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1-AMINOCYCLOPROPANE-1-CARBOXYLATE (ACC) ENRICHMENT: AN EFFECTIVE APPROACH TO SCREEN PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR MAIZE.

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By using enrichment medium containing 1-aminocyclopropane-1-carboxylic acid (ACC) as sole nitrogen source, 21 strains were isolated from the maize rhizosphere in two phases i.e., 9 isolates in first phase and 12 isolates in second phase. Two trials in glass jars were conducted under gnotobiotic conditions to select effective plant growth- promoting rhizobacteria (PGPR). All the 21 isolates tested in both the trials exhibited growth promoting activity in maize but with variable degree of efficacy. Among the 9 isolates tested in 1st trial, isolate Q14 caused an increase of 7.1- folds in root elongation over uninoculated control. Shoot length and seedling fresh weight (root + shoot) were increased up to 7.0- and 2.0- folds, respectively, over uninoculated control in response to inoculation with Q7. In the 2nd trial, rhizobacterial isolate Q30 was found to be the most effective as its inoculation resulted in 2.8-, 2.0- and 1.7- folds increase in root elongation, shoot length and seedling fresh weight (root + shoot weight), 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 use of ACC-enriched medium is an effective and efficient approach to select promising PGPR.
Key Words: 1-aminocyclopropane-1-carboxylate, enrichment, PGPR, maize.

INTRODUCTION

Ethylene is an important growth hormone that is produced by almost all plants and mediates a wide range of different plant responses and developmental processes. The presence of ethylene may be stimulatory or inhibitory depending upon its concentration, the 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 results in modified growth and development (Arshad and Frankenberger, 2002). Very recently, inoculation with specific bacteria has been shown to alter the endogenous levels of ethylene, which subsequently leads to changes in the growth and development of plants (Glick et. al., 1998). 1-Aminocyclopropane-1-carboxylate (ACC) is the immediate precursor of ethylene derived from methionine amino acid in plants (Yang and Hoffman, 1984). The synthesis of ethylene in plants is directly related with the concentration of ACC in the plant tissue (Macháčková et al., 1997). 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). A model describing the role of bacterial ACC-deaminase in facilitating plant growth implies that bacteria inhabiting roots or seeds cause decrease in the levels of endogenous ACC in plant roots and shoots. According to Glick et al., (1998) a significant proportion of ACC produced by plants may be exuded from plant roots or seeds, taken up by bacterium and hydrolyzed by ACC-deaminase on the surface of root. Decreased supply of ACC results in lower levels of endogenous ethylene, which eliminate the potential inhibitory effects of higher ethylene concentrations (Glick et. al., 1998).

Many studies have been conducted to investigate the effect of (PGPR) containing ACC-deaminase on plant growth. Glick et al. (1994) inoculated canola seeds with *Ps. putida* GR 12-2 and its ACC–deaminase minus mutants. They observed that only wild type strain promoted root growth of developing canola seedlings under gnotobiotic conditions. In another experiment Glick et al. (1997) studied the effect of *Ps. putida* GR12-2 (wild type and its ACC–deaminase minus mutants). They confirmed the results of previous studies that root and shoot growth were promoted in response to inoculation with wild type but not with the mutant. Hall et al. (1996) studied the effect of *Ps. putida* GR 12-2 and its ACC-deaminase mutants on canola, lettuce, tomato and wheat. They reported that wild type strain showed a positive effect on the root growth of inoculated plants. The results were comparable to those obtained with AVG (aminoethoxy vinylglycine) treatment, an inhibitor of ethylene biosynthesis in plants. Similar results have been reported by many other workers (Shah et al. 1998; Li et al.2000; Wang et al. 2000 and Pal et al 2000). These studies have indicated that the bacteria carrying ACC-hydrolyzing enzyme, ACC-deaminase could act as PGPR.

