

Full Length Research Paper

Efficacy of aminocyclopropane-1-carboxylic acid (ACC)-deaminase-producing rhizobacteria in ameliorating water stress in chickpea under axenic conditions

Palika Sharma, Veena Khanna* and Poonam Kumari

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India.

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To mitigate environmental stresses, use of aminocyclopropane-1-carboxylic acid (ACC)-deaminase containing plant growth promoting rhizobacteria (PGPR) as agricultural inputs for improved crop production is required. A total of 47 bacterial isolates from different rhizospheric soils of chickpea from Punjab were biochemically characterized and found to be representatives of genus *Bacillus* (25) and *Pseudomonas* (22). Ten (10) of the isolates were able to utilize ACC as a sole source of nitrogen, maximum growth (in terms of optical density $\lambda 600$) being recorded with *Bacillus* isolate 23-B (0.463) followed by *Pseudomonas* 6-P (0.317). Three isolates were P-solubilizers and their relative P-solubilization efficiency ranged from 14.6 to 21.6 mg/100 ml culture broth. All the isolates produced Indole-3-acetic acid (IAA) (4.4-22.8 $\mu\text{g/ml}$). Two PGPR's 23-B and 6-P alone and in combination with recommended (for Punjab state) *Mesorhizobium ciceris*, were evaluated for water stress mitigation and plant growth promotion under axenic conditions on *Cicer arietinum* varieties (*Kabuli* L-552 and *Desi* GPF-2). Both the rhizobacteria significantly improved germination, root and shoot length and fresh weight of chickpea seedlings under osmotic potential of up to 0.4 MPa over uninoculated control. Proline content was considerably higher in PGPR treated varieties of chickpea under water stress. Co-inoculation of 23-B with *Mesorhizobium* enhanced all growth parameters under water stress.

Key words: Aminocyclopropane-1-carboxylic acid (ACC)-deaminase, axenic, chickpea, polyethylene glycol (PEG), proline.

INTRODUCTION

Abiotic stresses caused by complex environmental conditions, for example, drought, salinity, high and low temperatures etc lead to substantial crop losses worldwide (Mittler et al., 2006). As a consequence of these stresses, plants typically stimulate 1-aminocyclo-propane 1-carboxylic acid (ACC) synthesis, a precursor to ethylene (Gamalero and Glick, 2012) which helps in inducing multifarious physiological changes in plants at molecular

level but at higher levels is usually deleterious, as it induces defoliation, changes cellular processes leading to growth inhibition, premature senescence, restricted nodulation, all of which reduce crop yield (Lie et al., 2005). Amongst the legumes, chickpea (*Cicer arietinum* L.) is third most important grain legumes in the world cultivated on 11.55 million hectares with production of 10.46 million tons, India being the largest producer

*Corresponding author. E-mail: veenadk@rediffmail.com.

(FAO STAT, 2010). To reduce the deleterious effects of ethylene stress, plant growth-promoting rhizobacteria (PGPR) that facilitate the proliferation of plants under stress conditions are a potentially viable option (Gamalero and Glick, 2012). These beneficial rhizobacteria with ACC deaminase activity sustain plant development by lowering ethylene levels by metabolizing ACC into α -ketobutyrate and ammonia (Mehta et al., 2010).

Under stress conditions, increased proline biosynthesis has been reported in various plant species inoculated with different PGPR (Vardharajula et al., 2011). Proline acts as osmoprotectant and reactive oxygen species scavenger thus supporting plant growth under stress. ACC-deaminase activity has been widely reported in numerous species of PGPR like *Azospirillum*, *Bacillus*, *Burkholderia*, *Pseudomonas* and *Rhizobium* etc (Shaharoon et al., 2006).

The result of adding PGPR to plants is a significant increase in seed germination and the biomass that the plants are able to attain under otherwise stressful and inhibitory conditions (Shaharoon et al., 2012). The present work was undertaken with the objective to screen rhizobacteria producing ACC-deaminase for plant growth promoting potential under water stressed conditions.

MATERIALS AND METHODS

Isolation and characterization of bacterial strains

Soil samples were collected from the depth of 10 to 15 cm from twenty different chickpea fields of Punjab. The soil samples were serially diluted up to 10^{-9} dilutions and were plated on nutrient agar (for *Bacillus* spp.) and King's B (*Pseudomonas* from spp). The plates were then incubated at 30°C for 24 h.

The colonies were picked up, sub-cultured and preserved on Nutrient agar slants at 4 to 5°C. The isolates were then characterised on the basis of colony morphology, Gram staining and biochemical tests namely: catalase production, nitrate reduction, starch hydrolysis and methyl red test (Cappuccino and Sherman, 1992).

Aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity

Bacterial isolates were grown in Luria Broth medium and cell pellet collected by centrifugation washed and resuspended in sterile water and spot inoculated on petriplates containing Dworkin and Foster (DF) salt minimal medium (Dworkin and Foster, 1958) supplemented with 3 mM ACC as a main source of Nitrogen.

In quantitative assay each selected isolate was grown individually in liquid DF minimal medium alone, DF+ACC and DF+ $(\text{NH}_4)_2\text{SO}_4$ and their growth measured (OD_{600}). ACC-deaminase producing rhizobacterial isolates were selected for evaluation of other PGP traits.

Phosphate-solubilization

Phosphate-solubilization was evaluated qualitatively (Mehta and Nautiyal, 2001) and solubilization index calculated

Phosphate solubilization Index =

$$\frac{\text{Total diameter (colony + halo zone)}}{\text{Diameter of colony}}$$

Quantitatively P-solubilization was recorded at intervals of 3 days up to 15 days (Jackson, 1973).

Production of Indole-3-acetic acid (IAA)

Bacteria were grown in Luria broth (72 h at 30°C) and IAA estimated by the method of Gordon and Weber (1951).

Induction of water stress by polyethylene glycol 6000 (PEG)

Seeds of two *Cicer arietinum* varieties (L-552 and GPF-2) obtained from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, were surface-sterilized and soaked in rhizobacterial culture (23-B and 6-P showing relatively higher ACC-deaminase activity, P-solubilization, IAA synthesis and compatibility with *Mesorhizobium ciceris*) alone and in combination with *Mesorhizobium* culture for 30 min. The experiment was conducted in completely randomized design with three replications. To simulate drought stress, PEG-6000 equivalent to water potential -0.2, -0.4 and -0.6 MPa (Mega Pascals) were used. Ten seeds of each genotype were germinated on sterilized filter paper lined petri dishes, moistened with 5 ml solution of PEG-6000 having appropriate osmotic pressure and kept in incubator at $22 \pm 2^\circ\text{C}$ with 90% humidity for duration of 10 days. Seeds germinated using distilled water only served as absolute control.

Proline content

Samples were extracted in Methanol: Chloroform: Water (12:5:1), centrifuged and the supernatant collected and made up to 10 ml with same solvent. To this 6 ml of chloroform and 4 ml of distilled water was added and allowed to stand till the two layers got separated. The final volume of upper layer was made 10 ml with distilled water, to 5 ml of this solution 2.5 ml of acid ninhydrin was added and mixture boiled for 45 min till pink colour developed and OD at 515 nm was recorded (Bates et al., 1973). Proline was used as standard to make standard curve (Sahu and Sindhu, 2011).

RESULTS AND DISCUSSION

Isolation and characterization of rhizobacteria

A total of 47 rhizobacteria were isolated from chickpea rhizospheric soils and out of these 22 were from Kings B medium, all showed yellowish green pigment characteristic of *Pseudomonas* spp whereas 25 were from nutrient agar medium showed predominantly off-white to creamish colonies, typical of the genus *Bacillus* (Figure 1). The predominance of *Pseudomonas* and *Bacillus* spp. in legume rhizosphere has been reported by many workers (Yadav et al., 2010). Most of the *Bacillus* isolates were indole and citrate negative, catalase and methyl red positive and reduced nitrate and hydrolysed starch. The other isolates were Gram negative rods and tested positive for MethylRed Voges-Proskauer, catalase and indole, negative for citrate test, reduced nitrate and

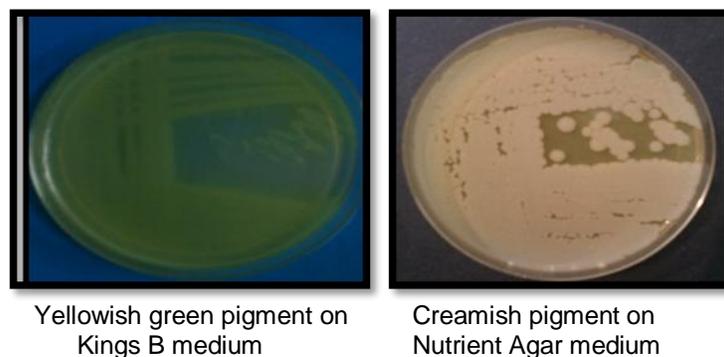


Figure 1. Cultural characteristics of rhizobacterial isolates.

Table 1. Comparative growth of rhizobacteria on media containing ACC/ Ammonium sulphate as sole nitrogen source.

