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RHIZOBACTERIA CONTAINING ACC-DEAMINASE FOR IMPROVING GROWTH AND YIELD OF WHEAT UNDER FERTILIZED CONDITIONS

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Abstract

Certain plant growth promoting rhizobacteria (PGPR) regulate the production of ethylene in plants and promote root elongation by hydrolyzing 1-aminocyclopropane-1-carboxylic acid, ACC (the immediate precursor of ethylene), through the action of ACC-deaminase. Rhizobacteria containing ACC-deaminase were isolated and screened for their growth promoting activity in wheat (*Triticum aestivum* L.) under axenic conditions. Three promising isolates (S5, S7 and S9) were selected on the basis of ACC-deaminase activity and growth promotion under axenic conditions. The performance of these selected PGPR isolates was evaluated with 50 and 75% of recommended (NPK: 120-100-60 kg ha^{-1}) chemical fertilizers for promoting growth and yield of wheat. Inoculated seeds were sown in the field, fertilized with NPK according to the treatments. All the isolates exhibited a significant increase in all the yield-contributing parameters at 50 and 75% of recommended chemical fertilizers compared with untreated control. It was observed that PGPR isolate S7, along with 75% of recommended chemical fertilizers, showed statistically similar results with recommended chemical fertilizers alone and it increased plant height, number of tillers $meter^{-2}$, spike length, number of spikelets $spike^{-1}$, total biomass, grain yield and 1000-grain weight by 11, 63, 22, 28, 98, 96 and 31%, respectively, over untreated control. Other attributes like NPK uptake were also increased (141, 160 and 174%, respectively) over untreated control. The growth promoting activity exhibited by the rhizobacterial isolate S7 might be due to its high *In vitro* IAA production, chitinase activity, P-solubilition and more intensive root colonization, besides ACC-deaminase activity. Results suggested that plant growth promoting rhizobacteria containing ACC-deaminase activity could be used as successful inoculant for improving wheat yield by reducing dependence on chemical fertilizers and saving ~ 25% of recommended chemical fertilizers. However, some other characteristics of the rhizobacteria such as root colonization ability and chitinase activity could also be used as an effective tool for selection/screening of efficient PGPR strains.

Introduction

Annually, more than 143.88 million nutrient tonnes of chemical fertilizers are used worldwide to increase the yield of crop plants (Anon., 2006). Despite their efficiency in promoting crop yield, they can, under certain circumstances, pollute the environment and contribute to a number of human and animal health problems.

The potential negative environmental impact of the large-scale use of chemical fertilizers together with their increased cost has prompted a number of scientists worldwide to consider the possibility of supplementing chemical fertilizers with microbial, self propagating sources of important plant nutrients. In recent years, researchers have turned their attention to promising bacterial species that occupy the rhizosphere. The use of bacterial inoculants in agriculture is predicted to increase, as the necessity to protect the environment and consumer leads to reduction in chemical inputs.

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Rhizobacteria that are beneficial to plants are often referred to as plant growth promoting rhizobacteria (Kloepper *et al*., 1989). They can affect plant growth either directly or indirectly through various mechanisms of action (Glick *et al*., 1998; Persello-Cartieaux *et al*., 2003; Mantelin & Touraine, 2004). Indirect growth promotion occurs when PGPR promote plant growth by improving growth restricting conditions (Glick *et al*., 1999). Direct promotion of plant growth by plant growth-promoting bacteria generally involves providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients.

There are several ways in which plant growth-promoting bacteria can directly facilitate the proliferation of their plant hosts. They may: fix atmospheric nitrogen; solubilize minerals such as phosphorus; produce siderophores, which can solubilize and sequester iron, and provide it to plant; synthesize phytohormones, including auxins, cytokinins, and gibberellins, which can enhance various stages of plant growth; and synthesize enzymes that can modulate plant growth and development (Brown, 1974; Davison, 1988; Kloepper *et al*., 1989; Lambert & Joos, 1989; Patten & Glick, 1996; Glick *et al*., 1999). A bacterium can affect plant growth by one or more of these mechanisms, and also use different abilities for growth promotion at various times during the life cycle of the plant (Glick *et al*., 1999).

