore, ammonia production, protease and glucanase activity at 40% PEG (Table 4).

Both selected PGPR isolates (IS-7 and IS-74) were experimentally analysed for their ability to ameliorate different drought severities (Table 5). Both the isolates could make the plant sustain even severe drought conditions (12.5 % WHC) whereas, control plant subjected to drought and without PGPR inoculation couldn't survive 50% WHC drought. There was significant decrease in plant growth parameters such as shoot/root length, fresh and dry weights and root volume is observed in plants stressed with drought stress conditions. This could be due to variations in physio biochemical levels because of water limitation. Similar observations were made in plants like *Helianthus annus* L (Abdel Razik et al 2021) and *Brassica napus* (Shafiq et al

**Table 1.** Characterization of PGPR traits of selected isolates from rhizosphere soils of *Capsicum annum*. L

| Area | Media | Isolates No. | IAA | Siderophore | N2Fixation | P' solubilization | HCN | GA | ACC deaminase |
|---|---|---|---|---|---|---|---|---|---|
| Avancha | CR-YEMA | IS-7 | + | + | -- | + | + | -- | + |
| | Jensen | IS-8 | -- | + | -- | -- | -- | -- | + |
| | Kings - B | IS-9 | + | + | -- | + | -- | -- | -- |
| Gangapur | CR-YEMA | IS-25 | -- | + | -- | + | -- | -- | + |
| | Jensen | IS-26 | + | + | + | -- | -- | -- | + |
| | Kings - B | IS-27 | -- | + | -- | -- | -- | -- | + |
| Rangareddyguda | CR-YEMA | IS-73 | -- | + | -- | -- | -- | -- | + |
| | Jensen | IS-74 | + | + | - | + | -- | -- | + |
| | Kings - B | IS-75 | + | + | -- | -- | -- | -- | -- |
| Alwanpally | CR-YEMA | IS-79 | -- | -- | -- | -- | -- | -- | -- |
| | Jensen | IS-80 | + | + | + | - | -- | + | + |
| | Kings - B | IS-81 | -- | -- | -- | -- | -- | -- | -- |
| Thimajipet | CR-YEMA | IS-91 | + | + | -- | -- | -- | -- | + |
| | Jensen | IS-92 | -- | -- | + | -- | -- | -- | + |
| | Kings - B | IS-93 | + | + | -- | -- | + | -- | + |

**Table 2.** Optical density values of different isolates at 600nm were measured in PEG-6000 amended nutrient broth

| Isolate No | Polyethyleneglycol | | | | |
|---|---|---|---|---|---|
| | 0% | 10% | 20% | 30% | 40% |
| IS-7 | 1.029 | 0.973 | 0.457 | 0.273 | 0.098 |
| IS-8 | 1.637 | 0.891 | 0.515 | 0.236 | 0.000 |
| IS-26 | 1.207 | 0.732 | 0.463 | 0.287 | 0.007 |
| IS-27 | 0.729 | 0.532 | 0.376 | 0.220 | 0.058 |
| IS-74 | 1.432 | 0.826 | 0.441 | 0.276 | 0.024 |
| IS-80 | 0.963 | 0.641 | 0.473 | 0.258 | 0.012 |
| IS-91 | 1.405 | 1.200 | 0.863 | 0.187 | 0.005 |
| IS-93 | 1.000 | 0.993 | 0.429 | 0.170 | 0.087 |

2014). In chilli total biomass reduction is due to decrease in turgor pressure (Chuyong and Acidri 2017). In general, there was not much significant difference between various morphological characters studied and control (un inoculated and no drought) up to 50% WHC severity which indicates that both the isolates could make the plant survive without much damage up to 50% WHC. The low shoot root ratio indicates higher transpiration and a higher ratio indicates more absorption of nutrients from soil and thereby increase shoot biomass and probably increase plant resistance to drought. This ratio is proportional to nutrient supply and fertilization (Kang and Van Iersel 2004). In the present study higher root shoot ratios were observed in plants treated with IS-7 compared to IS-74.