Rhizobacteria	OD ₆₀₀		
	ACC (+)	ACC(-)	Ammonium sulphate
4-B	0.424	0.020	0.621
8-B	0.406	0.035	0.644
23-B	0.463	0.015	0.730
24-B	0.260	0.051	0.572
25-B	0.351	0.015	0.574
1-P	0.217	0.019	0.248
4-P	0.273	0.013	0.670
6-P	0.317	0.053	0.693
10-P	0.180	0.022	0.250
15-P	0.176	0.060	0.347

hydrolysed starch. On the basis of these tests, the isolates were tentatively assigned to genera *Bacillus* (B) and *Pseudomonas* (P).

Aminocyclopropane-1-carboxylic acid (ACC)-deaminase activity

Of the 47 rhizobacteria, 10 were able to utilize ACC as sole N-source, however, variation in efficacy to utilize ACC was observed (Table 1). The *Bacillus* isolates (23-B, 4-B, 8-B, 25-B and 24-B) showed highest growth in terms of O.D λ_{600} (0.463, 0.424, 0.406, 0.351 and 0.260) whereas *Pseudomonads* (6-P, 4-P, 1-P 10-P and 15-P) showed medium growth (0.317, 0.273, 0.217, 0.180 and 0.176). Several bacterial species belonging to different genera have been reported to exhibit variable ACC-deaminase activity.

Phosphate-solubilization

Out of 10 ACC-deaminase positive isolates, three

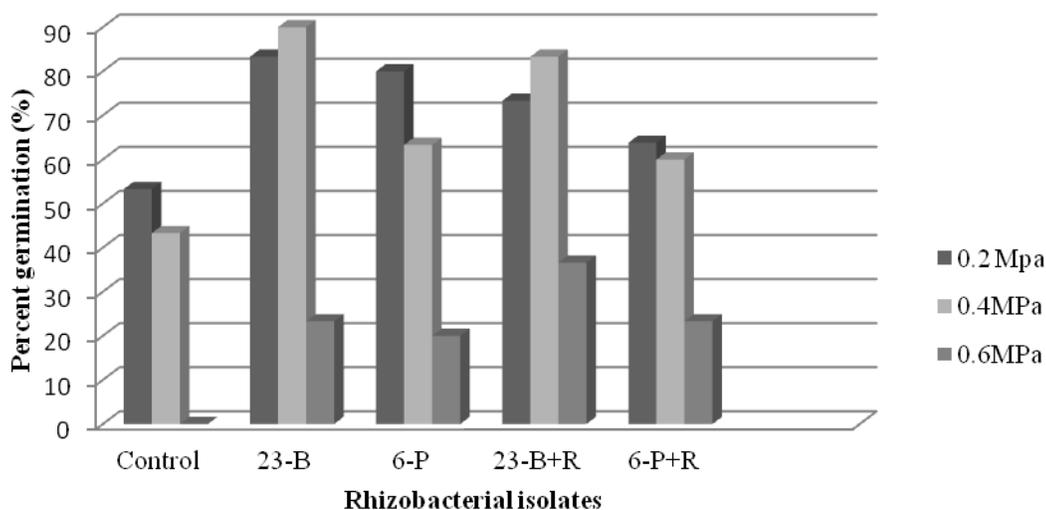
isolates showed varying P-solubilizing index of 2.2 to 2.7, highest being with isolate 23-B followed by 6-P. Under liquid culture P-solubilized ranged from 14.6 to 21.6 mg/100 ml. The rhizobacteria 6-P was a potent P-solubilizer showing maximum P-solubilization on 6th day of incubation (21.6 mg/100 ml) followed by 8-B (20.2 mg/100 ml) and 23-B (17.9 mg/100 ml) (Table 2). Mahalakshmi and Reetha (2009) reported that 83.3% of *Pseudomonas* isolates were P-solubilizers.

Indole-3-acetic acid (IAA) production

Diverse soil microorganisms are known to produce IAA, which is an important hormone for plant growth, root initiation and elongation (Yasmin et al., 2009). All the rhizobacteria produced IAA, *Bacillus* (11.2-22.8 $\mu\text{g/ml}$) and *Pseudomonas* isolates (4.4-21.6 $\mu\text{g/ml}$), highest being 23-B (22.8 $\mu\text{g/ml}$) (Table 2). In the present study *Bacillus* spp were found to be strong IAA producers. These results are in accordance to Etesami et al. (2009) they reported significant difference in IAA production amongst the isolates.

Table 2. Comparative performance of rhizobacteria in terms of plant growth promoting traits.