Ethylene, an important growth hormone, which is produced in almost all plants, mediates a wide range of different plant responses and developmental processes (Arshad & Frankenberger, 2002; Belimove *et al*., 2002). The presence of ethylene may be, in some instances stimulatory, while in others it is inhibitory depending upon its concentration, nature of physiological process and growth phase of plant. Any factor/stimulus, which causes a change in the endogenous levels of ethylene in a plant tissue, may modify growth and development (Arshad & Frankenberger, 2002).

In a number of different plants, ethylene stimulates germination and breaks the dormancy of the seeds (Esashi, 1991) but if the level of ethylene following germination is too high, root elongation is inhibited (Jackson, 1991). Glick *et al*., (1998) have proposed that certain PGPR can regulate the production of ethylene in developing seedlings through the action of ACC-deaminase and function as a sink for 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene in higher plants, by hydrolyzing it into α-ketobutyrate and ammonia, and consequently promote root growth.

The present study was aimed to investigate the potential of PGPR with ACC-deaminase activity as inoculant for the regulation of endogenous ethylene in wheat that could enhance the crop yield at different rates (50 and 75%) of recommended chemical fertilizers under field conditions.

Materials and Methods

Isolation and screening under axenic conditions: Rhizobacteria were isolated from the wheat rhizosphere by dilution plate technique using DF salt minimal medium (Dworkin & Foster, 1958) containing ACC as a sole source of nitrogen. Further streaking on fresh plates purified the rhizobacterial strains. These cultures were stored at $4 \pm 1^{o}C$ on slants and maintained by transferring them on fresh slants weekly. These isolates were tested for improving growth of wheat seedlings under axenic conditions. The inoculum was prepared by growing the rhizobacterial strains in 250-mL flasks containing DF minimal salt medium. The flasks were incubated at $28 \pm 1^{o}C$ for 48 h in the orbital shaking incubator at 100-rev min^{-1}. Gnotobiotic study was conducted for the screening of effective ACC-deaminating rhizobacterial isolates as described by Asghar *et al*., (2004).

Two sterilized filter paper sheets were soaked and saturated in suspension containing desired inoculum (OD_{550}: 0.5). Wheat seeds were surface sterilized by dipping in 95% ethanol solution for few seconds and 0.2% $HgCl_2$ solution for 3 min followed by washing thoroughly with sterilized water. These surface sterilized seeds were sandwiched in between soaked filter papers and were rolled and placed in sterilized glass jars. In case of uninoculated control, sterilized broth was used. Sterilized Hoagland solution was applied in the jars for providing nutrients to seedlings. Treatments in each jar were arranged using completely randomized design with three replications of each treatment. Jars were placed in growth chamber at 25 ± 1°C in dark and after germination adjusted to 14 hours light and 10 hours dark period. Data regarding root length, shoot length and fresh weights of seedling (root+shoot) were recorded after 15 days. Three promising isolates (S5, S7 and S9) showing prolific growth under axenic conditions were selected for further experimentation.

Characterization of the selected isolates: ACC-deaminase activity of the selected isolates was determined by monitoring the amount of ammonia generated due to hydrolysis of ACC by the rhizobacterial isolates containing ACC-deaminase (Shahaharoona *et al*., 2006). *In vitro* auxin production as indole acetic acid (IAA) equivalents by these isolates was determined in the presence and absence of L-tryptophan (an auxin precursor) by using the protocol described by Sarwar *et al*., (1992). Phosphorus solubilizing activity was determined according to qualitative method described by Mehta & Nautiyal (2001). Chitinase activity of the selected rhizobacteria was determined as described by Chernin *et al*., (1998). Root colonization ability of the isolates in wheat was studied under axenic conditions as described by Simons *et al*., (1996). The rhizobacterial isolate exhibiting the highest growth promoting activity under field conditions (S7) was identified as *Pseudomonas fluorescens* by Biolog ® identification system (Micro log ™ system Release 4.2, Hayward, CA, USA).

Field trial: A field trial was conducted in the research area, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad to assess the efficacy of selected rhizobacteria containing ACC-deaminase under fertilized conditions.

