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Screening rhizobacteria containing ACC-deaminase for growth promotion of chickpea seedlings under axenic conditions

S.M. Shahzad[1*], A. Khalid[2], M. Arshad[1] and Kalil-ur-Rehman[3]
[1]Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad
[2]Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi
[3]Department of Biochemistry, University of Agriculture, Faisalabad

Abstract

The use of beneficial bacteria as agricultural inputs for increasing crop production needs the selection of competent rhizobacteria with plant growth promoting attributes. Nodulation in chickpea plants is inhibited due to higher levels of ethylene production in the rhizosphere under stress conditions. Rhizobacteria having trait ACC-deaminase can facilitate plant growth to over come these deleterious effects. For this, isolation of rhizobacteria containing ACC-deaminase from the rhizosphere of chickpea plants grown in different districts of Punjab, were screened for growth promotion of chickpea seedlings under axenic conditions. All the rhizobacterial isolates had the potential to change the growth of chickpea seedlings under axenic conditions except few isolates which showed negative effect on root and shoot growth of chickpea seedlings. Results of jar study exhibited that inoculation with selected isolates increased the root length, shoot length, dry root weight, dry shoot weight, lateral root number, lateral root length and lateral root dry weight of chickpea seedlings up to 107.5, 57.4, 86.7, 83.5, 266.7, 286.6 and 121 %, respectively, over uninoculated control. It is concluded that the presence of ACC-deaminase enzyme activity could be a useful tool for screening effective rhizobacteria to promote seedlings growth of chickpea under controlled conditions before testing their effectiveness under natural environment.

Key words: rhizobacteria, ACC-deaminase, ethylene, chickpea

Introduction

The mechanisms through which rhizobacteria promote plant growth are not entirely understood, but are considered to include: the ability to produce phytohormones i.e. indoleacetic acid, gibberellic acid, cytokinins and ethylene (Mordukhova *et al.,* 1991; Arshad and Frankenberger, 1991; Glick *et al.,* 1995) asymbiotic nitrogen fixation (Kennedy *et al.,* 1997), production of siderophores (Scher and Baker, 1982), chitinase (Renwick *et al.,* 1991), antibiotics (Shanahan *et al.,* 1992) and cyanide (Flaishman *et al.,* 1996) and solubilization of mineral phosphates and other nutrients (De Freitas *et al.,* 1997; Nadeem *et al.,* 2006).

A number of studies on PGPR have shown growth promotion of plant but only under controlled (axenic) conditions (Glick *et al.,* 1995; Shaharoona *et al.,* 2006a) or in potted environment (Fuhrmann and Wollum, 1989; Shaharoona *et al.,* 2007a) where these bacteria do not compete with normal array of microbes. Recently, it has been revealed that certain plant growth promoting rhizobacteria having ACC-deaminase enzyme activity that changes ACC in to α-ketobutyrate and ammonia (Glick *et al.,* 1998; Tahir *et al.,* 2006; Arshad *et al.,* 2007) and reduce the amount of ACC as well as ethylene outside the germinating seeds. Reduced levels of ACC result in lowering the synthesis of endogenous ethylene, which lessen the inhibitory effects of higher ethylene levels (Glick *et al.,* 1998; Yuhashi *et al.,* 2000). However, ethylene is also identified to affect a number of features of root development and nodule formation (Ligero *et al.,* 1991), including its action as an inhibitor of nodulation (Hirsch and Fang, 1994).

Besides this, plants that are inoculated with rhizobacteria having ACC-deaminase are more resistant to the injurious effects of stress ethylene that is produced as a result of stressed environments such as flooding (Grichko and Glick, 2001), drought (Zahir *et al.,* 2007) and high salt concentration (Kausar and Shahzad, 2006; Nadeem *et al.,* 2007).