With the increase of drought severity there was decrease in the parameter studied (Table 6). Organic carbon is stored in the form of total soluble sugars which stands as an important feature for maintaining osmotic potential, plays a role in osmotic regulation and maintains osmotic pressure. Plants that accumulate more of the total soluble sugars in response to drought stress effectively regulate osmosis (Slama et al 2007). Chlorophyll a, b, total chlorophyll, total soluble carbohydrates and relative water content (RWC) significantly decreased compared with control at 100% WHC. However, inoculation of IS-7 and IS-74 could maintain adequate amounts of above said parameters even at severe drought (12.5% WHC) and sustain growth. Similar results were observed in *Zea mays*, where PGPR could increase the photosynthetic pigments compared to un inoculated control under drought stress (Yasmin et al 2017).

Drought stress triggers several complex mechanisms in order to defend from adversities caused by the ROS generated. Many protective and defensive physiological systems like scavenging of excessive ROS, production of low molecular weight nitrogenous compounds etc. are important in minimizing deleterious effects. Effective destruction of excess ROS require synchronous activity of many antioxidant enzymes (Gul et al 2022). SOD is a strong oxidant which oxidizes thiol groups in to OH radicals. Hydroxyl radicals are further converted to water and molecular oxygen by catalase and peroxisomes. Mittler (2002) has reported that dynamic levels of ROS are maintained in plants because of balance between SOD, POX and CAT etc. Enzymatic antioxidants like APOX, GPOX, SOD, CAT, MDA and non-enzymatic proline was estimated to understand the role of isolates in overcoming adversities from ROS (Table 6). In general, with the increase in drought there was an increase in quantities of all the enzymes possibly to overcome stress injury indicating the positive role of isolates inoculated. In contrast to this NR and GR decreased. Accumulation of proline and antioxidant enzymes in drought stressed plants was also recently reported in sweet pepper (Iqbal et al 2023). Similarly, enzyme activity of APOX, GPOX, SOD, MDA increased at the highest severity of water deficit and this was more seen in plants treated with IS 7 than IS 74 except for CAT activity. The degree of peroxidation of membrane lipids due to ROS is reflected in by product MDA (Lacan and Baccou 1998). Studies have shown that the levels of MDA increased with the increase in ROS in drought stressed phenotypes (Soureshjani et al 2019). Both GR and NR can be important indicators of metabolic and physiologic status of plant in water deficit conditions. Balance of redox potential in cells is achieved by the activity of GR which catalyse the conversion of reduced and oxidized glutathione and in turn helps in scavenging ROS and protect plant (Guo et al 2018). Nitrate reductase activity is involved in nitrate assimilation; plant acquisition of mineral nutrients helps in neutralizing adverse effects of drought stress (Caravaca et al 2003). In the present study decreased activity was observed even in higher severities, which may be due to decreased stress by the activity of inoculated isolates.

**Table 4.** PGPR properties at PEG 40%

| PGPR traits | IS - 7 | | IS - 74 | |
|---|---|---|---|---|
| | Control | 40% PEG | Control | 40% PEG |
| HCN production | + | -- | -- | -- |
| Siderophore production | + | + | + | -- |
| Ammonia production | -- | -- | + | -- |
| Chitinase activity | -- | -- | -- | -- |
| Protease activity | -- | -- | + | -- |
| β-1,3 glucanase activity | + | + | + | -- |

**Table 3.** PGPR properties at PEG 40%

| PGPR traits | IS- 7 | | | IS- 74 | | | CD at 0.05 | CD at 0.01 |
|---|---|---|---|---|---|---|---|---|
| | Control | 30 % PEG | 40% PEG | Control | 30% PEG | 40% PEG | | |
| Indole acetic acid (mg/ml) | 13.68 | 5.12 | 0.22 | 10.86 | 4.27 | 0.79 | 5.44 | 7.53 |
| P - solubilization (mg/100 ml $P_2O_5$) | 12.84 | 1.76 | 0.21 | 8.62 | 1.26 | 0.04 | 5.35 | 7.40 |
| ACC deaminase activity in µmol α- KBA (mg/h) | 8.52 | 4.25 | 1.02 | 9.34 | 5.12 | 2.12 | 3.36 | 4.65 |