Seed inoculation: For inoculation, the desired suspension of inoculum was mixed with sterile peat (seed to peat ratio 1.25:1 w/w) and incubated for 24 h at 28 ± 1°C before using it for seed coating. Seed dressing was done with the inoculated peat mixed with 10% sterilized sugar solution. In the case of uninoculated control, seeds were coated with the sterilized (autoclaved) peat treated with sterilized broth and 10% sterilized sugar solution. Inoculated seeds were placed over night for drying.

Recommended doses of NPK @ 120-100-60 kg ha^{-1} was applied as urea, single super phosphate (SSP) and muriate of potash (MOP) according to the treatments. Treatments were replicated four times using randomized complete block design. Fertilizers, P and K were applied as basal dose while N was applied in splits. Canal water was used for irrigation.

Inoculated and uninoculated seeds were sown in plots (5 m x 2 m) with sandy clay loam soil (typic haplocambids), having physico-chemical properties: pH 7.8; electrical conductivity, 3.5 dS m^{-1}; organic matter, 0.78%; total nitrogen, 0.05%; available phosphorus, 8.7 mg kg^{-1} and extractable potassium, 127 mg kg^{-1}.

The field trial included the following treatment plan with four replications of each.

Control (no fertilizers, no inoculation)
Recommended chemical fertilizers (NPK)
Isolate S5 + 50% of recommended chemical fertilizers
Isolate S7 + 50% of recommended chemical fertilizers
Isolate S9 + 50% of recommended chemical fertilizers
Isolate S5 + 75% of recommended chemical fertilizers
Isolate S7 + 75% of recommended chemical fertilizers
Isolate S9 + 75% of recommended chemical fertilizers

At maturity (172 days old), data regarding plant height, number of tillers meter^{-2}, spike length, number of spikelets spike^{-1}, total biomass, grain yield, straw yield and 1000-grain weight were recorded. Grain and straw samples were collected for NPK uptake. The data were statistically analyzed (Steel *et al*., 1997) and means were compared by Duncan's multiple range test (Duncan, 1955).

Results

Screening under axenic conditions: Results from axenic study revealed that all the ACC-deaminase enriched rhizobacterial isolates significantly increased root length, shoot length and seedling fresh weight as compared to control (Fig. 1a, b, c). Regarding root length (Fig. 1a), all the isolates significantly increased root length up to 2.6 fold, except S1 and S8, as compared to control. Isolate S9 proved to be the most effective in promoting root length and caused an increase of 2.6 fold over uninoculated control. Next effective isolates S7, S5 and S6 produced 2.5, 2.2 and 2.0 fold increase over uninoculated control, respectively.

Similarly, in case of shoot length (Fig. 1b) 2.4 fold increase was recorded by inoculation with isolate S5 in wheat seedlings under axenic conditions. It was followed by S7 and S9, which showed 1.95 and 1.8 fold increase over control.

Seedlings inoculated with isolates containing ACC-deaminase had significant increase in fresh weight of seedlings (root+shoot) i.e., 1.0-5.2 fold increase over uninoculated control (Fig. 1c). In this trial, 5.2 fold increase over control was observed by inoculation with isolate S7. Isolate S9 and S5 resulted in 5.0 and 4.2 fold higher seedling fresh weight over control. Isolates S5, S7 and S9 were selected on the basis of seedling growth under axenic conditions for field trial.

Field trial: Results of field trial revealed that inoculation of wheat seeds with PGPR containing ACC-deaminase activity significantly affected the growth and yield of wheat under fertilized conditions.

Maximum plant height (10.5% higher over uninoculated control) was recorded with isolate S7 at 75% of chemical fertilizers, where as 9.5% increase was recorded with recommended chemical fertilizers (Table 1). It was followed by isolates S9 and S5 along with 75% of chemical fertilizers, which was statistically similar with 100% chemical fertilizers. However, isolate S7 at 50% of chemical fertilizers showed 7.1% increase over uninoculated control that was statistically non- significant with isolate S5 at 75% of chemical fertilizers but significant with isolates S9 and S5 (5.3 and 4.9%, over uninoculated control, respectively) at 50% of chemical fertilizers.