Inoculation is commonly used practice to increase the population of effective PGPR in the rhizosphere, however, results under field conditions show inconsistency. One of the many reasons for inconsistent results is that most of the times, no valid basis of screening of rhizobacteria to select effective PGPR is being used. According to Cattelan et al.,(1999) ACC-deaminase trait is valid approach for screening of PGPR. Thus this study was under taken to screen the rhizobacteria to select most effective PGPR by using the ACC enrichment technique.

MATERIALS AND METHODS

Two trials were conducted in glass jars in a controlled temperature growth room (at 28 ± 1°C) under axenic conditions to screen the most efficient ACC-deaminase containing PGPR for improving growth of maize seedlings.

Isolation

Rhizosphere soils were collected from different fields. Rhizobacteria were isolated by dilution plate technique using salt minimal media containing ACC as sole nitrogen source (enrichment technique). The collected rhizobacterial strains were purified by further streaking on fresh plates. Nine strains showing prolific growth were isolated from research fields of University of Agriculture Faisalabad while twelve strains were isolated from farmer's fields at surrounding of Faisalabad. Since isolation was carried out in two phases, thus separate screening trials (trial 1 & trial 2) were conducted. These cultures were stored at 4±1 °C on slants and maintained by transferring them on fresh slants on regular basis.

Preparation of inoculum

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

Trials in glass jars

Two sterilized filter paper sheets were soaked and saturated with respective inoculum. Maize seeds were surface sterilized by dipping in 95% ethanol solution for 5 minutes and 0.2% $HgCl_2$ solution for 3 minutes, which were subsequently washed thoroughly with distilled 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 inoculum 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 adjusted to 16 hours light and 8 hours dark period. Data regarding root elongation, shoot length and fresh weights of seedlings (root+shoot) were recorded after ten days. The same procedure was used in both trials for screening of all rhizobacterial isolates for their growth-promoting activity in maize under axenic conditions. In 1st trial because of the power failure temperature rose to 40 °C and seedlings suffered from heat stress for eight hours.

