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INOCULATION WITH ACC-DEAMINASE CONTAINING RHIZOBACTERIA FOR IMPROVING GROWTH AND YIELD OF WHEAT

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Six strains (Q_{50}, N_3, Q_7, N_7, Q_{14} & Y) of plant growth promoting rhizobacteria showing ACC-deaminase activity were isolated from rhizosphere of wheat. Wheat seeds were inoculated and sown in pots filled with 12 kg soil fertilized with NPK @ 150-120-70 kg ha^{-1}, respectively. All the isolates exhibited a significant increase in grain yield, straw yield, root weight, root elongation, number of tillers, N, P & K uptake in straw and grain over uninoculated control. Root elongation, root weight, straw yield and grain yield increased up to 31, 83, 69 and 60%, respectively, over uninoculated control. The growth promoting activity exhibited by the rhizobacteria might be due to their ability to hydrolyze ACC, thus resulting in decreased endogenous ethylene synthesis, which eliminated the potential inhibitory effects of higher ethylene concentrations. Results showed that ACC-deaminase containing plant growth promoting rhizobacteria could be effectively used for growth promotion in wheat.
Key words: 1-Aminocyclopropane-1-carboxylate, rhizobacteria, wheat.

INTRODUCTION

Ethylene, a plant hormone, is required for seed germination by many plant species and rate of ethylene production increases during germination and seedling growth (Abeles et al., 1992). Although a low level of ethylene appears to enhance root initiation and growth, higher level of ethylene, produced by fast growing roots, can lead to the inhibition of root elongation (Mattoo and Shuttle, 1991; Ma et al., 1998). Growth promotion is not linked to the production of stimulatory hormones but to the reduction of the inhibitory hormone ethylene (Frankenberger and Arshad, 1995). Inoculation of seed or roots with specific inoculants could suppress the endogenous ethylene synthesis, which subsequently creates physiological response (Glick et al., 1998). 1-Aminocyclopropane-1-carboxylic acid (ACC) is the immediate precursor of ethylene derived from methionine amino acid in plants (Yang and Hoffman, 1984). It has been discovered that certain microorganisms contain an enzyme ACC-deaminase that hydrolyses ACC into ammonia and α-ketobutyrate (Glick et al., 1994 a, b; Mayak et al., 1999). The uptake and cleavage of ACC by ACC-deaminase containing rhizobacteria decreases the amount of ACC, resulting in the decreased level of ethylene, outside the germinating seeds, thereby acting as a sink for ACC. Decreased level of ACC results in lower levels of endogenous ethylene, which eliminates the potential inhibitory effect of higher ethylene concentration (Glick et al., 1998).

Keeping in view the above discussion, present study was conducted with an idea of using microbial strains containing ACC-deaminase as inoculant for the regulation of endogenous ethylene in wheat that could enhance the crop yield.

MATERIALS AND METHODS

A pot experiment was carried out in a wire house, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Six isolates (Q_{50}, N_3, Q_7, N_7, Q_{14} and Y) of plant growth promoting rhizobacteria showing ACC-deaminase activity were isolated from rhizosphere of wheat. Rhizosphere soils were collected from different fields of the University of Agriculture, Faisalabad. Rhizobacteria were isolated by dilution plate technique using salt minimal media (Dworkin and Foster, 1958) containing ACC as a sole nitrogen source (enrichment technique). Further streaking on fresh plates purified the rhizobacterial strains. Six isolates (Q_{50}, N_3, Q_7, N_7, and Q_{14} & Y) of plant growth promoting rhizobacteria showing prolific growth were selected for this pot trial. These cultures were stored at 4±1°C on slants and maintained by transferring them on fresh slants weekly.

Preparation of inoculum

Inoculum was prepared in minimal salt media containing ACC as sole nitrogen source. Each isolate was inoculated in 150 ml test tube containing 60 ml DF salt minimal medium and incubated at 28±1°C for three days. An optical density of 0.5 recorded at λ 535 nm was achieved by dilution with sterilized water to maintain uniform cell density (10^8-10^9 CFU/mL).

