***Original Research Article***

# WATER STRESS AMELIORATION AND PLANT GROWTH PROMOTION IN CAPSICUM PLANTS BY OSMOTIC STRESS TOLERANT BACTERIA


## ABSTRACT

The present study was initiated with testing of fifteen previously isolated indigenous plant growth promoting rhizobacteria for drought tolerance. Among all, two best isolates *Pseudomonas aeruginosa* ($JHA_6$) and *Bacillus amloliquefaciens* ($ROH_{14}$) were selected for in-vivo studies. A total of ten treatments comprising PGPR ($JHA_6$ and $ROH_{14}$) inoculated plants held at 80%, 60% and 40% field capacity (FC) soil moisture level was laid down in Completely Randomized Design with three replications. Un-inoculated plants held at various stress levels and non-stressed conditions (100% FC) served as control. In general, both the bacteria could promote capsicum growth in terms of increase in root and shoot biomass, height of plants, chlorophyll content as well as increase in nutrient content and uptake. Besides, the bacterial inoculated capsicum plants could withstand water stress more efficiently as indicated by increases in leaf area, total soluble proteins and relative water content of treated water stressed plants in comparison to untreated stressed ones. Enhanced antioxidant responses were evident as elevated activities of enzymes such as superoxide dismutase, catalase and peroxidase was recorded. Therefore, the ability of capsicum plants to withstand water stress is enhanced by application of the isolated bacteria which also function as plant growth promoting rhizobacteria.



**Keywords:** PGPR, drought, superoxide dismutase, peroxidase, catalase, relative water content.


## INTRODUCTION

With rapid increase in population, projected to be 9.7 billion by 2050, worldwide food production needs to be significantly increased to gear up for meeting demands on food in the coming years. This is extremely important in the Indian context as India's population is predicted to reach a staggering 1.7 billion by 2050 (Chakraborty et al.

2013). Abiotic stresses are considered to be the main source of plant growth stagnation or reduction in crop productivity. Water stress is perhaps the single most important limiting factor for crop production in many parts of the world (Passioura 2007).

A water deficit causes diminished water potential and turgor loss which results in stomatal closure, decline in the rate of photosynthesis, disruption of membrane integrity, protein denaturation and osmotic stress (Alcazar et al. 2011; Mi-Seon Hahm et al. 207). It also induces the generation of active oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals, which causes oxidation of lipids and proteins, chlorophyll bleaching, damage to nucleic acids, ultimately leading to cell death (Ashraf 2009).

Plants develop self defense mechanisms against stress induced adverse effects by producing antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase (Abdel 2011) and non-enzymatic antioxidants like cellular redox buffers, carotenoids, flavonoids, tocopherols, ascorbate, glutathione etc. (Amirjani 2012). In spite of the tolerance mechanisms of the plant including enhanced anti-oxidative activities, there is a significant reduction in yield due to the disturbed metabolic processes following water stress (Gill and Tuteja 2010).

In order to maintain or increase crop productivity it becomes necessary to evolve efficient low-cost technologies for abiotic stress management. It is now a priority area research for developing strategies to cope with abiotic stresses including development of stress tolerant varieties, shifting crop calendars, resource management practices etc (Venkateswarulu and Shanker 2009). However, most of these techniques are cost-intensive and time taking. Inoculation of plants with soil microorganisms can amplify productivity of crops under a drought stress environment (Chanway and Holl 1994). If such microorganisms have the ability to promote growth, they become extra beneficial. Plant growth promoting rhizobacteria (PGPR) mitigates the impact of drought stress on plants through a process called rhizobacterial-induced drought endurance and resilience (RIDER), which includes physiological and biochemical changes. Various RIDER mechanisms include modification in phytohormonal levels, antioxidant defense, bacterial exopolysaccharides (EPS) and accumulation of several compatible organic solutes like sugars, amino acids, polyamines etc. (Kaushal and Wani, 2015). Production of heat-shock proteins (HSPs), dehydrins and volatile organic compounds (VOCs) also plays significant role in the acquisition of drought tolerance (Rego MCF 2018). Increased length of lateral root as well as density and length of root hairs with PGPR strain led to a greater