Rhizobacteria	P-solubilization Index	IAA equivalents ($\mu\text{g/ml}$)		P-solubilization ($\text{mg}/100 \text{ ml}$)	
		L-TRP (-)	L-TRP(+)	6 th Day	9 th Day
4-B	-	1.4	19.4	-	-
8-B	2.2	8.8	18.2	20.2	17.2
23-B	2.7	4.7	22.8	14.6	17.9
24-B	-	3.5	13.6	-	-
25-B	-	8.8	11.2	-	-
1-P	-	1.7	11.9	-	-
4-P	-	4.2	10.0	-	-
6-P	2.4	5.6	21.6	21.6	18.2
10-P	-	0.5	4.4	-	-
15-P	-	3.4	8.2	-	-

**Figure 2.** Effect of ACC-deaminase positive rhizobacteria on germination of chickpea L-552 seeds under water stress.

Effect of aminocyclopropane-1-carboxylic acid (ACC)-deaminase positive rhizobacteria on germination and growth of chickpea L-552 under water stress

Germination of chickpea (L-552) seeds was affected by PEG induced water stress. At 0.2 MPa seeds inoculated with isolate 23-B showed maximum germination (83.3%) followed by 6-P (80%) as compared to uninoculated control (53%). Isolate 23-B in combination with *Mesorhizobium* showed better germination as compared to isolate 6-P. At 0.4 MPa, isolate 23-B showed 90% germination followed by 23-B+R (83.3%), 6-P (63.3%) and 6-P+R (60%) over control (43%), whereas at 0.6 MPa, 23-B+R (36.6%) and 23-B (23.3%), exhibited germination, however, no germination was observed in control. From the data it is evident that isolate 23-B

induced maximum water stress tolerance in chickpea L-552 as compared to isolate 6-P (Figure 2).

Root growth was also greatly affected by water stress at all the three levels (Table 3). At 0.2 and 0.4 MPa maximum increase in root elongation was recorded in seeds treated with PGPR 23-B (3.6 and 2.7 cm) whereas at 0.6 MPa, isolate 6-P+R (1.2 cm) was more effective. At 0.2 MPa application of isolate 23-B+R recorded root fresh weight of 70.3 mg/seedling followed by isolates 23-B, 6-P, 6-P+R (60, 57 and 52.7 mg/seedling), at 0.4 MPa isolate 23-B alone gave maximum root fresh weight (45 mg/seedling) but at 0.6 MPa isolate 23-B+R increased root fresh weight to maximum extent (18.3 mg/seedling). Shoot traits also exhibited significant decrease under stress, seeds showed no plumule emergence at 0.6 MPa stress. At 0.2 MPa, isolate 23-B+R showed maximum shoot length (1.2 cm) followed by 23-B (1 cm), at 0.4

Table 3. Effect of ACC-deaminase positive rhizobacteria on root and shoot traits of chickpea L-552 under water stress.

Rhizobacteria	PGP trait											
	Root length (cm)			Root fresh weight (mg/seedling)			Shoot length (cm)			Shoot fresh weight (mg/seedling)		
	Doses of PEG (MPa)											
	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
Control	2.1	1.6	-	28.3	27	-	0.53	-	-	15.6	-	-
23-B	3.6	2.7	0.6	60.0	45	12.7	1	0.6	-	22.7	25	-
23-B+R	2.1	2.3	0.8	70.3	36.3	18.3	1.2	0.5	-	24	21	-
6-P	2.7	1.9	0.6	57.0	30	16.7	-	-	-	-	-	-
6-P+R	2.1	1.6	1.2	52.7	22.3	13.3	-	-	-	-	-	-
Absolute control		5.9			100.0			1.3			52.7	
CD (P = 0.05)	1.7	1.9	1.3	34.6	25.5	13.2	0.48	0.52	-	28.1	26.6	-

MPa, 23-B showed maximum shoot length (0.6 cm) followed by 23-B+R (0.5 cm). Shoot fresh weight was maximum at 0.2 MPa when inoculated with isolate 23-B+R, followed by 23-B (24 & 22.7 mg/seedling) as compared to control (15.6 mg/seedling). At 0.4 MPa isolate 23-B showed maximum shoot fresh weight (25 mg/seedling) (Figure 3). It is clear from the data that 23-B alone and along with *Mesorhizobium* exhibited profound effect on chickpea growth under water stress. These results are in corroboration with results of Mayak et al. (2004) who reported that ACC-deaminase PGPR *Achromobacter piechaudii* ARV8 significantly increased the fresh and dry weights of tomato and pepper seedlings exposed to transient water stress.