Seed inoculation

Seeds were coated with slurry, which was prepared by mixing 5 ml of 15% sterilized sugar solution, 10 ml liquid culture and 35 g of sterilized peat plus clay. Control was treated with sterilized peat plus clay containing sterilized broth and sugar solution. Inoculated seeds were placed over night for drying.

Pot experiment

Ten inoculated seeds of wheat (*Triticum aestivum* L; vaiety, Watten) were sown in pots having 12 kg soil (having pH, 7.6; ECe,3 dSm^{-1}, organic matter, 0.65; total nitrogen, 0.04 %; available P, 6.8 mg kg^{-1}; and extractable K, mg kg^{-1},157) fertilized with NPK @ 150-120-70 kg ha^{-1}, using urea, single super phosphate & muriate of potash, respectively. After germination, the plants were thinned to allow four uniform seedlings in each pot. Pots were arranged randomly with three repeats using completely randomized design in wire house under ambient light and temperature conditions. Canal water was used for irrigation. Data regarding maximum tiller height, number of tillers, spike length, root length, root weight, 1000-grain weight, and straw and grain yields were collected at harvesting. Grain and straw samples were analyzed for NPK contents using standard methods. Data were analyzed statistically according to Steel and Torrie (1980) using completely randomized design with three replicates.

RESULTS

Root elongation

It is evident from the data (Figure 1) that inoculation with ACC deaminase containing rhizobacteria significantly increased root elongation. The increase in root elongation caused by different rhizobacteria ranged from 14 – 31% over uninoculated control. Inoculation with isolate N_7 was found to be most effective that caused 31% increase in root elongation over uninoculated control. The next effective isolates were Y, Q_{50} and N_3, which resulted in about 24, 18 and 16 % increase in root elongation, respectively.

Root weight

Data (Figure 2) revealed that inoculation with ACC deaminase containing rhizobacteria significantly increased dry root weight as compared to uninoculated control. The most effective isolate was found to be N_7 that caused an 83 % increase over uninoculated control. The next effective isolates were Q_{50}, Y and N_3. These were able to cause 41, 39 and 36.5% increase respectively, as compared to uninoculated control.

Straw yield

Inoculation with ACC deaminase containing rhizobacterial isolates caused a significant increase in straw yield over uninoculated control (Figure 3). The increase in straw yield caused by different rhizobacteria was up to 69% over uninoculated control. Maximum straw yield was increased by inoculation with isolate N_7. The next effective isolates were N_3, Q_7, and Y, which increased the straw yield by 53, 49 and 48% over control, respectively.

Grain yield

It is evident from the data (Figure 4) that inoculation with ACC deaminase containing rhizobacteria significantly increased grain yield. The maximum and minimum increase in grain yield caused by inoculation with different rhizobacteria was 60% and 18% respectively over uninoculated control. Again, isolate N_7 was found to be the most effective in increasing grain yield that was 60 % higher as compared to uninoculated control. Rhizobacterial isolates Y, N_3, Q_7 and Q_{50} caused statistically similar increase in grain yield.

Plant height

It is revealed from the data (Table 1) that inoculation with some of the ACC-deaminase containing rhizobacteria significantly increased plant height. Maximum plant height was recorded in Q_7, which was 8.2% higher than uninoculated control. It was followed in descending order by N_3 (7%), N_7 (5.8%) and Q_{14} (2.4%), respectively.

Table 1. Effect of ACC-deaminase containing rhizobacteria on plant height, number of tillers, number of spikelets and 1000-grain weight of wheat.

(Average of 3 replicates)

Isolate	Plant height (cm)	No. of tillers pot^{-1}	Spike lets $spike^{-1}$	1000- grain weight (g)
Control	50.0 c	12.6 c	15.6 d	28.8 d
Q50	50.3 c	15.6 ab	16.7 abc	34.2 ab
N3	53.5 ab	15.0 ab	16.5 bc	34.5 ab
Q7	54.1 a	15.6 ab	16.9 ab	35.8 a
N7	52.9 ab	17.0 a	17.0 a	33.5 bc
Q14	51.2 bc	14.6 b	16.4 bc	32.2 c
Y	51.1 bc	15.6 ab	16.3 c	33.7 bc