It is highly likely that presence of rhizobacteria having ACC-deaminase enzyme on the roots of legumes could reduce accelerated endogenous ethylene synthesis during the infection of rhizobium and thus may promote nodulation. So inoculation of legumes with competitive rhizobacteria having ACC-deaminase could be the most effective approach for growth promotion of chickpea seedlings under axenic as well as in field conditions. As the

*Email: crystalboy_uaf@yahoo.com

bacterial enzyme ACC-deaminase lowers the level of ethylene in roots, so inoculation with rhizobacteria having ACC-deaminase could be efficient and successful tool for promotion of growth and nodulation in chickpea. Keeping in view the above discussion, a study was conducted to isolate and screen the rhizobacteria under axenic conditions from different districts of Punjab.

Materials and Methods

Isolation of rhizobacteria containing ACC-deaminase

Rhizobacterial isolates were isolated from rhizosphere soil of the chickpea plants. Plants were collected from different locations of Faisalabad and Rawalpindi regions of Pakistan.

Plants of chickpea (45-60 days old) were uprooted and taken to the laboratory in polythene bags. Non-rhizosphere soil from chickpea plants was removed by gentle agitation of the roots and the soil strictly adhering to the roots (rhizosphere soil) was separated. The rhizobacteria were isolated by using minimal salt medium (MSM) containing ACC as sole nitrogen source (Glick *et al.,* 1994b). Dilution plate technique (Wollum II, 1982) was employed for isolation under aseptic conditions. The soil suspension was plated onto agar medium, and incubated at 30 ^{o}C. Twenty nine bacterial colonies with different growth characteristics (shape, size, colour and growth rate) were isolated and purified by further streaking on freshly prepared MSM plates and the pure cultures were used for further experiments.

Testing ability of rhizobacteria to use ACC as sole source of nitrogen

Five milliliter of ½ Tryptic soy broth (TSB) were inoculated with rhizobacterial isolates. The cultures were incubated for 48 h at 30 °C under shaking conditions (100 rpm). Cultures were diluted 10 times in sterilized 0.1 M $MgSO_4$ solution. In 96-well plate, 120 µL MSM were added to each well. In first 4 lanes, 15 µL 0.1 M $MgSO_4$, and in second 4 lanes, 15 µL 0.1 M $(NH_4)_2SO_4$ were added. The 3 mM ACC were filter sterilized with 0.2 µm membrane filter and was stored at -20 °C before the assay. This was allowed to thaw before use; 15 µL of thawed ACC were filled in the rest of the 4 lanes. For inoculation of each well, 15 µL bacterial culture were used. In untreated control wells, 15 µL 0.1 M $MgSO_4$ were used instead of inocula. Optical density (OD) was measured after 48 h at 600 nm using Biolog® identification system. The OD value of ACC and $(NH_4)_2SO_4$ wells were compared along with $MgSO_4$ wells to determine the ability of bacteria to utilize ACC for their growth as described by Jacobson *et al.* (1994).

The rhizobacterial isolates were grown on two N sources [ACC and $(NH_4)_2SO_4$], and one mineral source ($MgSO_4$) to observe the growth rate of each isolate for ACC substrate in parallel to $(NH_4)_2SO_4$. The rhizobacterial isolates were categorized into three groups, as isolates with higher (Group A), medium (Group B), and lower (Group C) ACC utilizing rate depending upon their OD values at 600 nm for ACC substrate as compared to $(NH_4)_2SO_4$. The isolates with higher ACC-utilization rate possessed OD value for the wells of ACC substrate similar/near to the OD value for the wells of $(NH_4)_2SO_4$ in the initial 48 h of growth. Similarly, isolates with medium ACC-utilization rate possessed lower OD value for ACC wells as compared to those for $(NH_4)_2SO_4$ in the initial 48 h of growth. Isolates with lower ACC-metabolic rate possessed the lowest OD value for ACC wells (near to the OD value of wells for $MgSO_4$) in the same time.

Screening rhizobacteria for their abilities to promote growth of chickpea

The inoculum of selected rhizobacteria was prepared by using MSM. For this purpose, MSM broth was prepared in 250 mL flasks separately and autoclaved at 121 ^{o}C for 20 min. Each broth after cooling was inoculated with respective bacterial isolates. All the flasks were incubated at 30 ^{o}C for 48 h in the orbital shaking incubator at 100 rpm. Optical density was measured at 600 nm by spectrophotometer and uniform cell density containing 10^8-10^9 CFU mL^{-1} was achieved. For all experiments, the inocula containing 10^8-10^9 CFU mL^{-1} were used however; fresh inoculum was prepared for each experiment.