Effect of aminocyclopropane-1-carboxylic acid (ACC)-deaminase positive rhizobacteria on germination and growth of chickpea GPF-2 under water stress

Chickpea variety GPF-2 also showed significant reduction in plant growth under water stress conditions and germination was inhibited at osmotic potential (0.6 MPa) which is considered as threshold potential (Table 4). Only radicle emergence was noticed in GPF-2 variety whereas plumules failed to emerge at all the three water stress levels. Here too, at 0.2 MPa isolates 23-B and 23-B+R showed maximum germination (63.3%) and at 0.4 MPa 23-B+R showed maximum germination (56.6%) followed by isolate 23-B (40%) over uninoculated control (Figure 4). At 0.2 MPa and 0.4 MPa seed inoculation with 6-P+R gave maximum root elongation (3.0 and 3.1 cm) over control (1.8 cm). At 0.2 MPa isolate 23-B exhibited profound increase in root fresh weight whereas, at 0.4 MPa, 6-P+R showed higher root fresh weight ((53 & 32 mg/seedling) as compared to uninoculated control (18.3 mg/seedling) (Figure 5). This study shows that water stress inhibited coleoptile emergence more than the radicle which is in accordance to results of Macar et al.

(2008). The ACC-deaminase containing rhizobacteria probably reduced the endogenous ethylene levels in chickpea at early stage of development thus enhanced their growth and root elongation under stress. *Bacillus* isolate 23-B was found to be more effective in plant growth promotion probably due to its higher ACC-deaminase activity. Treatment with ACC deaminase-producing bacteria typically reduces ethylene levels by about 2-4 fold (Reed and Glick, 2005). It has been reported ACC deaminase-PGPR protects the plants against damage from drought, high salt and polyaromatic hydrocarbons (Mayak et al., 2004; Glick et al., 2007).

Proline accumulation

The proline content shows an increasing trend in both chickpea varieties under water stress which is in accordance to the result of Madhurendra (2009). Treatment with *Bacillus* isolate 23-B shows maximum proline accumulation (0.80 and 0.66 mg/g fresh weight radicle) in comparison to *Pseudomonas* isolate 6-P (0.32 and 0.31) in both chickpea varieties as compared to control and absolute control (Table 5). Co-inoculation of isolate 23-B with *Mesorhizobium* also gave similar results. These results are in corroboration with work of Singh (2004) who reported accumulation of proline in saline stress tolerant chickpea genotypes. Slow utilization of proline for protein synthesis during stress results in its accumulation. Proline is known to act as a compatible osmolyte, antioxidant and maintains cytosolic pH (Verbruggen and Hermans, 2008).

Conclusion

The present study indicates that co-inoculation of ACC-deaminase producing PGPR with *Mesorhizobium* significantly promoted growth of chickpea by positively influencing seed germination and other growth factors under