**Table 5.** Effect of selected PGPR isolates for plant growth promotion in *Capsicum annum*. L subjected to drought stress

| Isolate No. | WHC % | Shoot length (mm) | Root length (mm) | Shoot Wt. Wt. (gm) | Shoot dry Wt. (gm) | Root Wt Wt. (gm) | Root dry Wt. (gm) | Root volume (cm3) | Root shoot ratio |
|---|---|---|---|---|---|---|---|---|---|
| IS-7 | 75 | 30.62$^{a}$ | 15.62$^{a}$ | 58.6$^{bc}$ | 26.32$^{b}$ | 8.23$^{ab}$ | 3.98$^{a}$ | 0.31$^{ab}$ | 0.15 (22.79)$^{a}$ |
| | 50 | 20.42$^{c}$ | 12.88$^{b}$ | 52.82$^{bc}$ | 21.81$^{b}$ | 6.87$^{bc}$ | 3.02$^{b}$ | 0.24$^{b}$ | 0.13 (21.13)$^{ab}$ |
| | 25 | 19.86$^{c}$ | 10.26$^{c}$ | 45.21$^{c}$ | 20.16$^{b}$ | 5.23$^{c}$ | 2.81$^{b}$ | 0.18$^{bc}$ | 0.13 (21.13)$^{ab}$ |
| | 12.5 | 18.42$^{c}$ | 9.74$^{c}$ | 22.94$^{d}$ | 9.72$^{c}$ | 4.28$^{c}$ | 1.74$^{c}$ | 0.10$^{c}$ | 0.17 (24.35)$^{a}$ |
| IS-74 | 75 | 29.64$^{a}$ | 12.41$^{b}$ | 65.66$^{ab}$ | 30.12$^{b}$ | 9.64$^{a}$ | 3.34$^{ab}$ | 0.25$^{b}$ | 0.11 (19.37)$^{b}$ |
| | 50 | 26.72$^{ab}$ | 11.86$^{bc}$ | 62.02$^{bc}$ | 29.44$^{b}$ | 8.2$^{ab}$ | 3.02$^{b}$ | 0.20$^{bc}$ | 0.1 (18.43)$^{b}$ |
| | 25 | 20.46$^{c}$ | 10.22$^{c}$ | 54.68$^{b}$ | 23.62$^{b}$ | 5.29$^{bc}$ | 2.08$^{bc}$ | 0.12$^{c}$ | 0.08 (16.43)$^{b}$ |
| | 12.5 | 18.86$^{c}$ | 8.64$^{c}$ | 24.25$^{d}$ | 10.86$^{c}$ | 3.14$^{c}$ | 1.16$^{c}$ | ND | 0.1 (18.43)$^{b}$ |
| Control | FC | 32.68$^{a}$ | 12.94$^{b}$ | 80.74$^{a}$ | 39.88$^{a}$ | 8.68$^{ab}$ | 3.92$^{ab}$ | 0.38$^{a}$ | 0.09 (17.46)$^{b}$ |
| CD at 0.05 | | 5.53 | 2.08 | 18.29 | 9.23 | 2.18 | 0.94 | 0.12 | 2.53 |
| CD at 0.01 | | 7.49 | 2.81 | 24.79 | 12.50 | 2.95 | 1.27 | 0.16 | 3.43 |

*Values are significant at P<0.05 as per Fisher's test, values super scribed by same alphabet are not significantly different at P<0.05, Values in the parenthesis are arc sin transformed