Germination assay

Germination tests were carried out to determine the effect of inoculation with rhizobacteria having different ACC-utilization rates on seed germination. The chickpea (*Cicer arietinum* L.) cv. Bittal-98 seeds were surface-disinfected by dipping in 95% ethanol and 0.2% (w/v) $HgCl_2$ solutions as described by Russel *et al.* (1982) and Khalid *et al.* (2004a). The treated seeds were rinsed with sterilized water thoroughly. These seeds were inoculated by dipping in the rhizobacterial culture for 20 min. Six inoculated seeds were placed in each petri-plate containing soaked (with distilled water) filter papers. Uninoculated control was used for comparison. The Petri-plates were incubated at 25±2 ^{o}C for 10 days. Each treatment was replicated three times. Percent germination was calculated.

Jar experiment

Jar experiment was conducted in the growth room under axenic conditions for screening rhizobacteria containing ACC-deaminase for promoting root and shoot

growth of chickpea seedlings. Sterilized glass jars were used for the experiment.

The surface disinfected seeds of chickpea were placed in petri-plate containing soaked (with sterilizer distilled water) filter paper for germination. The Petri-plates were incubated at 25±2 ^{o}C for 4 days. Slightly germinated seeds were inoculated with relevant bacterial cell suspension (OD at 600 nm, 10^{8}-10^{9} CFU mL^{-1}) by dipping them for 10 min. Two sterilized filter paper sheets were saturated with the sterilized distilled water and then three inoculated slightly germinated seeds were placed in between the filter papers, which were rolled and put in sterilized glass jars. Uninoculated control was included for comparison. Jars were placed at 20±2 ^{o}C in the growth room using completely randomized design (CRD) with three replications for each treatment. Nutrients were supplied by adding 20 mL of sterilized Hoagland solution (½ strength) (Hoagland and Arnon, 1950). Light and dark period was adjusted to 10 and 14 h, respectively. After 15 days, the data regarding root and shoot growth were recorded. On the basis of screening experiment, six rhizobacterial isolates (J107, J108, J112, J118, J119 and J120) were selected based on their potential to promote root growth (root length, lateral root length and numbers, and dry root biomass) for further studies.

Statistical procedures were applied to analyze the data (Steel *et al.,* 1997) and means were compared by Duncan's Multiple Range Tests (Duncan, 1955).

Results

First isolated rhizobacteria were tested for their ability to utilize ACC as source of N and later on these were screened for improving growth of chickpea seedlings under axenic conditions.

Utilization of ACC as source of N by rhizobacteria

The ability of rhizobacterial isolates to utilize ACC as a source of N was assessed on the basis of bacterial growth. Test isolates utilized ACC as N source (i.e. positive for ACC-deaminase enzyme activity) but with different degrees of efficacy. These isolates were divided into three groups on the basis of their growth measured in terms of cell density (OD_{600}), (Table 1). Rhizobacterial isolates (J16, J18, J107, J108, J112, J118, J119 and J120) showing highest growth (OD > 0.75) by utilizing ACC as a N source were categorized as Group-H. Similarly, eleven isolates showing medium growth (OD 0.75-0.50), were placed in Group-M while ten isolates exhibiting least growth (OD < 0.50) were placed in Group-L.

Effect of rhizobacteria on germination of chickpea seeds

Inoculation with rhizobacterial isolates did not affect the germination percentage of chickpea seeds significantly; however, there was a strong effect of inoculation with some rhizobacterial isolates on the root growth of germinated seedlings (Table 2). Up to 75.2% increase in root length was observed in case of inoculation as compared to uninoculated control.