exchange surface area with soil and thus a higher water flux through the whole root system up to the leaves of the plant (Kechid et al. 2013). PGPR increased the active accumulation of organic and inorganic maintain cellular turgor and help plants lower water potential without decreasing actual water content, thereby, protects enzymes, proteins, cellular organelles and membranes against oxidative damage and helps plants tolerate drought-induced damage (Huang et al. 2014; Khan et al. 2019). The most predominant rhizosphere colonizing bacteria belongs to *Pseudomonas* and *Bacillus* species because of their association with soil organic matter, nutritional diversity and rapid growth rate (Vijayapal et al. 1998).

Capsicum (*Capsicum annum* L.; Solanaceae) is a remunerative crop in India and cultivated in greenhouses and fields for a number of years, a practice that can increase soil salinity through the accumulation of organic fertilizers and pesticides, and as a result, many agricultural lands have become highly saline, thus reducing plant yield and causing major economic losses (Gupta et al. 2017). Although many studies have reported that the application of PGPR is an eco-friendly and sustainable agricultural strategy, only a few studies have examined the ability of PGPR to induce salt-stress tolerance in pepper (Siddikee et al. 2011).

Keeping in view all the above points, the present investigations were undertaken to select bacteria from previously isolated PGPR strains from capsicum rhizosphere, for ability to grow in high salt medium, possessing various traits for plant growth promotion and alleviation of water stress.

## MATERIAL AND METHODS

The experiment was conducted at laboratory of Department of Basic sciences, Dr. YS Parmer University of Horticulture & Forestry Sloan, Himachal Pradesh (India) during 2013-2016. Effect of isolated PGPR strains for physiological efficacy under water stress conditions on growth of capsicum variety California Wonder was studied. The rhizobacteria used were isolated previously from the rhizospheric soil and root samples of Capsicum plants obtained from different agro-climatic zones of Himachal Pradesh, India.

### Testing of plant growth promoting traits, salt and drought tolerance of bacteria.

Previously isolated fifteen indigenous plant growth promoting rhizobacteria (PGPR) were evaluated for various plant growth promoting activities. Phosphate solubilization and siderophore production was assessed as per method described by Bray and Kurtz (1945) and Schwyn and Neilands (1987). The ability to fix nitrogen on nitrogen free Jensen media and to produce indole-3-acetic acid (IAA) on Luria Brentani Broth were determined by the method of Jensen (1987) and Gorden and Palleg (1957), respectively. ACC (1-aminocyclopropane-1-carobylic acid) deaminase

activity was tested as per method described by Dworkin and Foster (1958). The selected bacteria were tested for their tolerance to water stress in vitro as described by Sandhya et al. (2011). The bacteria were grown in NA medium supplemented with different concentrations of PEG6000 to achieve varying levels of water potentials. Addition of 25 % PEG 6000 gave a water potential of -0.73 MPa and the ability of any bacterium to grow in such a medium was considered as drought tolerant.

**Selection of the bacterial strains for in vivo plant growth promotion**

Among fifteen isolates, two isolates $JHA_6$ and $ROH_{14}$ possessing maximum plant growth promoting traits, salt and drought tolerance in vivo studies were selected for further studies. On the basis of 16S rDNA the bacterial isolates $JHA_6$ and $ROH_{14}$ were identified as *Pseudomonas aeruginosa* and *Bacillus amloliquefaciens*.