**Table 6.** Effect of selected PGPR isolates for plant growth promotion in *Capsicum annum*. L subjected to drought stress

| Isolate No. | WHC % | No. of leaves ($Plt^{-1}$) | Leaf area ($mm^2$) | Chlorophyll A (mg $g^{-1}$ FW) | Chlorophyll B (mg $g^{-1}$ FW) | Total chlorophyll (mg $g^{-1}$ FW) | Total soluble carbohydrates (mg $g^{-1}$ DW) | Relative water content (RWC) |
|---|---|---|---|---|---|---|---|---|
| IS-7 | 75 | 20.64$^{b}$ | 1798.62$^{b}$ | 18.36$^{ab}$ | 1.94$^{a}$ | 42.88$^{ab}$ | 0.49$^{a}$ | 52.96$^{a}$ |
| | 50 | 19.82$^{bc}$ | 1424.26$^{bc}$ | 12.42$^{c}$ | 1.86$^{a}$ | 30.76$^{b}$ | 0.34$^{bc}$ | 30.42$^{bc}$ |
| | 25 | 13.64$^{c}$ | 1064.22$^{bc}$ | 9.78$^{cd}$ | 1.34$^{b}$ | 17.74$^{bc}$ | 0.3$^{bc}$ | 29.92$^{bc}$ |
| | 12.5 | 9.82$^{c}$ | 746.86$^{c}$ | 6.84$^{d}$ | 1.08$^{b}$ | 10.86$^{c}$ | 0.23$^{c}$ | 17.84$^{c}$ |
| IS-74 | 75 | 27.78$^{a}$ | 2964.32$^{a}$ | 19.38$^{a}$ | 1.66$^{ab}$ | 46.76$^{a}$ | 0.5$^{a}$ | 51.68$^{a}$ |
| | 50 | 20.42$^{bc}$ | 1924.82$^{b}$ | 13.64$^{c}$ | 1.09$^{b}$ | 38.42$^{ab}$ | 0.42$^{b}$ | 42.24$^{b}$ |
| | 25 | 16.68$^{bc}$ | 1730.2$^{bc}$ | 9.86$^{cd}$ | 0.94$^{bc}$ | 18.74$^{bc}$ | 0.37$^{b}$ | 23.78$^{c}$ |
| | 12.5 | 12.14$^{c}$ | 842.16$^{bc}$ | 7.64$^{d}$ | 0.53$^{c}$ | 12.46$^{c}$ | 0.34$^{bc}$ | 21.48$^{c}$ |
| Control | FC | 28.24$^{a}$ | 4024.12$^{a}$ | 14.42$^{bc}$ | 1.8$^{ab}$ | 51.86$^{a}$ | 0.56$^{a}$ | 60.95$^{a}$ |
| CD at 0.05 | | 6.58 | 1033.18 | 4.31 | 0.50 | 14.59 | 0.13 | 15.18 |
| CD at 0.01 | | 8.91 | 1400.10 | 5.84 | 0.67 | 19.78 | 0.18 | 20.57 |

* Figures with same alphabet are not significantly different at P<0.05

**Table 7.** Effect of selected PGPR isolates for plant growth promotion in *Capsicum annum*.L subjected to drought stress