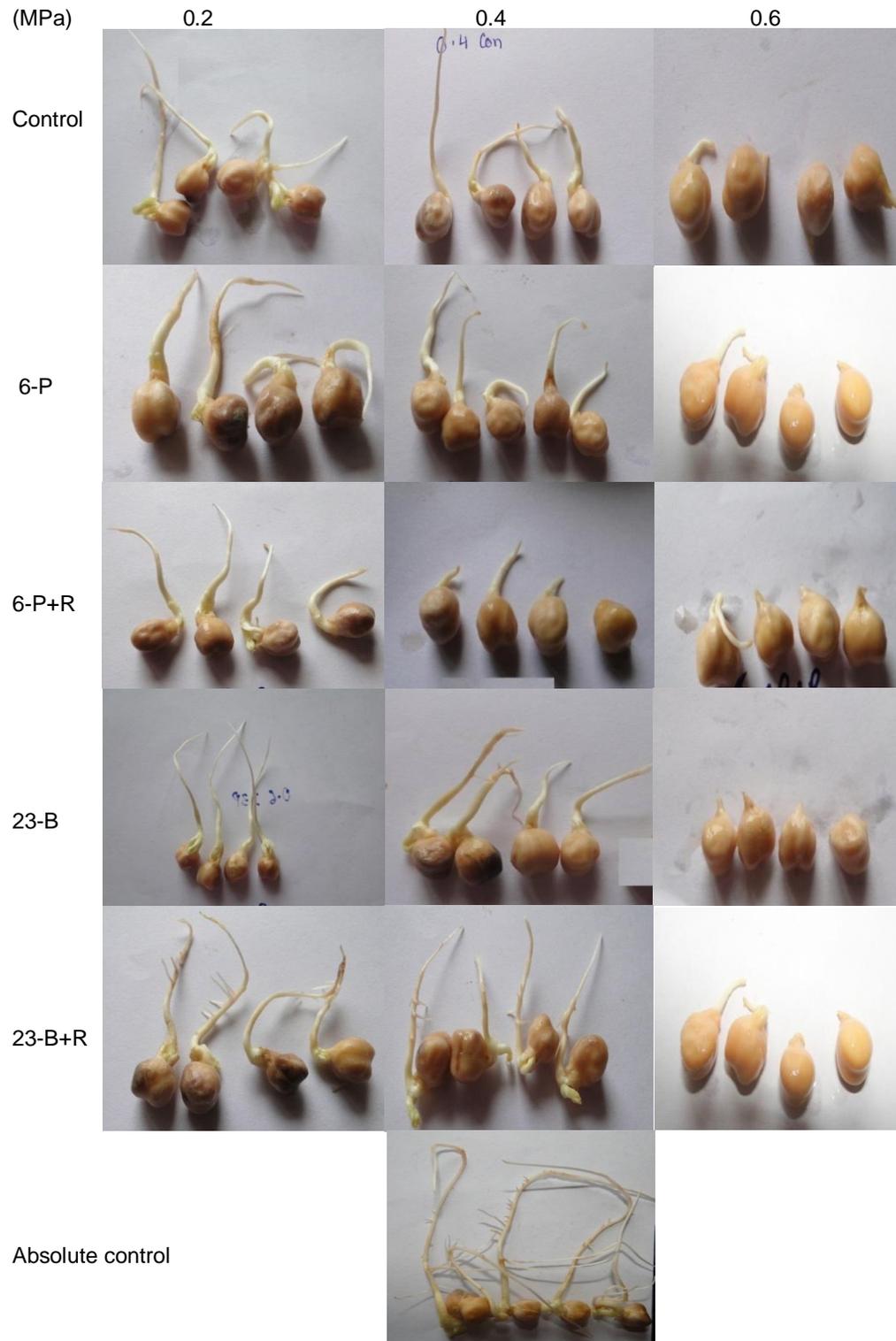


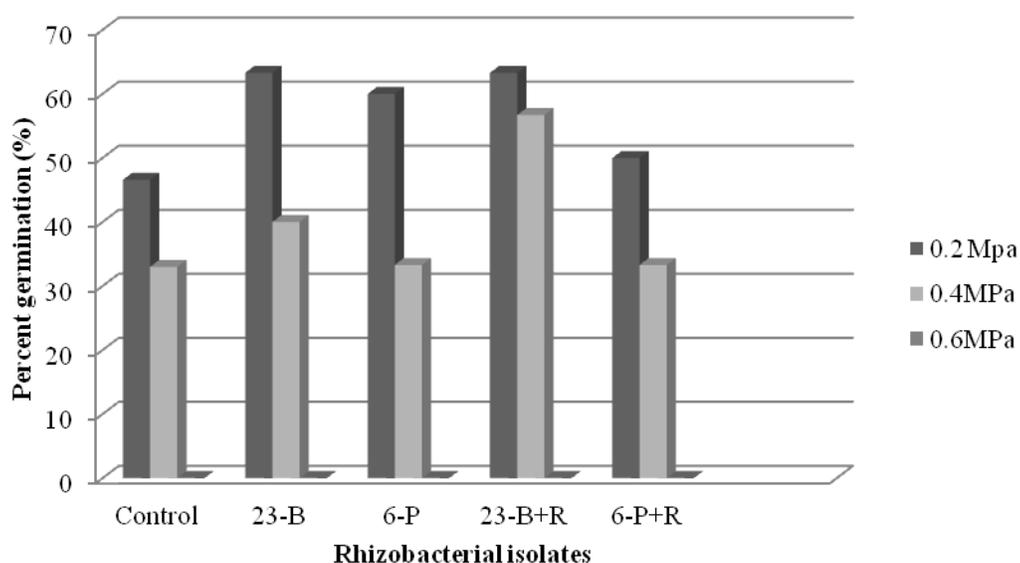
Figure 3. Effect of ACC-deaminase positive rhizobacteria on germination and growth of chickpea (L-552) under water stress

water stressed conditions, most probably through lowering of ethylene. The efficiency of application of such

inoculants under field conditions would further elucidate their potential in stress amelioration. Screening efficient

Table 4. Effect of ACC-deaminase positive rhizobacteria on root traits of chickpea GPF-2 under water stress.

Rhizobacteria	PGP trait					
	Root length (cm)			Root fresh weight (mg/seedling)		
	Doses of PEG (MPa)					
	0.2	0.4	0.6	0.2	0.4	0.6
Control	1.8	1.5	-	38.7	18.3	-
23-B	2.7	1.7	-	53.0	20.0	-
23B+R	2.8	2.3	-	45.3	21.0	-
6-P	2.3	-	-	49.7	-	-
6-P+R	3.0	3.1	-	42.0	32.0	-
Absolute control		2.6			40.7	
CD (P = 0.05)	NS	1.8	-	NS	13.7	-

**Figure 4.** Effect of ACC-deaminase positive rhizobacteria on germination of chickpea GPF-2 seeds under water stress.**Table 5.** Proline content in water stress induced radicles of chickpea.