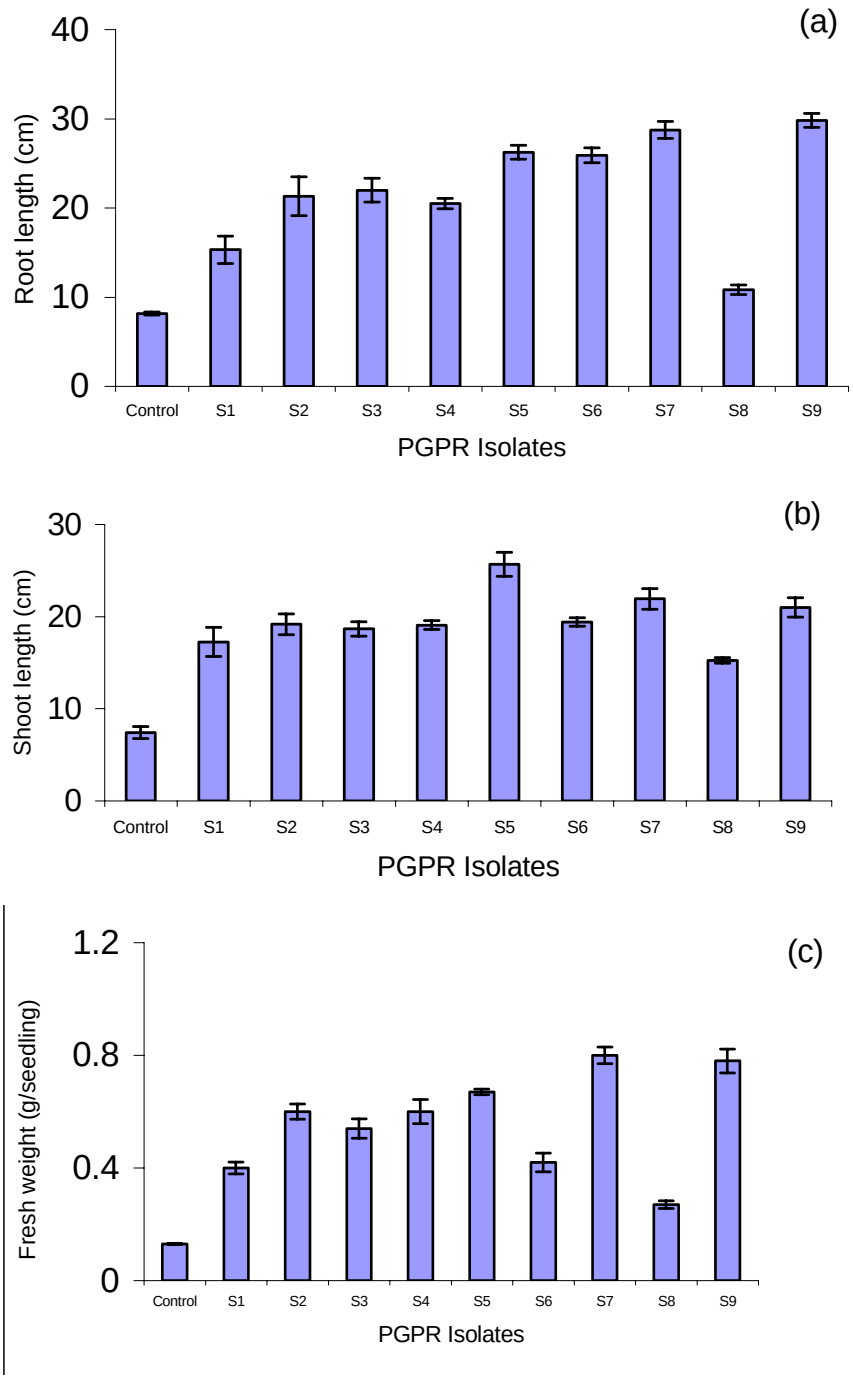

Fig. 1. Effect of inoculation with ACC-deaminase containing rhizobacteria on a) root length, b) shoot length and c) seedling fresh weight in wheat seedlings under axenic conditions.

Table 1. Effect of inoculation with rhizobacteria containing ACC-deaminase on plant height, tillers m^{-2}, spike length and number of spikelets $spike^{-1}$ of wheat (Average of four replications).