Means sharing similar letter(s) do not differ significantly at p=0.05

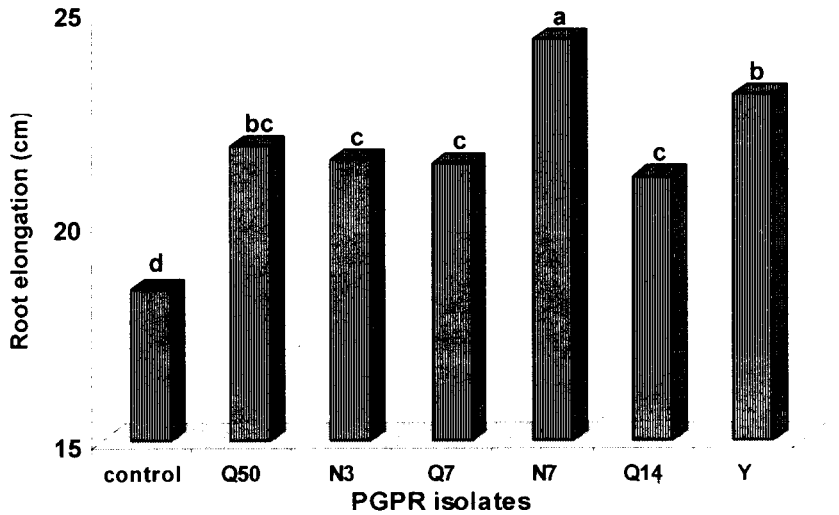

Figure 1. Effect of ACC-deaminase containing rhizobacteria on root elongation of wheat (Average of 3 replicates). Bar(s) sharing similar letter(s) do not differ significantly at p=0.05

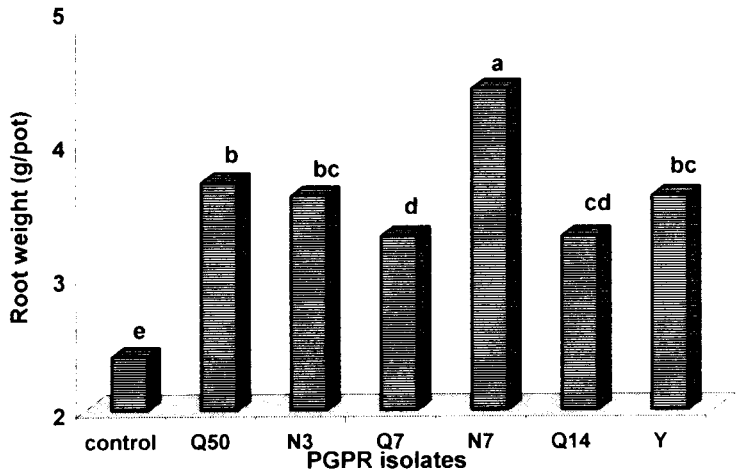

Figure 2. Effect of ACC-deaminase containing rhizobacteria on root weight of wheat (Average of 3 replicates).Bar(s) sharing similar letter(s) do not differ significantly at p=0.05

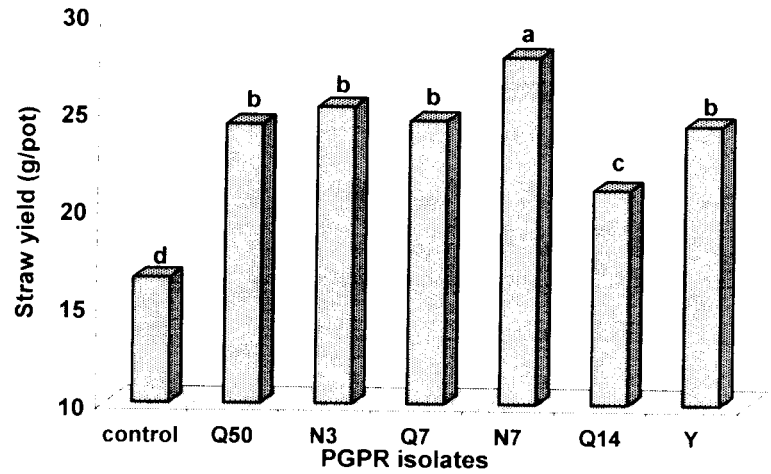

Figure 3. Effect of ACC-deaminase containing rhizobacteria on straw yield of wheat (Average of 3 replicates). Bar(s) sharing similar letter(s) do not differ significantly at p=0.05

