

**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* (JHA₆) and *Bacillus amyloliquefaciens* (ROH₁₄) were selected for in-vivo studies. A total of ten treatments comprising Plant growth promoting rhizobacteria (PGPR) (JHA₆ and ROH₁₄) 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 tolerate 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.

27 2013). Abiotic stresses are considered the main source of plant growth stagnation or reduction in crop productivity.
28 Water stress is perhaps the single most important limiting factor for crop production in many parts of the world
29 (Passioura 2007).

30 A water deficit causes diminished water potential and turgor loss which results in stomatal closure, decline in the
31 rate of photosynthesis, disruption of membrane integrity, protein denaturation and osmotic stress (Alcazar et al.
32 2011; Mi-Seon Hahm et al. 2007). It also induces the generation of Reactive Oxygen Species (ROS) such as
33 superoxide, hydrogen peroxide and hydroxyl radicals, which causes oxidation of lipids and proteins, chlorophyll
34 bleaching, damage to nucleic acids, ultimately leading to cell death (Ashraf 2009).

35 Plants develop self defense mechanisms against stress induced adverse effects by producing antioxidant enzymes
36 like superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase (Abdel 2011) and non-
37 enzymatic antioxidants like cellular redox buffers, carotenoids, flavonoids, tocopherols, ascorbate, glutathione etc.
38 (Amirjani 2012). In spite of the tolerance mechanisms of the plant including enhanced anti-oxidative activities, there
39 is a significant reduction in yield due to the disturbed metabolic processes following water stress (Gill and Tuteja
40 2010).

41 In order to maintain or increase crop productivity it becomes necessary to evolve efficient low-cost technologies for
42 abiotic stress management. It is now a priority area research for developing strategies to manage with abiotic
43 stresses including development of stress tolerant varieties, shifting crop calendars, resource management practices
44 etc (Venkateswarulu and Shanker 2009). However, most of these techniques are cost-intensive and time taking.
45 Inoculation of plants with soil microorganisms can amplify productivity of crops under a drought stress environment
46 (Chanway and Holl 1994). If such microorganisms have the ability to promote growth, they become extra beneficial.
47 Plant growth promoting rhizobacteria (PGPR) mitigates the impact of drought stress on plants through a process
48 called rhizobacterial-induced drought endurance and resilience (RIDER), which includes physiological and
49 biochemical changes. Various RIDER mechanisms include modification in phytohormonal levels, antioxidant
50 defense, bacterial exopolysaccharides (EPS) and accumulation of several compatible organic solutes like sugars,
51 amino acids, polyamines etc. (Kaushal and Wani, 2015). Production of heat-shock proteins (HSPs), dehydrins and
52 volatile organic compounds (VOCs) also plays significant role in the acquisition of drought tolerance (Rego MCF
53 2018). Increased length of lateral root as well as density and length of root hairs with PGPR strain led to a greater
54 exchange surface area with soil and thus a higher water flux through the whole root system up to the leaves of the

55 plant (Kechid et al. 2013). PGPR increased the active accumulation of organic and inorganic maintain cellular turgor
56 and help plants lower water potential without decreasing actual water content, thereby, protects enzymes, proteins,
57 cellular organelles and membranes against oxidative damage and helps plants tolerate drought-induced damage
58 (Huang et al. 2014; Khan et al. 2019). The most predominant rhizosphere colonizing bacteria belongs to
59 *Pseudomonas* and *Bacillus* species because of their association with soil organic matter, nutritional diversity and
60 rapid growth rate (Vijayapal et al. 1998).

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

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

70 **MATERIAL AND METHODS**

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

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

77 Previously isolated fifteen indigenous plant growth promoting rhizobacteria (PGPR) were evaluated for various
78 plant growth promoting activities. Phosphate solubilization and siderophore production was assessed as per method
79 described by Bray and Kurtz (1945) and Schwyn and Neilands (1987). The ability to fix nitrogen on nitrogen free
80 Jensen media and to produce indole-3-acetic acid (IAA) on Luria Brentani Broth were determined by the method of
81 Jensen (1987) and Gorden and Palleg (1957), respectively. ACC (1-aminocyclopropane-1-carobylic acid) deaminase
82 activity was tested as per method described by Dworkin and Foster (1958). The selected bacteria were tested for

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

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

88 Among fifteen isolates, two isolates JHA₆ and ROH₁₄ possessing maximum plant growth promoting traits, salt and
89 drought tolerance in vivo studies were selected for further studies. On the basis of 16S rDNA the bacterial isolates
90 JHA₆ and ROH₁₄ were identified as *Pseudomonas aeruginosa* and *Bacillus amloliquefaciens*.