**Pot experiment**

The potting mixture was prepared by mixing sand, soil and well rotten farm yard manure (FYM) in a ratio of 1:1:2 having pH. 6.6, Electrical conductivity 0.42 $dSm^{-1}$ and organic carbon 0.92 %. Available N, P and K contents were 298.7, 24.6 and 194.9 Kg $ha^{-1}$, respectively. Field capacity was determined by draining the soil for 72 h after saturation. Three levels of water stress i.e. 80, 60 and 40 per cent of the field capacity (FC) were determined and maintained as described by Ghorbanpour et al. (2013). Surface sterilized capsicum seeds were dipped into individual culture broth of selected isolates ($JHA_6$ and $ROH_{14}$) (cell density about $10^8$ cells/ml) for four hours. The control seeds were treated with sterilized culture broth. The experiment was carried under net house conditions by taking the following ten treatments: $T_1$: 100% of field capacity (control); $T_2$: 80% of field capacity; $T_3$: 80 % of field capacity + $JHA_6$; $T_4$: 80 % of field capacity + $ROH_{14}$; $T_5$: 60% of field capacity; $T_6$: 60% of field capacity + $JHA_6$; $T_7$: 60% of field capacity + $ROH_{14}$; $T_8$: 40% of field capacity; $T_9$: 40% of field capacity + $JHA_6$; $T_{10}$: 40% of field capacity+$ROH_{14}$ in Completely Randomized Block Design with 3 replications. The observations on root/shoot length and biomass were recorded following standard methods. Plant nitrogen (N), phosphorus (P) and potassium (K) content were determined as per Jackson (1973). Nutrient uptake (mg $plant^{-1}$) was worked out by multiplying total NPK concentration of whole plant with total dry matter content. Leaf area ($cm^2$) was measured using leaf area meter (LI-Cor-3100). The chlorophyll content and relative leaf water content were determined by following the methods given by Withem et al. (1971) and Jeon et al. (2006), respectively.

Superoxide dismutase (SOD) activity was assayed described by Beauchamp and Fridovich (1971). Total catalase activity (CAT) and peroxidase (POD) assay was carried out as described by Chandlee and Scandalios (1984); Addy

and Goodman (1972), respectively. Soluble protein was estimated as described by Lowry et al. (1951).

**Statistical Analysis**

The data obtained were subjected to statistical analysis using SPSS (16v) and MS excel at 5% level of significance.

**RESULTS**

*Screening of isolated strains for PGPR traits, salt and drought tolerance in vitro*

The bacteria isolated from capsicum rhizosphere were screened for various PGP traits and fifteen isolates possessing maximum of plant growth promoting traits were then tested for ACC deaminase production, drought and salt tolerance. Results revealed that all isolates possess one or more PGP traits (Table 1). However, two isolates $JHA_6$ and $ROH_{14}$ showed positive result in all tests such as phosphate solubilization, IAA and ACC deaminase production as well as siderophore production, growth on high salt (10%NaCl) medium and in water stressed conditions. These two bacteria were taken up for further in vivo tests.

Table 1 Plant growth promoting characteristics of the selected bacterial isolates.

| Isolates | P-solubilisation efficiency (%) | Siderophore production effiency (%) | IAA production (µg/ml) | ACC-deaminase activity | Ammonia | HCN | Salt tolerance (8%NaCl) | Drought tolerance (25 % PEG 6000) |
|---|---|---|---|---|---|---|---|---|
| $RAK_9$ | 36.47 | 85.71 | 10.33 | + | + | + | - | + |
| $MAT_8$ | 93.17 | 55.56 | 23.67 | + | + | - | + | - |
| $NER_4$ | 86.19 | 40.94 | 13.00 | + | + | + | - | - |
| $PAR_2$ | 78.02 | 80.59 | 19.00 | - | - | - | - | + |
| $PAO_2$ | 82.59 | 75.40 | 0.00 | - | - | - | + | - |
| $SIH_6$ | 84.92 | 85.71 | 12.33 | + | - | - | - | - |
| $PAL_7$ | 58.52 | 77.98 | 21.67 | + | + | - | + | - |
| $KAN_{11}$ | 90.11 | 78.57 | 25.00 | + | - | - | - | - |
| $BHAR_4$ | 81.06 | 81.62 | 22.33 | + | - | + | - | - |
| $PAT_9$ | 72.94 | 78.27 | 0.00 | + | - | - | + | - |
| $PAT_{13}$ | 34.34 | 79.80 | 0.00 | - | + | - | + | - |
| $SARA_9$ | 82.74 | 74.90 | 31.33 | + | - | - | - | + |
| $JHA_6$ | 94.87 | 86.67 | 21.00 | + | - | + | + | + |
| $ROH_6$ | 92.31 | 37.82 | 21.33 | + | - | - | + | - |
| $ROH_{14}$ | 95.24 | 84.44 | 23.67 | + | + | - | + | + |