Effect of rhizobacteria on growth of chickpea seedlings under axenic conditions

Results of jar trial revealed that inoculation with rhizobacteria containing ACC-deaminase increased the root length up to 107.5% as compared to uninoculated control (Table 3). Six, the most effective rhizobacterial isolates (J107, J108, J112, J118, J119 and J120) increased root length ranging from 63.8 to 107.5% over uninoculated control. Three isolates had negative effect on root length (up to 8.1%) compared to uninoculated control while rest of the test isolates increased root length up to 59.6% compared with uninoculated control.

The maximum increase (86.7% greater than control) in root dry weight of chickpea seedlings was observed in response to inoculation with J119 (Table 3). Overall, 25 isolates had positive effect on root dry weight of chickpea seedlings ranging from 12.2 to 86.7% more than uninoculated control. Four isolates (J10, J17, J109 and J122) had negative effect on root dry weight and a decrease in root dry weight up to 8.8% was observed upon inoculation, compared to uninoculated control.

Inoculation with rhizobacteria containing ACC-deaminase increased the shoot length of chickpea seedlings up to 57.4% over uninoculated control (Table 3). The isolates J107, J108, J112, J118, J119 and J120 showed substantial increases in shoot length that were 20.9 to 57.4% higher than uninoculated control. Four isolates (J4, J105, J109 and J122) decreased the shoot length up to 8.3% as compared to control. The remaining isolates caused maximum increase in shoot length up to 23.9% over uninoculated control.

Increase in shoot dry weight in response to inoculation with rhizobacteria containing ACC-deaminase was found up to 83.5% as compared to uninoculated control (Table 3). The most promising increase was observed in case of inoculation with the rhizobacteria such as J107, J108, J112, J118, J119 and J120 that ranged from 67.1 to 83.5% over control. Isolate J119 was the most effective isolate among the tested 29 isolates. However, three isolates (J10, J109

and J122) gave negative effect and decreased the shoot dry weight up to 4.5% as compared to uninoculated control.

number of lateral roots of chickpea seedlings up to 266.7% more than uninoculated control. Rhizobacteria isolates J107, J108, J112, J118, J119 and J120 showed the most

Table 1. Rhizobacterial isolates showing variable growth (measured as OD) on the media containing ACC as sole N source

(Average of seven replications)

Rhizobacterial isolates	Grouping of isolates based on their ACC-utilization rate		
	Group-H O.D>0.75	Group-M O.D=0.75-0.50	Group-L O.D<0.50
J4			✓
J5		✓	
J6			✓
J7		✓	
J10		✓	
J14			✓
J15		✓	
J16	✓		
J17		✓	
J18	✓		
J19		✓	
J24		✓	
J26			✓
J27		✓	
J28			✓
J41			✓
J105			✓
J107	✓		
J108	✓		
J109		✓	
J112	✓		
J114			✓
J115		✓	
J117			✓
J118	✓		
J119	✓		
J120	✓		
J122		✓	
J127			✓

Table 2. Germination percentage and root elongation of chickpea as affected by inoculation with rhizobacterial isolates

(Average of six replications)

Bacterial isolate	Germination (%)	Root length (cm)	Means ± S.E.
		Range	
Control	88.0	-	1.69 ± 0.06
High	82.3	1.34 - 4.43	2.96 ± 0.08
Medium	85.0	1.32 - 2.98	2.14 ± 0.11
Low	82.1	1.35 - 3.90	2.58 ± 0.05

±: S.E (Standard error of means)

Results regarding number of lateral roots are presented in table 4. Overall, it was observed that inoculation with rhizobacteria containing ACC-deaminase increased the

prolific increases in number of lateral roots that were 133.3 to 266.7% higher in comparison to uninoculated control. Two isolates (J109 and J122) decreased the number of

lateral roots up to 50% as compared to control. Rest of the isolates caused increase in number of lateral roots that ranged from up to 33.3 to 116.7% over control.

J122) showed negative effect and decreased the lateral root length up to 55.8% as compared to uninoculated control.