91 **Pot experiment**

92 The potting mixture was prepared by mixing sand, soil and well rotten farm yard manure (FYM) in a ratio of 1:1:2
93 having pH. 6.6, Electrical conductivity 0.42 dSm⁻¹ and organic carbon 0.92 %. Available N, P and K contents were
94 298.7, 24.6 and 194.9 Kg ha⁻¹, respectively. Field capacity was determined by draining the soil for 72 h after
95 saturation. Three levels of water stress i.e. 80, 60 and 40 per cent of the field capacity (FC) were determined and
96 maintained as described by Ghorbanpour et al. (2013). Surface sterilized capsicum seeds were dipped into individual
97 culture broth of selected isolates (JHA₆ and ROH₁₄) (cell density about 10⁸ cells/ml) for four hours. The control
98 seeds were treated with sterilized culture broth. The experiment was carried under net house conditions by taking the
99 following ten treatments: T₁: 100% of field capacity (control); T₂: 80% of field capacity; T₃: 80 % of field capacity
100 + JHA₆; T₄: 80 % of field capacity + ROH₁₄; T₅: 60% of field capacity; T₆: 60% of field capacity + JHA₆; T₇: 60%
101 of field capacity + ROH₁₄; T₈: 40% of field capacity; T₉: 40% of field capacity + JHA₆; T₁₀: 40% of field
102 capacity+ROH₁₄ in Completely Randomized Block Design with 3 replications. The observations on root/shoot
103 length and biomass were recorded following standard methods. Plant nitrogen (N), phosphorus (P) and potassium
104 (K) content were determined as per Jackson (1973). Nutrient uptake (mg plant⁻¹) was worked out by multiplying
105 total NPK concentration of whole plant with total dry matter content. Leaf area (cm²) was measured using leaf area
106 meter (LI-Cor-3100). The chlorophyll content and relative leaf water content were determined by following the
107 methods given by Withem et al. (1971) and Jeon et al. (2006), respectively.

108 Superoxide dismutase (SOD) activity was assayed described by Beauchamp and Fridovich (1971). Total catalase
109 activity (CAT) and peroxidase (POD) assay was carried out as described by Chandlee and Scandalios (1984); Addy
110 and Goodman (1972), respectively. Soluble protein was estimated as described by Lowry et al. (1951).

111 **Statistical Analysis**

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

113 **RESULTS**

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

115 The bacteria isolated from capsicum rhizosphere were screened for various PGP traits and fifteen isolates possessing
 116 maximum of plant growth promoting traits were then tested for ACC deaminase production, drought and salt
 117 tolerance. Results revealed that all isolates possess one or more PGP traits (Table 1). However, two isolates JHA₆
 118 and ROH₁₄ showed positive result in all tests such as phosphate solubilization, IAA and ACC deaminase production
 119 as well as siderophore production, growth on high salt (10%NaCl) medium and in water stressed conditions. These
 120 two bacteria were taken up for further in vivo tests.

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

Isolates	P-solubilisation efficiency (%)	Siderophore production efficiency (%)	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	+	+	-	+	+

122
 123 *In vivo plant growth promotion*
 124 Inoculation of capsicum plants with both the PGPR strains resulted in a significant increase in growth variables,
 125 leaves parameters and NPK content of plants exposed to drought stress (Table 2). Plants inoculated with isolate

126 ROH₁₄ and subjected to 80% FC soil moisture level (T₄ treatment) resulted in maximum shoot length and biomass
 127 (39.1 cm and 10.55 g), however, was statistically at par with T₁ (non-stressed, uninoculated plants) and T₃ (plants
 128 inoculated with JHA₆ and grown under 80% FC soil moisture level) treatments. Maximum root length (16.4 cm) was
 129 observed for T₃, which was statistically at par with T₁. Plants subjected to 80% FC soil moisture level inoculated
 130 with either of two bacterial isolates (T₃ and T₄ treatment) recorded maximum root biomass (10.9 mg), which was
 131 statistically at par with the treatment T₁ (non-stressed, uninoculated plants) (Table 2).

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

134

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 ^c	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

135 *Number followed by same letters within a column was not significantly different but statistically significant over

136 other treatment combinations based on CD_{0.05}.