*In vivo plant growth promotion*

Inoculation of capsicum plants with both the PGPR strains resulted in a significant increase in growth variables, leaves parameters and NPK content of plants exposed to drought stress (Table 2). Plants inoculated with isolate $ROH_{14}$ and subjected to 80% FC soil moisture level ($T_4$ treatment) resulted in maximum shoot length and biomass (39.1 cm and 10.55 g), however, was statistically at par with $T_1$ (non-stressed, uninoculated plants) and $T_3$ (plants inoculated with $JHA_6$ and grown under 80% FC soil moisture level) treatments. Maximum root length (16.4 cm) was observed for $T_3$, which was statistically at par with $T_1$. Plants subjected to 80% FC soil moisture level inoculated with either of two bacterial isolates ($T_3$ and $T_4$ treatment) recorded maximum root biomass (10.9 mg), which was statistically at par with the treatment $T_1$ (non-stressed, uninoculated plants) (Table 2).

Table 2. Influence of PGPR isolates on growth variables, leaves parameters and NPK content of capsicum under varied levels of drought stress.

| Treatments | Shoot length (cm) | Root length (cm) | Shoot biomass (g) | Root biomass (g) | Leaf area ($cm^2$) | Relative water content in leaves (%) | Total chlorophyll content | Total soluble protein (mg/g fresh leaves) |
|---|---|---|---|---|---|---|---|---|
| $T_1$: 100% of field capacity | $38.3^{abc}$ | $16.1^{ab}$ | $10.33^{abc}$ | $1.07^{ab}$ | $22.14^{bc}$ | $92.47\ (80.51)^{a}$ | $2.027^{abc}$ | $0.233^{j}$ |
| $T_2$: 80% of field capacity | $32.9^{d}$ | $13.8^{d}$ | $8.88^{d}$ | $0.92^{c}$ | $19.90^{d}$ | $76.13\ (61.64)^{abcd}$ | $1.848^{d}$ | $0.254^{i}$ |
| $T_3$: 80 % of field capacity + $JHA_6$ | $38.9^{ab}$ | $16.4^{a}$ | $10.51^{ab}$ | $1.09^{a}$ | $22.73^{b}$ | $94.45\ (78.79)^{ab}$ | $2.036^{ab}$ | $0.273^{h}$ |
| $T_4$: 80 % of field capacity + $ROH_{14}$ | $39.1^{a}$ | $15.2^{bc}$ | $10.55^{a}$ | $1.09^{a}$ | $24.07^{a}$ | $91.90\ (76.37)^{abc}$ | $2.041^{a}$ | $0.274^{g}$ |
| $T_5$: 60% of field capacity | $28.1^{ghi}$ | $11.8^{g}$ | $7.59^{ghi}$ | $0.79^{f}$ | $13.10^{ghi}$ | $62.27\ (57.19)^{abc}$ | $1.689^{fg}$ | $0.294^{f}$ |
| $T_6$: 60% of field capacity + $JHA_6$ | $31.8^{de}$ | $13.4^{de}$ | $8.60^{de}$ | $0.89^{cd}$ | $16.83^{e}$ | $68.41\ (55.91)^{abc}$ | $1.713^{fg}$ | $0.317^{e}$ |
| $T_7$: 60% of field capacity + $ROH_{14}$ | $30.6^{ef}$ | $12.9^{def}$ | $8.26^{ef}$ | $0.86^{de}$ | $15.60^{f}$ | $71.15\ (57.86)^{abc}$ | $1.747^{ef}$ | $0.347^{cd}$ |
| $T_8$: 40% of field capacity | $23.3^{j}$ | $7.8^{i}$ | $6.28^{j}$ | $0.65^{g}$ | $11.27^{j}$ | $50.87\ (45.44)^{d}$ | $1.657^{g}$ | $0.352^{c}$ |
| $T_9$: 40% of field capacity + $JHA_6$ | $28.8^{fg}$ | $10.1^{g}$ | $7.79^{fg}$ | $0.81^{ef}$ | $13.83^{g}$ | $54.17\ (47.40)^{d}$ | $1.671^{g}$ | $0.384^{a}$ |
| $T_{10}$: 40% of field capacity + $ROH_{14}$ | $28.2^{gh}$ | $9.9^{gh}$ | $7.62^{gh}$ | $0.79^{f}$ | $13.23^{gh}$ | $51.76\ (46.00)^{d}$ | $1.685^{g}$ | $0.379^{ab}$ |
| $CD_{0.05}$ | 2.02 | 1.04 | 0.55 | 0.06 | 1.21 | 28.82 (23.45) | 0.068 | 0.02 |

* Number followed by same letters within a column was not significantly different but statistically significant over other treatment combinations based on $CD_{0.05}$.