Rhizobacteria	Proline content (mg/g fresh weight)	
	GPF-2	L-552
	6-P	0.32
6-P+R	0.52	0.60
23-B	0.80	0.66
23-B+R	0.38	0.42
Control*	0.19	0.21
Absolute control**	0.29	0.24

Data recorded at 0.2 MPa PEG induced water stress. *Uninoculated seeds under water stress. **Uninoculated seeds under stress free conditions.

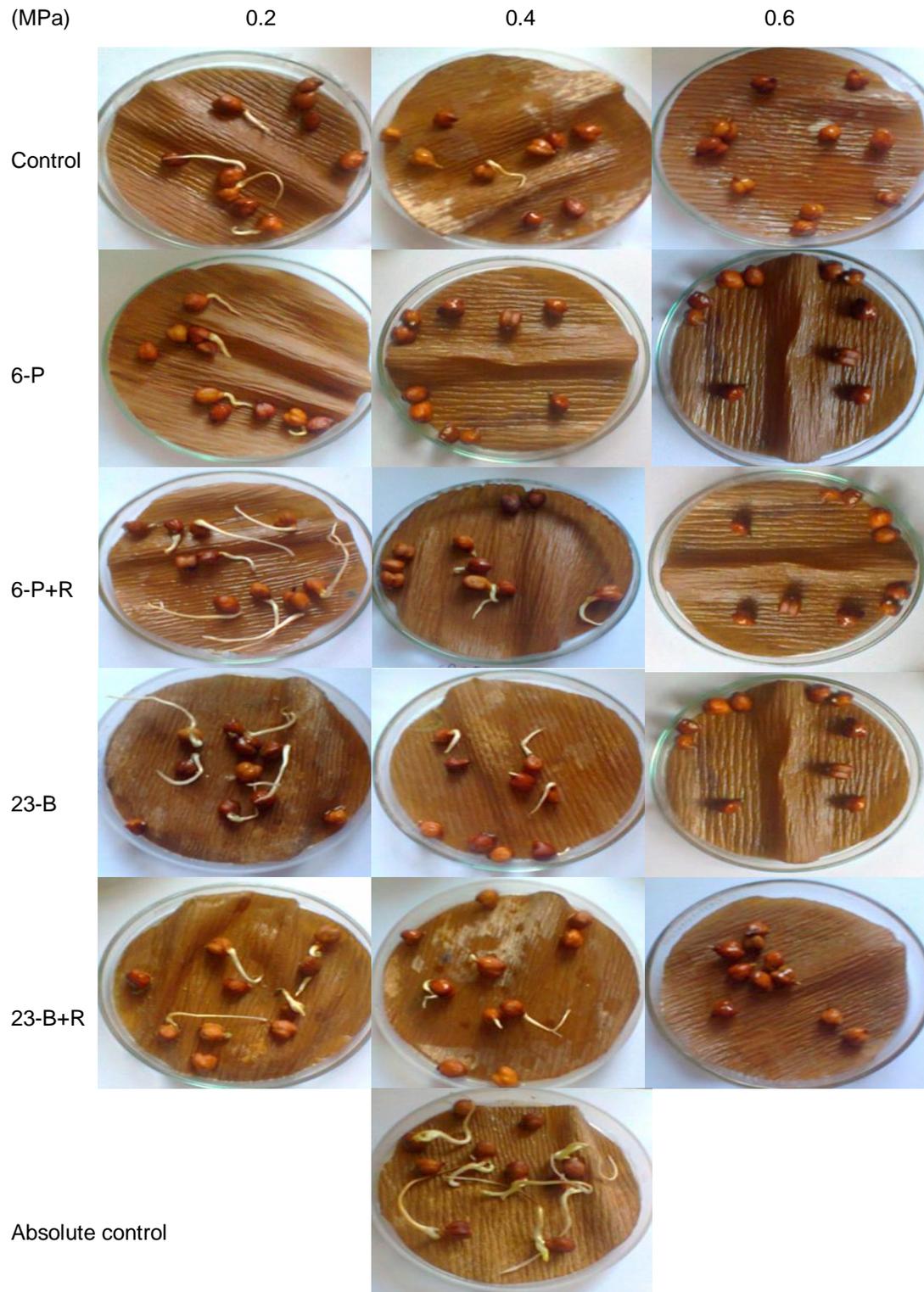


Figure 5. Effect of ACC-deaminase positive rhizobacteria on germination and growth of chickpea (GPF-2) under water stress.

PGPR's containing ACC-deaminase activity compatible with the environment and plant hostis thus a very useful

approach to enhance the nodulation and growth in legumes under stress conditions.