137 *Elevation of water stress*

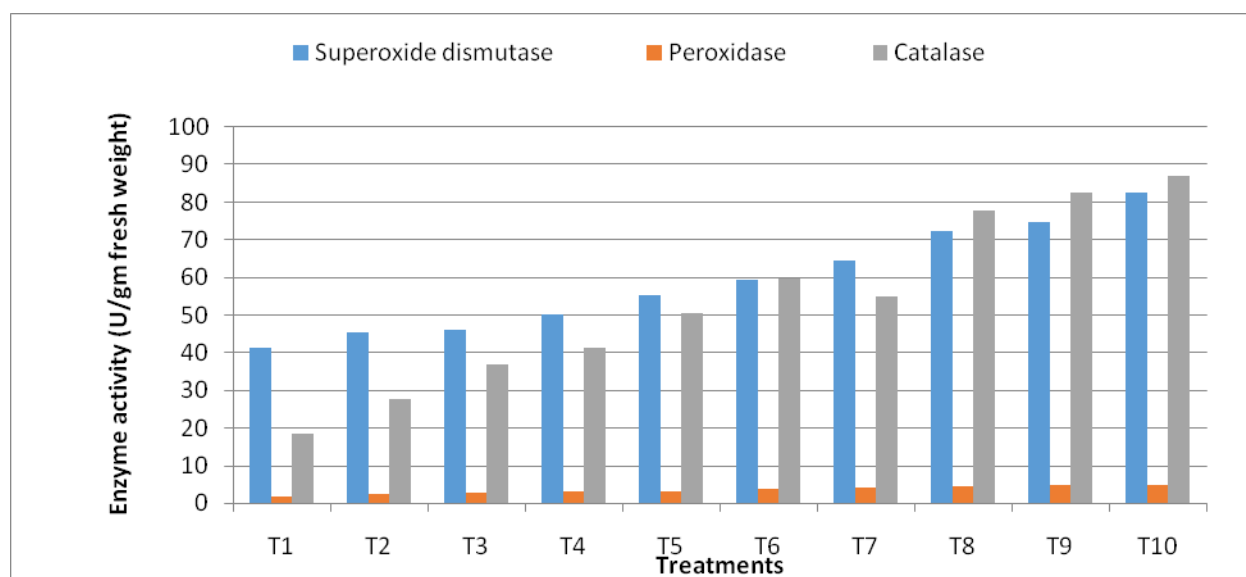
138 The effect of water stress, bacterial strain and their interactions on leaf area revealed that drought stress substantially
139 reduced the leaf area of the plants as compared to non-stressed plants. However, PGPR inoculated plants, mitigated
140 the drought stress effect by increasing leaf area (10-12%; 16-22% and 15-18%) over uninoculated treatments with
141 80%, 60% and 40% FC soil moisture level, respectively.

142 On comparison of various uninoculated water stress treatments (T₂, T₅ and T₈) with their inoculated counter-part
143 treatments it can be concluded that PGPR has increased the RWC, thereby improving drought tolerance of capsicum
144 plants. Maximum RWC (94.45%) has been noticed for T₃ treatment (plants inoculated with JHA₆ and subjected to
145 80% FC soil moisture level), followed by T₄ treatment. Maximum total chlorophyll content (2.041) has been noticed
146 for T₄ treatment (plants inoculated with ROH₁₄ and grown under 80% FC soil moisture level), however, was
147 statistically at par with T₁ (uninoculated non-stressed plants) and T₃ (plants inoculated with JHA₆ and grown under
148 80% FC soil moisture level) treatments.

149 *Total soluble proteins and antioxidative enzymes*

150 The concentration of total soluble proteins was higher in plants grown under drought than well watered conditions.
151 Further the leaves of un-inoculated capsicum plants, which suffered from drought stress, had significant and
152 substantial lower soluble proteins as compared to their respective PGPR inoculated stressed plants. This tended to
153 occur regardless of bacterial strain. Maximum total soluble protein (0.384 mg/g fresh leaves) has been noticed in
154 ROH₁₄ inoculated plants subjected to 40% of the field capacity (T₁₀ treatment). The water stress treatment caused a
155 significant increase in the concentrations of antioxidant enzymes in all comparisons (Fig 1). Maximum (82.62, 4.94
156 and 87.16 U/gm fresh weight) SOD, POD and CAT enzyme activities, respectively, was recorded for plants
157 inoculated with isolate ROH₁₄ (T₁₀ treatment) and subjected to 40% FC soil moisture level followed by JHA₆

158 inoculated plants grown under same stress level (T₉ treatment).



159
160 Fig. 1 Influence of PGPR isolates on antioxidant enzymes (SOD, POD and CAT) of capsicum under varied levels of
161 drought stress.

162 *NPK content and uptake in plants*

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

167 Treatment with bacterial strains in the water stress treatment increased NPK content and uptake per plant as
168 compared to un-inoculated stressed plants. Maximum (5.08%) N content and its uptake (5.79 mg/plant) was
169 recorded in T₁ treatment (un-inoculated, non-stressed plants) followed by T₄ treatment. Similar results were recorded
170 for K content and its uptake. However, maximum (0.31% and 0.36 mg/plant) P content and uptake, respectively,
171 was recorded for T₃ and T₄ treatments followed by T₁ treatment. PGPR inoculated plants subjected to various
172 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
173 cent) and K-uptake (18-19, 16-22 and 10-14 per cent) over respective control treatments (uninoculated plants held at
174 80%, 60% and 40% FC soil moisture level, respectively).