Table 3. Effect of rhizobacterial inoculation on root and shoot growth of chickpea seedlings under axenic conditions

(Means of three replications)

Rhizobacterial Isolate	Root length (cm)	Root dry weight (g plant^{-1})	Shoot length (cm)	Shoot dry weight (g plant^{-1})
Control	13.64 ± 1.07†	0.178 ± 0.05†	15.60 ± 1.19†	0.167 ± 0.04†
J4	14.78 ± 1.10	0.200 ± 0.03	15.57 ± 1.06	0.177 ± 0.02
J5	19.04 ± 1.31	0.208 ± 0.05	17.33 ± 1.12	0.219 ± 0.07
J6	21.14 ± 1.43	0.280 ± 0.08	19.07 ± 1.21	0.268 v 0.04
J7	22.47 ± 1.29	0.215 ± 0.06	18.50 ± 1.10	0.199 ± 0.06
J10	20.04 ± 1.23	0.167 ± 0.03	19.03 ± 2.01	0.163 ± 0.07
J14	19.54 ± 1.18	0.287 ± 0.05	19.33 ± 1.16	0.267 ± 0.02
J15	17.04 ± 1.13	0.217 ± 0.03	17.67 ± 0.89	0.172 ± 0.03
J16	20.84 ± 1.37	0.256 ± 0.07	18.53 ± 1.03	0.200 ± 0.04
J17	21.54 ± 1.53	0.162 ± 0.04	19.32 ± 1.24	0.252 ± 0.06
J18	22.84 ± 1.83	0.273 ± 0.05	18.47 ± 1.13	0.258 ± 0.07
J19	19.74 ± 1.61	0.281 ± 0.04	18.77 ± 0.96	0.266 ± 0.08
J24	17.44 ± 0.97	0.281 ± 0.03	17.93 ± 1.39	0.269 ± 0.05
J26	21.04 ± 1.08	0.295 ± 0.02	19.47 ± 1.41	0.236 ± 0.03
J27	18.84 ± 1.31	0.291 ± 0.02	18.22 ± 1.31	0.255 ± 0.04
J28	15.34 ± 1.12	0.285 ± 0.01	19.83 ± 1.51	0.271 ± 0.06
J41	15.94 ± 1.21	0.251 ± 0.06	18.10 ± 0.93	0.231 ± 0.01
J105	13.30 ± 0.86	0.258 ± 0.09	15.57 ± 1.03	0.238 ± 0.03
J107	**28.30 ± 2.31**	**0.314 ± 0.05**	**18.87 ± 1.43**	**0.289 ± 0.04**
J108	**21.34 ± 2.14**	**0.321 ± 0.06**	**19.67 ± 1.63**	**0.292 ± 0.06**
J109	12.90 ± 1.03	0.167 ± 0.07	14.30 ± 1.01	0.159 ± 0.03
J112	**22.54 ± 2.37**	**0.297 ± 0.09**	**19.60 ± 2.11**	**0.274 ± 0.05**
J114	20.34 ± 2.01	0.288 ± 0.04	18.93 ± 2.03	0.247 ± 0.06
J115	17.64 ± 1.10	0.268 ± 0.03	16.17 ± 1.16	0.231 ± 0.02
J117	14.34 ± 1.13	0.257 ± 0.03	16.31 ± 1.13	0.211 ± 0.04
J118	**23.63 ± 2.59**	**0.300 ± 0.02**	**19.42 ± 1.89**	**0.280 ± 0.07**
J119	**27.80 ± 2.68**	**0.332 ± 0.08**	**24.55 ± 1.96**	**0.306 ± 0.08**
J120	**27.38 ± 1.97**	**0.318 ± 0.04**	**21.97 ± 1.51**	**0.293 ± 0.03**
J122	12.54 ± 0.96	0.167 ± 0.05	15.30 ± 1.31	0.160 ± 0.04
J127	15.12 ± 1.10	0.243 ± 0.03	18.81 ± 1.41	0.221 ± 0.06

†: Standard error of mean

Standard error of difference between two means: Root length: 2.163; Root dry weight: 0.032; Shoot length: 1.137; Shoot dry weight: 0.029; P value: 0.05