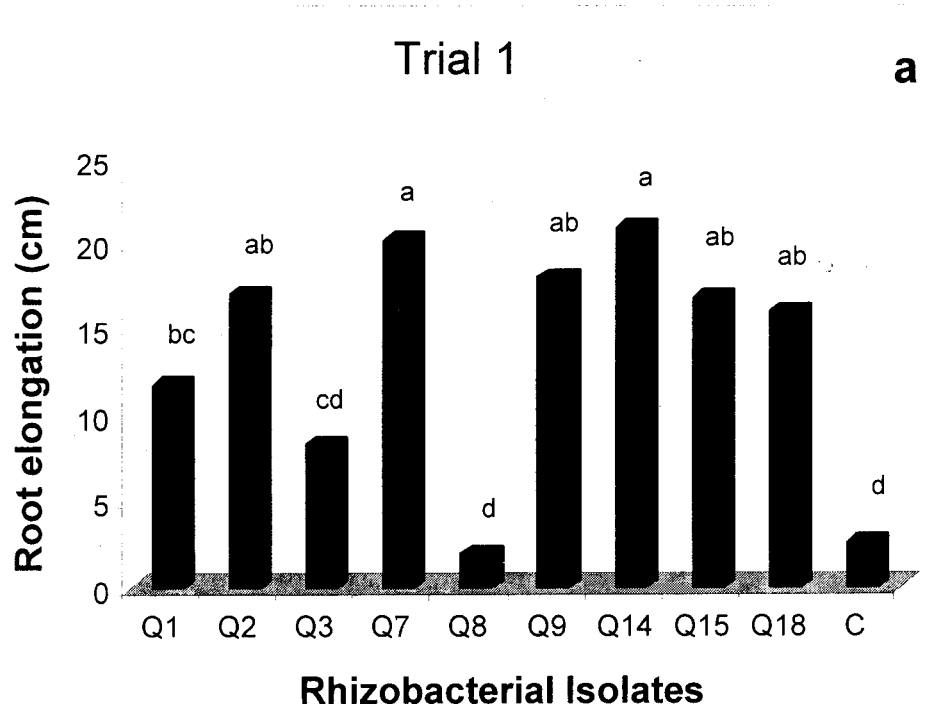
RESULTS

Root elongation

Inoculation of maize seedlings with ACC-deaminase containing rhizobacterial isolates significantly increased root elongation in both trials except Q8 and Q31 (Fig.1). In 1st trial, the increase in root elongation caused by different rhizobacteria ranged from –0.2- to 7.1- folds over uninoculated control (Fig.1a). In this trial isolate Q14 was found to be the best that caused 7.1-folds increase in root elongation over uninoculated control. Isolates Q7 & Q9 were next in promoting root elongation by 6.8- & 6.0-folds over uninoculated control. It was highly interesting to observe that the uninoculated seedlings were the most seriously affected by the heat stress while all inoculated seedlings tolerated the stress very well. Isolate Q8 was found to be the least effective in promoting root elongation (20% less) than uninoculated control. In 2nd trial, inoculation with ACC enriched isolates increased root elongation ranging from –0.4- to 2.8- folds over uninoculated control (Fig.1b). Isolate Q30 was found to be the most effective which resulted in 2.8-folds higher root elongation over uninoculated control. The other effective isolates were Q26, Q21 and Q41 that caused 2.4-, 1.5- and 1.3-folds higher root elongation over uninoculated control. The least effective isolate was Q31 that caused 40% less root elongation over uninoculated control.

Shoot length

Data revealed that shoot length of maize seedlings was significantly increased due to inoculation with ACC-deaminase containing rhizobacteria except Q8 and Q31 (Fig.2). In 1st trial, increase in shoot length due to inoculation with isolated rhizobacteria ranged from –0.1- to 7.0- folds over uninoculated control (Fig.2a). The most effective isolate was Q7 that caused 7.0- folds increase in shoot length over uninoculated control. Isolates Q9 & Q14 were the next effective



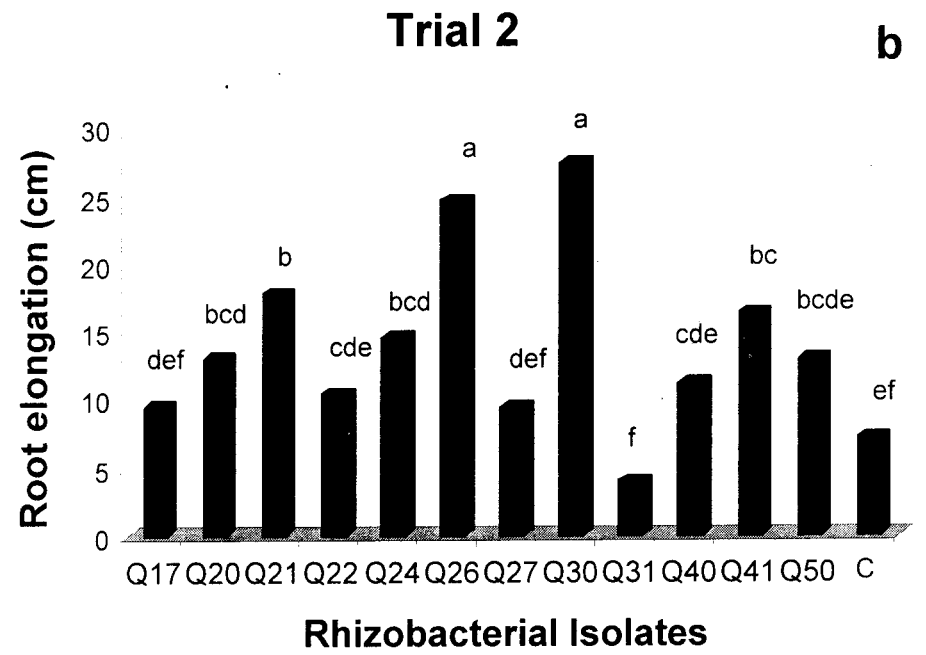


Fig. 1. Effect of ACC-enriched rhizobacteria on root elongation of maize seedlings under gnotobiotic conditions. (Average of 3 replicates)