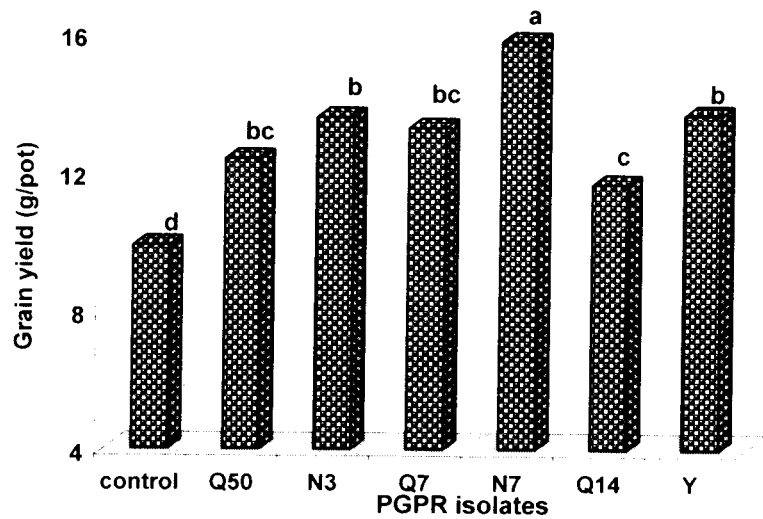

Figure 4. Effect of ACC-deaminase containing rhizobacteria on grain yield of wheat (Average of 3 replicates). Bar(s) sharing similar letter(s) do not differ significantly at p=0.05

Number of tillers

Data (Table 1) revealed that all the rhizobacterial isolates containing ACC-deaminase enzyme, significantly increased the number of tillers per plant compared with control. Maximum number of tillers per pot (17) was recorded in case of inoculation with isolate N_7 that was 34% higher than control. All the isolates produced statistically similar number of tillers although significantly higher than control.

Number of spikelets per spike

Data (Table 1) showed that inoculation with ACC-deaminase containing rhizobacteria significantly increased number of spikelets per spike compared with uninoculated control. Maximum numbers of spikelets

per spike were produced by N_7 that was 10% more than control. It was followed in descending order by Q_7 (8%), Q_{50} (7%) & N_3 (6%), respectively.

1000-grain weight

It is evident from the data (Table 1) that inoculation with ACC-deaminase containing rhizobacteria had significantly increased the 1000 grain weight from 12 – 24% with Q_7 being the most effective isolate which caused a 24% increase in 1000 grain weight compared with uninoculated control. Next to it was N_3 that resulted in 20% increase in 1000-grain weight.

Nutrient uptake

It was observed that the inoculation with ACC-deaminase containing rhizobacteria also significantly increased the nutrients uptake (N, P and K) over uninoculated control.

N uptake in straw and grain

Data in Table 2 shows significant increase in N uptake in straw and grain due to inoculation with ACC-deaminase containing rhizobacteria. Different rhizobacteria increased N uptake by 8 – 74% in straw. Isolate N_7 performed outstandingly and produced 74 increase in N uptake in straw over uninoculated control. Isolates N_3, Y & Q_7 were the next which resulted in 70, 64 & 45% increase in N uptake in straw. The maximum increase in N uptake in grain was observed in case of inoculation with isolate N_7 that was 60% over uninoculated control. While, isolates Q_7, Y & N_3 were the next which resulted in 43, 41 & 35% increase in N uptake in grain over uninoculated control respectively.