The increase in lateral root length caused by inoculation with rhizobacteria containing ACC-deaminase was recorded up to 286.6% as compared to uninoculated control (Table 4). The most prominent increase was found in response to inoculation with the rhizobacteria such as J107, J108, J112, J118, J119 and J120 that ranged from 198.3 to 286.6% over uninoculated control. Isolate J119 was the most effective isolate of rhizobacteria among the tested 29 isolates. However, three isolates (J105, J109 and

The maximum increase (121.1% higher than control) in lateral root dry weight of chickpea seedlings was recorded through inoculation with J119 (Table 4). In general, 27 isolates had positive effect on lateral root dry weight ranging from 5.3 to 121.1% higher compared to uninoculated control. Two isolates (J109 and J122) had negative effect on lateral root dry weight and a decrease in lateral root dry weight up to 26.3% was found upon inoculation compared to uninoculated control.

Table 4: Effect of rhizobacterial inoculation on lateral root growth of chickpea seedlings under axenic conditions

(Means of three replications)

Rhizobacterial Isolate	Number of lateral roots	Lateral root length (cm)	Lateral root dry weight (cm)
Control	6 ± 1.02†	2.31 ± 0.10†	0.019 ± 0.02†
J4	8 ± 1.05	3.52 ± 0.13	0.025 ± 0.03
J5	9 ± 1.01	4.12 ± 0.09	0.024 ± 0.02
J6	12 ± 1.41	5.02 ± 0.02	0.035 ± 0.04
J7	9 ± 1.32	2.62 ± 0.11	0.027 ± 0.03
J10	10 ± 1.40	3.12 ± 0.10	0.022± 0.01
J14	14 ± 1.01	5.79 ± 0.13	0.033 ± 0.05
J15	11 ± 1.56	4.29 ± 0.14	0.021 ± 0.03
J16	12 ± 1.61	3.85 ± 0.09	0.032 ± 0.04
J17	10 ± 1.06	3.45 ± 0.11	0.020 ± 0.01
J18	13 ± 1.23	4.56 ± 0.15	0.030 ± 0.05
J19	12 ± 1.11	5.12 ± 0.08	0.029 ± 0.03
J24	11 ± 1.08	3.28 ± 0.10	0.035 ± 0.06
J26	13 ± 1.32	4.09 ± 0.07	0.034 ± 0.04
J27	11 ± 1.41	4.15 ± 0.11	0.028 ± 0.03
J28	14 ± 1.54	4.39 ± 0.09	0.036 ± 0.05
J41	10 ± 1.29	2.91 ± 0.10	0.030 ± 0.02
J105	9 ± 1.02	1.94 ± 0.07	0.032 ± 0.03
J107	**14 ± 1.65**	**6.89 ± 0.13**	**0.039 ± 0.06**
J108	**16 ± 2.01**	**7.12 ± 0.11**	**0.038 ± 0.04**
J109	3 ± 0.62	1.02 ± 0.05	0.014 ± 0.03
J112	**16 ± 1.68**	**7.63 ± 0.09**	**0.037 ± 0.02**
J114	12 ± 0.96	2.89 ± 0.11	0.033 ± 0.02
J115	10 ± 1.12	3.16 ± 0.10	0.029 ± 0.03
J117	11 ± 1.05	3.06 ± 0.09	0.032 ± 0.04
J118	**17 ± 2.13**	**7.23 ± 0.14**	**0.038 ± 0.05**
J119	**22 ± 1.89**	**8.93 ± 0.11**	**0.040 ± 0.04**
J120	**20 ± 1.64**	**8.51 ± 0.13**	**0.042 ± 0.03**
J122	4 ± 0.72	1.89 ± 0.07	0.016 ± 0.02
J127	10 ± 0.89	3.08 ± 0.10	0.023 ± 0.04

†: Standard error of mean;

Standard error of difference between two means: Number of lateral roots: 2.637; Lateral root length: 0.020; Lateral root dry weight: 0.0037; P value: 0.05