Treatments	Plant height (cm)	No. of tillers m^{-2}	Spike length (cm)	No. of spikelets $spike^{-1}$
Control[a]	94.51 e*	235 d	13.34 e	14.24 e
Recommended NPK[b]	103.5 ab	388.5 a	15.73 ab	18.10 a
Isolate S5 + 50% NPK	99.53 d	333.3 c	14.63 d	16.38 d
Isolate S7 + 50% NPK	101.2 c	348.3 bc	15.12 bcd	17.15 bc
Isolate S9 + 50% NPK	99.19 d	330.3 c	14.81 cd	17.0 cd
Isolate S5 + 75% NPK	102.2 bc	365.3 b	15.28 bc	17.85 ab
Isolate S7 + 75% NPK	104.5 a	384.8 a	16.25 a	18.25 a
Isolate S9 + 75% NPK	102.9 ab	370.0 ab	15.33 bc	18.05 a

*Means sharing similar letter(s) do not differ significantly at p = 0.05
[a]No fertilizer, No inoculation
[b]NPK @ 120-100-60 kg ha^{-1}

Table 2. Effect of inoculation with rhizobacteria containing ACC-deaminase on grain yield, plant biomass, straw yield and 1000-grain weight of wheat (Average of four replications).

Treatments	Grain yield (t ha^{-1})	Total biomass (t ha^{-1})	Straw yield (t ha^{-1})	1000-grain weight (g)
Control[a]	2.82 e*	6.69 e	3.87 f	35.42 d
Recommended NPK[b]	5.42 ab	13.30 a	7.88 a	45.45 ab
Isolate S5 + 50% NPK	4.83 d	11.16 d	6.33 e	40.59 c
Isolate S7 + 50% NPK	5.19 c	12.17 c	6.98 d	44.24 b
Isolate S9 + 50% NPK	4.97 d	11.53 d	6.55 e	41.20 c
Isolate S5 + 75% NPK	5.35 bc	12.62 bc	7.24 cd	45.14 ab
Isolate S7 + 75% NPK	5.54 a	13.24 a	7.70 ab	46.42 a
Isolate S9 + 75% NPK	5.39 ab	12.85 ab	7.46 bc	45.16 ab

*Means sharing similar letter(s) do not differ significantly at p = 0.05
[a]No fertilizer, No inoculation
[b]NPK @ 120-100-60 kg ha^{-1}

The effectiveness of all the treatments for increasing number of tillers m^{-2} is clearly evident from Table 1. Recommended chemical fertilizers gave maximum number of tillers m^{-2} (65% more than untreated control), which was statistically equal with isolate S7 (63% more than uninoculated control) and also isolate S9 (57.45% more than uninoculated control) at 75% of chemical fertilizers. Isolate S7 at 50% of chemical fertilizers showed 48.2% increase over uninoculated control that was non-significant with isolates S9 and S5 at 50 and 75% of chemical fertilizers.

Data regarding spike length are given in (Table 1). Maximum spike length (22% more than uninoculated control) was observed with isolate S7 at 75% of chemical fertilizers, whereas statistically similar (18% increase over untreated control) spike length was recorded with 100% chemical fertilizers alone. Next better results were observed with isolates S9 and S5 (i.e., 14.9 and 14.5% increase over uninoculated control) at 75% of chemical fertilizers. Isolate S9 and S5 with 50% of chemical fertilizers statistically remained non-significant with each other (11 and 9.67% increase over uninoculated control) while isolate S7 even at 50% of chemical fertilizers showed statistically non-significant results with isolates S9 and S5 at 75% of chemical fertilizers.

Data in Table 1 showed that all the treatments significantly increased number of spikelets spike^{-1} of wheat over untreated control. In general, statistically similar effect was observed in case of isolates S7, S9 and S5 at 75% of chemical fertilizers and at 100% chemical fertilizers alone. Isolate S7 statistically remains non-significant with S9 (20.4 and 19.38% increase over uninoculated control, respectively) at 50% of chemical fertilizers while isolate S9 remains non-significant with S5 (15% increase over uninoculated control) at 50% of chemical fertilizers.

A significant increase (71-96%) in grain yield was observed by different treatments over uninoculated and unfertilized control (Table 2). The effect of inoculation with isolate S7 at 75% of chemical fertilizers was found more pronounced (96% more than untreated control) as compared with all other treatments. Isolate S9 with 75% of chemical fertilizers showed statistically similar effect compared to 100% chemical fertilizers (91.13 and 92% more than untreated control, respectively). However, isolate S7 even with 50% of chemical fertilizers showed statistically non-significant effect compared with S5 at 75% of chemical fertilizers (84 and 89% more than untreated control, respectively). Isolates S9 and S5 at 50% of chemical fertilizers showed statistically similar results i.e. 76.24 and 71.28% more than untreated control, respectively.