*Elevation of water stress*

The effect of water stress, bacterial strain and their interactions on leaf area revealed that drought stress substantially reduced the leaf area of the plants as compared to non-stressed plants. However, PGPR inoculated plants, mitigated the drought stress effect by increasing leaf area (10-12%; 16-22% and 15-18%) over uninoculated treatments with 80%, 60% and 40% FC soil moisture level, respectively.

On comparison of various uninoculated water stress treatments ($T_2$, $T_5$ and $T_8$) with their inoculated counter-part treatments it can be concluded that PGPR has increased the RWC, thereby improving drought tolerance of capsicum plants. Maximum RWC (94.45%) has been noticed for $T_3$ treatment (plants inoculated with $JHA_6$ and subjected to 80% FC soil moisture level), followed by $T_4$ treatment. Maximum total chlorophyll content (2.041) has been noticed for $T_4$ treatment (plants inoculated with $ROH_{14}$ and grown under 80% FC soil moisture level), however, was statistically at par with $T_1$ (uninoculated non-stressed plants) and $T_3$ (plants inoculated with $JHA_6$ and grown under 80% FC soil moisture level) treatments.

*Total soluble proteins and antioxidative enzymes*

The concentration of total soluble proteins was higher in plants grown under drought than well watered conditions. Further the leaves of un-inoculated capsicum plants, which suffered from drought stress, had significant and substantial lower soluble proteins as compared to their respective PGPR inoculated stressed plants. This tended to occur regardless of bacterial strain. Maximum total soluble protein (0.384 mg/g fresh leaves) has been noticed in $ROH_{14}$ inoculated plants subjected to 40% of the field capacity ($T_{10}$ treatment). The water stress treatment caused a significant increase in the concentrations of antioxidant enzymes in all comparisons (Fig 1). Maximum (82.62, 4.94 and 87.16 U/gm fresh weight) SOD, POD and CAT enzyme activities, respectively, was recorded for plants inoculated with isolate $ROH_{14}$ ($T_{10}$ treatment) and subjected to 40% FC soil moisture level followed by $JHA_6$ inoculated plants grown under same stress level ($T_9$ treatment).