REFERENCES

- Bates LS, Waldeen RP, Teare ID (1973). Rapid determination of free proline in water stress studies. *Pl. Soil.* 39:205-207.
- Cappuccino JC, Sherman N (1992). In: *Microbiology: A Laboratory Manual*, New York. pp. 125-179.
- Dworkin M, Foster J (1958). Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.* 75:592-601.
- Etesami H, Alikhani HA, Jadidi M, Aliakbari A (2009). Effect of superior IAA producing rhizobia on N, P, K uptake by wheat grown under greenhouse condition. *World Appl. Sci. J.* 6:1629-1633.
- Food and Agricultural Organisation of the United Nations (2010). *FAO Statistical Database*. Available at <http://faostat.fao.org/FAO>, Rome.
- Gamalero E, Glick BR (2012). Ethylene and abiotic stress tolerance in plants. In: Ahmad P and Prasad MNV (eds) *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*, Springer-Verlag, Berlin. pp. 395-412.
- Glick BR, Cheng ZY, Czarny J, Duan J (2007). Promotion of plant growth by ACC deaminase producing soil bacteria. *Eur. J. Pl. Pathol.* 119:329-339.
- Gordon AS, Weber RP (1951). Calorimetric estimation of Indole acetic acid. *Plant Physiol.* 25:192-195.
- Jackson ML (1973). Phosphorus determination for soils. In: *Soil Chemical Analysis*. Prentice Hall India Pvt. Ltd, New Delhi. pp. 134-182.
- Lie Q, Saleh-Lakha S, Glick BR (2005). The effect of native and ACC deaminase-containing *Azospirillum brasilense* Cd1843 on the rooting of carnation cuttings. *Can. J. Microbiol.* 51:511-514.
- Macar TK, Turan O, Ekmekçi Y (2008). Effects of water deficit induced by PEG and NaCl on Chickpea (*Cicer arietinum* L.) cultivars and lines at early seedling stages. *G. U. J. Sci.* 22:5-14.
- Madhurendra PN (2007). Changes in certain organic metabolites during seedling growth of chickpea under salt stress and along with exogenous proline. *Leg. Res.* 30:41-44.
- Mahalakshmi M, Reetha D (2009). Assessment of plant growth promoting activities of bacterial isolates from rhizosphere of tomato (*Lycopersicon esculantum* L.). *Rec. Res. Sci. Technol.* 1:26-29.
- Mayak S, Tirosh T, Glick BR (2004). Plant growth promoting bacteria confers resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42:565-572.
- Mehta P, Chauhan A, Mahajan R, Mahajan PK, Shirkot CK (2010). Strain of *Bacillus circulans* isolated from apple rhizosphere showing plant growth promoting potential. *Curr. Sci.* 98:538-542.
- Mehta S, Nautiyal CS (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr. Microbiol.* 43:51-56.
- Mittler R, Kim Y, Song LH, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Steveson B, Zhu JK (2006). Gain and loss of function mutations in *Zat 10* enhance the tolerance of plants to abiotic stress. *FEBS Lett.* 580:6537-6542.
- Reed MLE, Glick BR (2005). Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. *Can. J. Microbiol.* 51:1061-1069.
- Sahu GK, Sindhu SS (2011). Disease control and plant growth promotion of green gram by siderophore producing *Pseudomonas* sp. *J. Microbiol. Res.* 6:735-749.
- Shaharouna B, Muhammad A, Rashid W, Khalid A (2012). Role of ethylene and plant growth-promoting rhizobacteria in stressed crop plants. In: Venkateswarlu B, Shanker AK, Shanker C, Maheswari M. (eds) *Crop stress and its management: Perspectives and Strategies*. Springer Science+ Business Media B.V. pp. 429-446.
- Shaharouna B, Arshad M, Zahir ZA, Khalid (2006). A Performance of *Pseudomonas* spp containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L) in the presence of nitrogenous fertilizer. *Soil Biol. Biochem.* 38:2971-2975.
- Singh AK (2004). The physiology of salt tolerance in four genotypes of chickpea during germination. *J. Agric. Sci. Technol.* 6:87-93.
- Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011). Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact.* 6:1-14.
- Verbruggen N, Hermans C (2008). Proline accumulation in plants: a review. *Amino Acids* 35:753-759.
- Yadav J, Verma JP, Tiwari KN (2010). Effect of plant growth promoting rhizobacteria on seed germination and plant growth Chickpea (*Cicer arietinum* L.) under *in vitro* conditions. *Biol. Forum* 2:15-18.
- Yasmin F, Othman R, Sijam K, Saad MS (2009). Characterization of beneficial properties of plant growth promoting rhizobacteria isolated from sweet potato rhizosphere. *Afr. J. Microbiol. Res.* 3:815-821.