Integrated use of PGPR isolates with chemical fertilizers significantly increased total biomass (Table 2) of wheat. However, maximum biomass (99% increase over untreated control) was recorded in response to the application of 100% chemical fertilizers that was statistically non-significant to the response of isolates S7 and S9 at 75% of chemical fertilizers (98 and 92% increase over untreated control, respectively). Similar to grain yield, isolate S7 at 50% chemical fertilizer showed that total biomass was statistically non- significant compared to isolate S5 at 75% of chemical fertilizers (81.91 and 88.64% increase over untreated control, respectively). Similar results were recorded with isolates S9 and S5 at 50% of chemical fertilizers (72.35 and 66.82% more than untreated control, respectively) that were non-significant with each other but highly significant compared to uninoculated control.

Maximum increase 103% over untreated control in straw yield was recorded by chemical fertilizers that was statistically similar with 99% increase over uninoculated control by isolate S7 at 75% of chemical fertilizers (Table 2). Isolate S5 at 75% of chemical fertilizers showed statistically non-significant increase (87.1 and 93.28% over untreated control, respectively) with S9 at 75% of chemical fertilizers and also with S7 at 50% of chemical fertilizers (80.36% increase over untreated control). Isolates S9 and S5 at 50% of chemical fertilizers remained statistically similar (69.25 and 63.56% increase over uninoculated control, respectively).

The increase in 1000-grain weight with different treatments ranged from 15-31% (Table 2), with S7 at 75% of chemical fertilizers being the most effective isolate which produced 31% increase in 1000-grain weight compared with uninoculated control. Next to it were chemical fertilizers alone, isolates S9 and S5 at 75% of chemical fertilizers (28.3, 27.5 and 27.4% increase over untreated control, respectively), which were statistically non-significant with each other. Isolate S7 at 50% of chemical fertilizers showed 24.9% increase over uninoculated control that was statistically significant compared with isolates S9 and S5 at 50% of chemical fertilizers i.e., 16.4 and 15.8% increase over uninoculated control but non-significant even with chemical fertilizers alone, S9 and S5 at 75% of chemical fertilizers.

Table 3. Effect of inoculation with rhizobacteria containing ACC-deaminase on nitrogen, phosphorus and potassium uptake by shoot (Average of four replications).

Treatments	N-uptake (kg ha^{-1})	P-uptake (kg ha^{-1})	K-uptake (kg ha^{-1})
Control[a]	42.56 e*	15.82 e	60.17 f
Recommended NPK[b]	103.1 a	39.40 ab	159.3 b
Isolate S5 + 50% NPK	77.51 d	30.70 d	117.9 e
Isolate S7 + 50% NPK	88.27 c	34.14 cd	132.2 d
Isolate S9 + 50% NPK	79.20 d	31.81 d	121.9 e
Isolate S5 + 75% NPK	95.22 bc	37.50 bc	150.7 c
Isolate S7 + 75% NPK	102.7 a	41.20 a	165.4 a
Isolate S9 + 75% NPK	99.15 ab	38.52 ab	156.6 b

*Means sharing similar letter(s) do not differ significantly at p = 0.05
[a]No fertilizer, No inoculation
[b]NPK @ 120-100-60 kg ha^{-1}

Statistical behavior of N uptake by shoot (Table 3) depicted that similar results were observed with 100% chemical fertilizers, isolates S7 and S9 at 75% of chemical fertilizers (142.2, 141.3, and 133% more than untreated control, respectively). However, isolate S7 at 50% of chemical fertilizers showed statistically non-significant results with isolate S5 at 75% of chemical fertilizers (107.5 and 123% increase over uninoculated control, respectively). Isolates S5 and S9 remained statistically similar regarding N uptake however, significant than uninoculated control.

Maximum P uptake was observed with isolate S7 at 75% of chemical fertilizers (160% increases over uninoculated control). It was followed by 100% chemical fertilizers and isolate S9 at 75% of chemical fertilizers producing 149 and 143.5% increase over untreated control, respectively. Isolate S7 at 50% of chemical showed statistically non-significant results with isolate S5 at 75% of chemical fertilizers and S5 and S9 at 50% of chemical fertilizer.