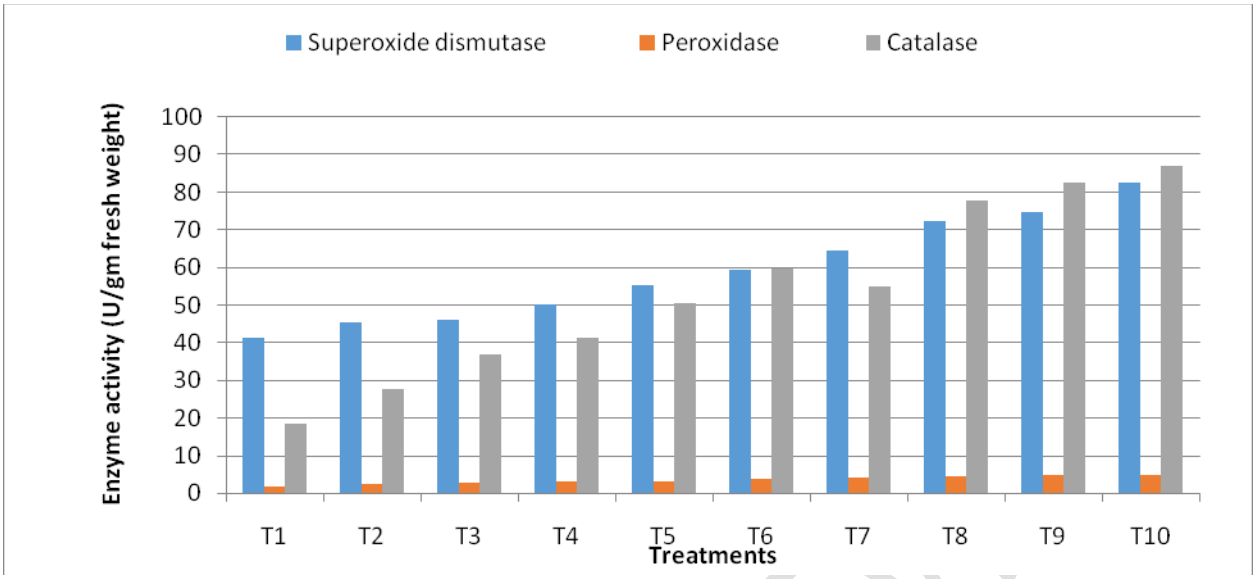


Fig. 1 Influence of PGPR isolates on antioxidant enzymes (SOD, POD and CAT) of capsicum under varied levels of drought stress.

*NPK content and uptake in plants*

Growth and nutrient concentrations usually determine the performance of plants growing in any environment. The effects of water stress and bacterial strains on NPK content and their uptake per plant (Table 3) revealed that mineral content and their uptake under water stress treatments in capsicum was significantly decreased compared to the non-water stress treatment.

Treatment with bacterial strains in the water stress treatment increased NPK content and uptake per plant as compared to un-inoculated stressed plants. Maximum (5.08%) N content and its uptake (5.79 mg/plant) was recorded in $T_1$ treatment (un-inoculated, non-stressed plants) followed by $T_4$ treatment. Similar results were recorded for K content and its uptake. However, maximum (0.31% and 0.36 mg/plant) P content and uptake, respectively, was recorded for $T_3$ and $T_4$ treatments followed by $T_1$ treatment. PGPR inoculated plants subjected to various drought stress levels increased N-uptake (24-27, 15-24 and 33-45 per cent), P- uptake (27-28, 13-20 and 31-35 per cent) and K-uptake (18-19, 16-22 and 10-14 per cent) over respective control treatments (uninoculated plants held at 80%, 60% and 40% FC soil moisture level, respectively).

Table 3. Influence of PGPR isolates on NPK content and uptake of capsicum under varied levels of drought stress.

| Treatments | N (%) | P (%) | K (%) | NU (mg/plant) | PU (mg/plant) | KU (mg/plant) |
|---|---|---|---|---|---|---|
| $T_1$: 100% of field capacity | 5.08 (2.25)$^{a}$ | 0.31 (0.55)$^{b}$ | 1.84 (1.35)$^{a}$ | 5.79$^{a}$ | 0.34$^{b}$ | 2.10$^{a}$ |
| $T_2$: 80% of field capacity | 3.85 (1.96)$^{c}$ | 0.26 (0.51)$^{c}$ | 1.58 (1.26)$^{bc}$ | 3.78$^{d}$ | 0.26$^{c}$ | 1.55$^{d}$ |
| $T_3$: 80 % of field capacity + $JHA_6$ | 4.32 (2.08)$^{bc}$ | 0.31 (0.56)$^{a}$ | 1.63 (1.28)$^{b}$ | 5.00$^{c}$ | 0.36$^{ab}$ | 1.89$^{bc}$ |
| $T_4$: 80 % of field capacity + $ROH_{14}$ | 4.48 (2.12)$^{ab}$ | 0.31 (0.56)$^{a}$ | 1.64 (1.28)$^{b}$ | 5.20$^{bc}$ | 0.36$^{ab}$ | 1.91$^{ab}$ |
| $T_5$: 60% of field capacity | 3.36 (1.83)$^{ef}$ | 0.22 (0.47)$^{g}$ | 1.35 (1.16)$^{f}$ | 2.81$^{gh}$ | 0.19$^{fg}$ | 1.13$^{g}$ |
| $T_6$: 60% of field capacity + $JHA_6$ | 3.50 (1.87)$^{de}$ | 0.25 (0.50)$^{d}$ | 1.53 (1.24)$^{cd}$ | 3.31$^{ef}$ | 0.24$^{cd}$ | 1.45$^{de}$ |
| $T_7$: 60% of field capacity + $ROH_{14}$ | 4.07 (2.02)$^{bcd}$ | 0.24 (0.49)$^{e}$ | 1.47 (1.21)$^{de}$ | 3.71$^{de}$ | 0.22$^{de}$ | 1.34$^{ef}$ |
| $T_8$: 40% of field capacity | 2.54 (1.59)$^{h}$ | 0.19 (0.43)$^{h}$ | 1.47 (1.21)$^{de}$ | 1.76$^{i}$ | 0.13$^{h}$ | 1.02$^{g}$ |
| $T_9$: 40% of field capacity + $JHA_6$ | 3.77 (1.94)$^{cd}$ | 0.23 (0.48)$^{f}$ | 1.38 (1.18)$^{ef}$ | 3.24$^{fg}$ | 0.20$^{ef}$ | 1.19$^{fg}$ |
| $T_{10}$: 40% of field capacity + $ROH_{14}$ | 3.17 (1.78)$^{efg}$ | 0.23 (0.48)$^{f}$ | 1.36 (1.16)$^{f}$ | 2.65$^{h}$ | 0.19$^{fg}$ | 1.14$^{fg}$ |
| $CD_{0.05}$ | 0.57 (0.16) | 0.016 (0.015) | 0.10 (0.04) | 0.46 | 0.035 | 0.21 |