Discussion

A series of laboratory studies were conducted under controlled (axenic) conditions to screen effective strains of rhizobacteria with ACC-deaminase activity for promoting growth and nodulation of chickpea. First of all, ability of rhizobacterial isolates to utilize ACC as sole source of N was confirmed by monitoring their growth in ACC-deaminase assay. All the rhizobacterial isolates, originated from the rhizosphere soil of chickpea were capable of utilizing ACC and variable growth rates were shown on MSM containing ACC as sole N source (Table 1). This may imply that ACC-deaminase enzyme in different rhizobacteria might have variable potential to hydrolyze ACC and, thus could have differential affect on growth of inoculated plants. Several bacterial species belonging to different genera such as *Azospirillum, Agrobacterium, Achromobacter, Burkholderia, Enterobacter, Pseudomona*s and *Ralstonia* have been reported to possess variable ACC-deaminase activity (Nukui *et al.,* 2000; Arshad *et al.,* 2008).

Plate (germination assay) and jar (root and shoot growth) experiments were conducted for evaluating growth promoting activities of rhizobacteria containing ACC-deaminase under axenic conditions. Inoculation with rhizobacteria containing ACC-deaminase did not affect germination of chickpea significantly (Table 2). Since ethylene is required for germination of chickpea seeds (Gallardo *et al.,* 1995; Matilla and Matilla-Vazquez, 2008), so germination was slightly decreased in response to inoculation with rhizobacteria containing ACC-deaminase compared to uninoculated control. However, in this study, a

significant increase in root length and weight was observed in the case of inoculated plants compared with uninoculated control. It is highly likely that rhizobacteria promoted root growth by lowering ethylene levels in plant and/or in the vicinity of roots because of their ACC-deaminase activity. This premise is further supported by the results obtained from jar experiment (Table 3) in which observed root growth was much better in the plants inoculated with the rhizobacteria containing ACC-deaminase than the uninoculated control. These results are in agreement with the findings of many researchers who reported better root growth in plants inoculated with bacteria containing ACC-deaminase (Glick *et al.,* 1995; Mayak *et al.,* 2004a; Shaharoona *et al.,* 2006a). A direct correlation has been found between in vitro bacterial ACC-deaminase activity and root growth (Mayak *et al.,* 2004a; Shaharoona *et al.,* 2006a).

In this study, it was also observed that rhizobacteria containing ACC-deaminase were highly effective in promoting number of lateral roots, lateral root length and root dry weight of chickpea seedlings, in addition to improving roots length. These changes in root architecture of the inoculated plants could be attributed to bacterial ACC-deaminase activity as described earlier. Furthermore, a tremendous positive effect on shoot growth by rhizobacteria containing ACC-deaminase was also observed. A significant direct relationship has been observed between root and shoot growth (Shaharoona *et al.,* 2006a). Ghosh *et al.* (2003) reported that rhizobacteria containing ACC-deaminase promoted plant growth, while root length was significantly increased under axenic conditions. Similar kind of findings have been documented by other researchers (Dodd *et al.,* 2004; Sergeeva *et al.,* 2006). These findings may imply that rhizobacteria with ACC-deaminase activity could prove to be effective inoculants for improving growth of chickpea plants.

Some rhizobacterial isolates were not capable of promoting growth of chickpea seedlings. It means that the presence of rhizobacteria in the rhizosphere can have a neutral, detrimental or beneficial effect on plant growth. The presence of neutral rhizobacteria in the rhizosphere probably has no effect on plant growth. Deleterious rhizobacteria are presumed to adversely affect plant growth and development through the production of metabolites like phytotoxins but also through competition for nutrients (Nehl *et al*., 1996). However, Klopper *et al.* (2004) also reported the problems associated with early research work on deleterious rhizobacteria, resulting from the use of soil-less systems lacking competition from native soil and rhizosphere bacteria, and from the use of a very high number of bacteria to inoculate plants.

It is concluded that presence of ACC-deaminase enzyme activity could be a useful tool for screening effective inoculants (i.e. rhizobacteria) to promote seedlings growth of chickpea under controlled conditions before testing their efficacy under natural environment.

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