| Isolate No. | WHC % | APOX (Unit $mg^{-1}$ protein) | GPOX (Units $mg^{-1}$ protein) | SOD (Units $mg^{-1}$ protein) | catalase (Units $mg^{-1}$ protein) | MDA (n mol $g^{-1}$FW) | NR ($mgN0_2\ g^{-1}\ h^{-1}$) | GR (m mol NADPH $min^{-1}g^{-1}$FW) | Total Proline ($uMg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| IS-7 | 75 | 12.68$^{cd}$ | 0.42$^{c}$ | 152$^{c}$ | 1.57$^{c}$ | 18.8$^{b}$ | 1876$^{ab}$ | 5$^{ab}$ | 5.2$^{cd}$ |
| | 50 | 14.34$^{c}$ | 0.57$^{ab}$ | 176$^{bc}$ | 1.84$^{bc}$ | 21.9$^{b}$ | 1042$^{bc}$ | 4.2$^{ab}$ | 6.24$^{c}$ |
| | 25 | 18.26$^{a}$ | 0.64$^{ab}$ | 204$^{bc}$ | 2.32$^{ab}$ | 32.6$^{ab}$ | 987$^{bc}$ | 2.8$^{b}$ | 7.04$^{ab}$ |
| | 12.5 | 20.42$^{a}$ | 0.73$^{a}$ | 274$^{a}$ | 2.6$^{ab}$ | 35.2$^{a}$ | 727$^{c}$ | 1.2$^{c}$ | 7.52$^{a}$ |
| IS-74 | 75 | 11.68$^{d}$ | 0.39$^{c}$ | 148$^{c}$ | 1.34$^{c}$ | 18.4$^{b}$ | 1465$^{b}$ | 4.6$^{ab}$ | 5.16$^{cd}$ |
| | 50 | 12.94$^{c}$ | 0.5$^{b}$ | 190$^{bc}$ | 1.86$^{b}$ | 22.7$^{b}$ | 935$^{bc}$ | 3.4$^{b}$ | 5.88$^{bc}$ |
| | 25 | 14.66b$^{c}$ | 0.62$^{ab}$ | 224$^{ab}$ | 2.14$^{b}$ | 26.2$^{b}$ | 867$^{c}$ | 1.8$^{bc}$ | 6.42$^{b}$ |
| | 12.5 | 17.24$^{ab}$ | 0.68$^{a}$ | 268$^{a}$ | 2.76$^{a}$ | 30.4$^{ab}$ | 625$^{c}$ | 0.9$^{c}$ | 6.89$^{ab}$ |
| Control | FC | 9.28$^{d}$ | 0.23$^{c}$ | 132$^{c}$ | 1.13$^{c}$ | 16.2$^{b}$ | 2426$^{a}$ | 5.9$^{a}$ | 4.62$^{d}$ |
| CD at 0.05 | | 3.41 | 0.17 | 50 | 0.56 | 6.59 | 579 | 1.74 | 0.99 |
| CD at 0.01 | | 4.61 | 0.23 | 68 | 0.76 | 8.94 | 785 | 2.36 | 1.34 |

* Figures with same alphabet are not significantly different at P<0.05

Proline is reported to act as an OH radical scavenger, as a solute to protect macromolecules and reduces acidity in cells (Mundada et al 2021). With the gradual increase in drought, proline content also increased and this was more pronounced in IS-7 compared to IS-74. Accumulation of more soluble total sugars and proline could be responsible to less stress injury owing to evolving a mechanism to maintain favourable water gradient and water entry in roots (Bouremani et al 2023). The pure cultures of IS-7 and IS-74 subjected for genomic DNA isolation for 16s r RNA characterization and sequence data was obtained with the help of universal primers. The obtained sequences are set for BLAST analysis in NCBI website and established phylogenetic relationship IS-7 as *Bacillus halotolerans* (Accession No. OR593309) and IS-74 as *Enterobacter hormaechei* (Accession No. OR593312).

## CONCLUSIONS

Plant growth promoting isolates with ACC deaminase activity is an added advantage to mitigate drought stress conditions. The presence of multiple PGPR traits helped the plants for plant growth promotion and helping the plant overcome negative effects by way of acquiring nutrients and maintaining proper balance of redox potentials. The selected isolates, IS-7 (*Bacillus halotolerans*) and IS-74 (*Enterobacter hormaechei*) were found to ameliorate deleterious effects of drought stress even under 12.5% WHC of pot soil which is remarkable. Both the isolate were copious producers of exopolysaccharides which helped in retaining water for longer periods and requires further evaluation. Both the isolates were found to be efficient for sustenance of capsicum under severe drought conditions at green house experiments. However, field level evaluation experiments are underway to determine their full potential.

## ACKNOWLEDGEMENT

The authors are thankful to the Vice Chancellor, Palamuru University and head department of microbiology for providing necessary facilities and grants and constant support to accomplish these results.

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*Received 22 November, 2023; Accepted 30 April, 2024*