## DISCUSSION

The soil plant interaction is a highly complex one as the soil with its edaphic and biological components provides most of the necessary nutrients and water to the plant besides the interaction of the microbial community with the root system which again depends on the plant as well as the soil components. With fast growing population in the World, and India, in particular, leading to urbanization and decrease in cultivable land, it is but natural that hitherto uncultivable lands such as saline or desert areas have to be brought into cultivation. On the other hand, plants are continuously exposed to various abiotic stresses of which drought or water scarcity is the most severe. While many attempts are being made to evolve techniques for making plants more tolerant to such stresses, enabling them to grow in adverse environments, most of them are time consuming and not cost effective. In this scenario, the last two decades have witnessed several studies where salt or water stress tolerant soil bacteria have been utilized for amelioration of abiotic stresses in plants (Dimkpa et al. 2009; Grover et al. 2010; Saleem et al. 2018). Another property of several soil bacteria is their ability to promote growth of plants and the reports of such bacteria, known as plant growth promoting bacteria (PGPR) are numerous (Kloepper et al. 2005; Erdogan et al. 2007; Woyessa and

192 Assefa 2011). It is important to select bacteria having multi-functional traits such as tolerance to abiotic stresses and
193 in vitro PGPR characteristics so that these may be tested for alleviation of abiotic stresses in plants. The present
194 study was thus taken to initially isolate bacteria with PGP traits from capsicum rhizosphere and further tested for
195 draught and salt tolerance.
196 Based upon PGP traits and draught tolerance two isolates $JHA_6$ and $ROH_{14}$ were taken for in vivo studies.
197 Identification of the two bacteria on the basis of morphological, biochemical and 16SrDNA sequencing revealed
198 them to be *Pseudomonas aeruginosa* ($JHA_6$) and *Bacillus amloliquefaciens* ($ROH_{14}$). Alleviation of water stress by
199 bacteria which also possessed PGPR traits have been reported earlier by some workers (Yang et al. 2009; Dimkpa et
200 al. 2009).
201 The two selected bacteria were next tested for their ability to promote growth of capsicum plants and these were
202 applied to the soil as soil drench. Both the bacteria promoted growth significantly as evidenced by significant
203 increases in root and shoot length and biomass. However, growth promotion varied with the bacteria. *Pseudomonas*
204 *aeruginosa* ($JHA_6$) promoted shoot growth better than *Bacillus amloliquefaciens* ($ROH_{14}$). The results are in
205 confirmation with Lim and Kim (2013), who reported that pepper plants treated with *Bacillus licheniformis* K11 and
206 exposed to drought stress had 50% higher shoot length and biomass than non-treated plants. The reduced drought
207 stress imposed effects on various growth variables with PGPR inoculation might be contributed to asymbiotic $N_2$
208 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients (Heidari et
209 al. 2011), modifying the phytohormone content like decreasing ethylene production by the ACC-deaminase activity
210 (Belimov et al. 2009).
211 Plant growth is dependent on water status of leaf, as drought stress can create a water deficit inside plant tissues.
212 Measuring RWC indicates stress response of plant (Gonzalez and Gonzalez-Vilar 2003), as higher RWC may help
213 plants counteract the oxidative and osmotic stresses caused by draught stress. In this study, we observed that in cases
214 where bacterial application had been done, RWC was not lowered. As both strains used in the present study produce
215 ACC deaminase, it is likely that the stress-induced accelerated synthesis of ethylene was reduced by inoculant
216 strains having ACC deaminase activity resulting in longer roots, which might be helpful in the uptake of relatively
217 more water from deep soil (Dodd et al. 2010). Our findings are confirmatory to other studies (Rakshapal et al. 2013)
218 which suggest that the PGPR-inoculated plants not only reduce stress but also help to fetch higher water quantity
219 from sources inaccessible to control plants.