Regarding K uptake, 174.8% increase over untreated control was observed with isolate S7 at 75% of chemical fertilizers. Next to it, chemical fertilizers and isolate S9 at 75% of chemical fertilizers remained non-significant with each other (167.7 and 160% more than untreated control, respectively). Isolates S9 and S5 remain statistically similar in NPK uptake at 50% of chemical fertilizers but significant over untreated control.

Characterization of selected PGPR strains: Characterization of the selected PGPR strains showed that these strains were positive for ACC-deaminase activity, IAA production, chitinase activity, P-solubilization and root colonization ability but with different degree of efficacy. Results revealed that strain S7 possessed high IAA production both in the absence and presence of L-tryptophan (10.8, 20.8 mg L^{-1}) and root colonization ability (4.0 x 10^6 cfu g^{-1}), respectively followed by S9 strain. All the strains were positive for P-solubilization. Only strain S7 that performed better was positive for chitinase activity and medium in ACC-deaminase activity.

Discussion

In this study, 9 isolates of PGPR containing ACC-deaminase activity were screened for their growth promoting activity under axenic conditions. Three promising ACC-deaminase enriched strains of PGPR (S5, S7 and S9) were further evaluated in the

presence of 50 and 75% of recommended chemical fertilizers in a field trial. It was observed that inoculation with these three rhizobacterial strains containing ACC-deaminase activity significantly promoted growth and yield of wheat at 75% of chemical fertilizers and also one of them even at 50% of chemical fertilizers showed surprising results compared to the other strains. This growth promotion might be attributed to the decreased ethylene levels due to inoculation with ACC-deaminase containing rhizobacteria. Because production of ethylene is accelerated during seed germination which may have inhibitory effects on seed germination and root growth (Glick *et al*., 1998). So, it is highly likely that inoculation with these rhizobacterial isolates might have decreased endogenous inhibitory levels of ethylene in developing seedlings and roots and thus resulted in formation of longer roots because of their ACC-deaminase activity. This premise is supported by the ability of ACC-deaminase containing PGPR strains to eliminate "classical triple response" in etiolated pea seedlings, partially or completely, exposed to 5 mmol L^{-1} ACC, added to the rooting medium (Shaharoona *et al*., 2006). This may imply that the inoculation with rhizobacteria containing ACC-deaminase could result in the development of much better germination of seeds and longer roots, which subsequently affects shoot growth and yield positively. This contention is strongly supported by the work reported by several other researchers (Glick *et al*., 1998; Mayak *et al*., 1999; Wang *et al*., 2000; Belimove *et al*., 2002; Shaharoona *et al*., 2003, 2007; Zafar-ul-Hye *et al*., 2007).

Under field condition, there is a complex system and various biotic and abiotic factors may cause modification in the behavior of particular strains. As we observed that out of three selected strains, inoculation with isolate S9 showed promising results in promoting root growth that might be due to its high ACC-deaminase activity and S5 showed better results in improving shoot growth under axenic conditions while regarding seedling fresh weight isolate S7 performed very well under axenic conditions. However, under field conditions, isolate S7 was highly effective in improving growth and yield of wheat at both levels (i.e., 50 and 75%) of chemical fertilizers. It is highly likely that greater effectiveness of this strain might be related to its high root colonization ability and chitinase activity in addition to ACC-deaminase activity, which made this strain more competitive than other strains. Other traits for growth promotion like production of IAA, phosphate solubilization and chitin production by this PGPR isolate S7 might have helped in better nutrient mobilization, availability and thus uptake by the plants (Zahir *et al*., 2004). This resulted in healthy plant due to efficient and balanced nutrient availability and uptake even at 75% of chemical fertilizers, which increased plant biomass, N, P and K uptake and thus saved ~ 25% of chemical fertilizers. This suggested that rhizobacteria containing ACC-deaminase activity do have potential for improving growth and yield of wheat even at reduced fertilizers application; however, some other characteristics of the rhizobacteria such as root colonization ability and chitinase activity could also be used as an effective tool for selection/screening of efficient PGPR strains.

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