P uptake in straw & grain

Data (Table 2) revealed that P uptake in grain and straw was significantly increased by inoculation with ACC-deaminase containing rhizobacteria. The increase in P uptake caused by different rhizobacterial isolates ranged from 29 – 101% in straw and 12 – 56% in grain over uninoculated control. Again, isolate N_7 was found to be the most effective among all the isolates that caused 101 & 56% increase in P uptake in straw and grain over uninoculated control, respectively. It was followed in descending order by Q_{50} (65%), Q_7 (54%) & N_3 (52%) in straw and Q_7 (54%), Y (51%) & N_3 (40%) in grain, respectively.

K uptake in straw and grain

It is revealed from data (Table 2) that all the isolates of ACC-deaminase containing rhizobacteria significantly increased K uptake in both straw and grains over the uninoculated control. The increase in K uptake due to inoculation with these rhizobacteria ranged from 31-77% in straw and 33 – 122% in grain over uninoculated control. Isolate N_7 was found to be the best that caused 77 & 122% increase in P uptake in straw and grain over uninoculated control respectively.

DISCUSSION

Six rhizobacterial isolates containing ACC-deaminase activity were evaluated for growth promotion in wheat in pot trial. All the rhizobacterial isolates increased root elongation, root weight, straw yield, grain yield, number of tillers and 1000 grain weight significantly as compared to uninoculated control. This growth promotion might be attributed to the decreased ethylene levels due to inoculation with ACC-deaminase containing rhizobacteria. Since no efforts have been successful in isolating a bacterium capable of utilizing ACC as a precursor of ethylene (Arshad and Frankenberger, 1990). This implies that the rhizobacteria grown on ACC utilize it as a nitrogen source via deaminase trait i.e. ACC is converted into NH_3 and α-ketobutyrate instead of ethylene (Arshad and Frankenberger, 2002). Therefore, the ability of ACC enriched rhizobacteria isolates to deaminate ACC might be the responsible mechanism of action for promoting growth and yield of wheat. Because production of ethylene is accelerated during seed

Table 2. Effect of ACC-deaminase containing rhizobacteria on nutrient uptake in wheat.
(Average of 3 replicates)

Isolate	Nutrient uptake in grain (mg pot^{-1})			Nutrient uptake in straw (mg pot^{-1})		
	N	P	K	N	P	K
Control	209.7 d	62.1 d	28 e	59.8 e	9.3 d	519.2 c
Q50	311.2 b	81.4 bc	37.4 d	98.5 d	15.3 b	827.2 a
N3	315.8 b	87.1 ab	45.0 c	146.8 b	14.3 bc	902.1 a
Q7	333.0 b	95.4 ab	41.3 cd	125.2 c	12.8 bc	854.1 a
N7	372.1 a	96.9 a	62.3 a	171.7 a	18.7 a	917.9 a
Q14	260.7 c	69.3 cd	45.4 c	93.1 d	12 cd	678.2 b
Y	329.6 b	93.8 ab	55.3 b	153.5 b	14.1 bc	812.6 a

Means sharing similar letter(s) do not differ significantly at p=0.05

germination which may have inhibitory effects on seed germination and root growth (Glick et al., 1998). So inoculation with ACC-deaminase containing rhizobacteria might have decreased ethylene levels in developing seeds and roots and thus prevented its inhibitory effects on their growth which resulted in formation of longer roots. Better germination rate of seeds and longer roots helped the plant to grow more efficiently and produce more yields.

This contention is strongly supported by the work reported by several other researchers (Hall et al., 1996; Glick et al., 1998; Mayak et al., 1999; Shaharoona et al., 2003).

Although all the rhizobacterial isolates promoted growth and yield of wheat but with variable efficacy. This might be due to the fact that different isolates have different ACC-deaminase activity, which might have produced better root growth in initial stages of crop growth. Shaharoona et al. (unpublished data) also reported positive correlation between *in vitro* ACC-deaminase activity of different rhizobacteria and the effect on root elongation due to inoculation with these rhizobacteria in maize under axenic conditions. Other traits for growth promotion like production of IAA and siderophores, and phosphate solubilization by PGPR isolates 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 balanced nutrient availability and uptake, which increased plant biomass, N, P and K uptake in straw and grain. Thus ACC-deaminase activity is a unique trait of PGPR for improving growth and yield of wheat and other crops.

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