In order to determine the influence of these applied bacteria on oxidative stress and antioxidant mechanism in leaves of capsicum, total soluble proteins, total chlorophyll content and the activities of three different antioxidative enzymes were assayed. Results clearly revealed that total soluble protein accumulation was enhanced by bacterial application, which may be attributed to the increased total chlorophyll content as a result of increased leaf area in PGPR inoculated plants. A similar result was reported by Vivas et al. (2003) who showed that inoculation of bacterial strain increased stomatal conductance and chlorophyll content of lettuce compared to a non-drought control. One of the mechanisms of alleviation of water stress seems to be the ability to tilt the balance from oxidatively stressed condition to a more antioxidative state, thereby resisting the effects of stress. Our results reveal that application of the two bacteria helped to maintain higher levels of antioxidant enzymes i.e. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), thus helped alleviate drought stress. It has been reported in several instances that water or salt stress tolerance in plants is related to maintaining of higher antioxidative status for prolonged period (Foyer and Noctor 2003; Nair et al. 2008; Iqbal and Bano 2009).

The results are in confirmation with Gururani et al. (2013) who reported that treatment of potato plants with two PGPR strains, *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40, induced an increase in the levels of ROS-scavenging enzymes including ascorbate peroxidase and catalase under drought stress in PGPR-treated plants compared with that in non-treated plants. Elevated accumulation of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), serves to minimize oxidative injury and contributes to the drought tolerance (Tank and Saraf 2010).

Similar growth promotion and stress tolerance effects of PGPR application on plants were also observed by Jay et al. (2013) who reported *Mesorhizobium* sp. and *Pseudomonas aeruginosa* to increase the N, P and K uptake of chickpea plants, under draught stress conditions. Further, the results are in confirmation with Vivas et al. (2003), they also reported that N, P and K concentrations in lettuce inoculated by *Bacillus* sp. under drought stress conditions were increased by about 5, 70 and 50%, respectively, compared to the non-water stress control. Bacterial-induced alterations in root architecture may lead to an increase in total root surface area and consequently lead to improved water and nutrient uptake, with positive effects on plant growth as a whole (Timmusk et al. 2014; Kanwal et al. 2017).

## CONCLUSIONS

The result of the present study suggests that PGPR isolates *Pseudomonas aeruginosa* ($JHA_6$) and *Bacillus amloliquefaciens* ($ROH_{14}$) have ameliorative effects on capsicum growth, which resulted in better survival, root/shoot biomass and water content compared to the non-inoculated control. PGPR strains enhance stress tolerance as a consequence of increasing activity of some antioxidant enzymes and soluble proteins during stress periods. The PGPR strains also improve the NPK content and their uptake in plants by increasing the shoot/root length and biomass. Therefore, inoculation with selected PGPR could serve as a useful tool for alleviating drought stress on capsicum. Our study suggests that the two PGPR strains could be efficiently used as bio-fertilizer and bio-stimulants for capsicum production in sustainable and ecological agricultural systems.

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