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

176

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

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

180 DISCUSSION

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

192 as plant growth promoting bacteria (PGPR) are numerous (Kloepper et al. 2005; Erdogan et al. 2007; Woyessa and
193 Assefa 2011). It is important to select bacteria having multi-functional traits such as tolerance to abiotic stresses and
194 in vitro PGPR characteristics so that these may be tested for alleviation of abiotic stresses in plants. The present
195 study was thus taken to initially isolate bacteria with PGP traits from capsicum rhizosphere and further tested for
196 draught and salt tolerance.

197 Based upon PGP traits and draught tolerance two isolates JHA₆ and ROH₁₄ were taken for in vivo studies.
198 Identification of the two bacteria on the basis of morphological, biochemical and 16SrDNA sequencing revealed
199 them to be *Pseudomonas aeruginosa* (JHA₆) and *Bacillus amloliquefaciens* (ROH₁₄). Alleviation of water stress by
200 bacteria which also possessed PGPR traits have been reported earlier by some workers (Yang et al. 2009; Dimkpa et
201 al. 2009).

202 The two selected bacteria were next tested for their ability to promote growth of capsicum plants and these were
203 applied to the soil as soil drench. Both the bacteria promoted growth significantly as evidenced by significant
204 increases in root and shoot length and biomass. However, growth promotion varied with the bacteria. *Pseudomonas*
205 *aeruginosa* (JHA₆) promoted shoot growth better than *Bacillus amloliquefaciens* (ROH₁₄). The results are in
206 confirmation with Lim and Kim (2013), who reported that pepper plants treated with *Bacillus licheniformis* K11 and
207 exposed to drought stress had 50% higher shoot length and biomass than non-treated plants. The reduced drought
208 stress imposed effects on various growth variables with PGPR inoculation might be contributed to asymbiotic N₂
209 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients (Heidari et
210 al. 2011), modifying the phytohormone content like decreasing ethylene production by the ACC-deaminase activity
211 (Belimov et al. 2009).

212 Plant growth is dependent on water status of leaf, as drought stress can create a water deficit inside plant tissues.
213 Measuring RWC indicates stress response of plant (Gonzalez and Gonzalez-Vilar 2003), as higher RWC may help
214 plants counteract the oxidative and osmotic stresses caused by draught stress. In this study, we observed that in cases
215 where bacterial application had been done, RWC was not lowered. As both strains used in the present study produce
216 ACC deaminase, it is likely that the stress-induced accelerated synthesis of ethylene was reduced by inoculant
217 strains having ACC deaminase activity resulting in longer roots, which might be helpful in the uptake of relatively
218 more water from deep soil (Dodd et al. 2010). Our findings are confirmatory to other studies (Rakshapal et al. 2013)

219 which suggest that the PGPR-inoculated plants not only reduce stress but also help to fetch higher water quantity
220 from sources inaccessible to control plants.

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

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

239 Similar growth promotion and stress tolerance effects of PGPR application on plants were also observed by Jay et al.
240 (2013) who reported *Mesorhizobium* sp. and *Pseudomonas aeruginosa* to increase the N, P and K uptake of
241 chickpea plants, under draught stress conditions. Further, the results are in confirmation with Vivas et al. (2003),
242 they also reported that N, P and K concentrations in lettuce inoculated by *Bacillus* sp. under drought stress
243 conditions were increased by about 5, 70 and 50%, respectively, compared to the non-water stress control. Bacterial-
244 induced alterations in root architecture may lead to an increase in total root surface area and consequently lead to
245 improved water and nutrient uptake, with positive effects on plant growth as a whole (Timmusk et al. 2014; Kanwal
246 et al. 2017).

247

248

249 CONCLUSIONS

250 The result of the present study suggests that PGPR isolates *Pseudomonas aeruginosa* (JHA₆) and *Bacillus*
251 *amyloliquefaciens* (ROH₁₄) have ameliorative effects on *Capsicum* growth, which resulted in better survival,
252 root/shoot biomass and water content compared to the non-inoculated control. PGPR strains enhance stress tolerance
253 as a consequence of increasing activity of some antioxidant enzymes and soluble proteins during stress periods. The
254 PGPR strains also improve the NPK content and their uptake in plants by increasing the shoot/root length and
255 biomass. Therefore, inoculation with selected PGPR could serve as a useful tool for alleviating drought stress on
256 capsicum. Our study suggests that the two PGPR strains could be efficiently used as bio-fertilizer and bio-stimulants
257 for capsicum production in sustainable and ecological agricultural systems.

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