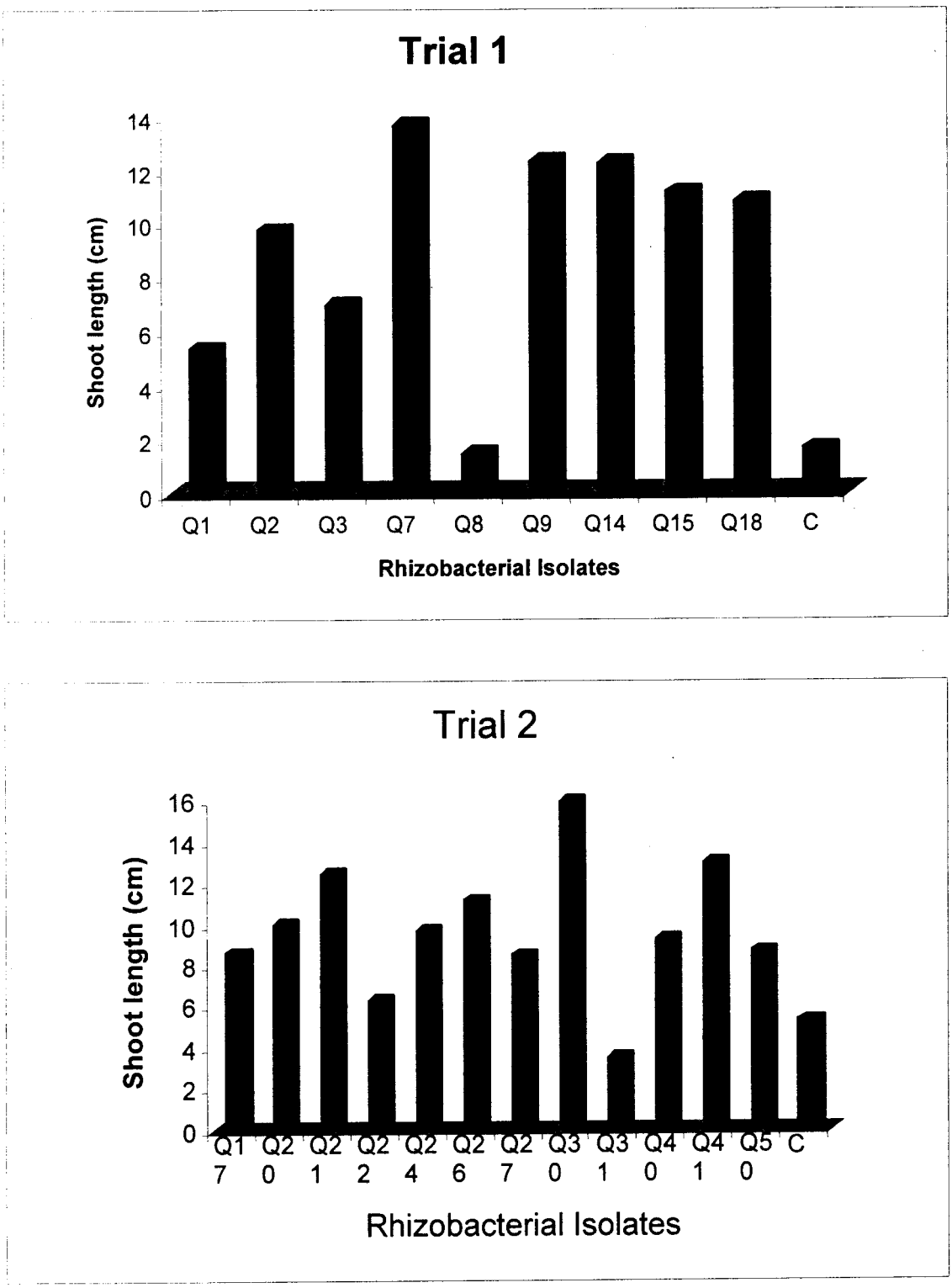
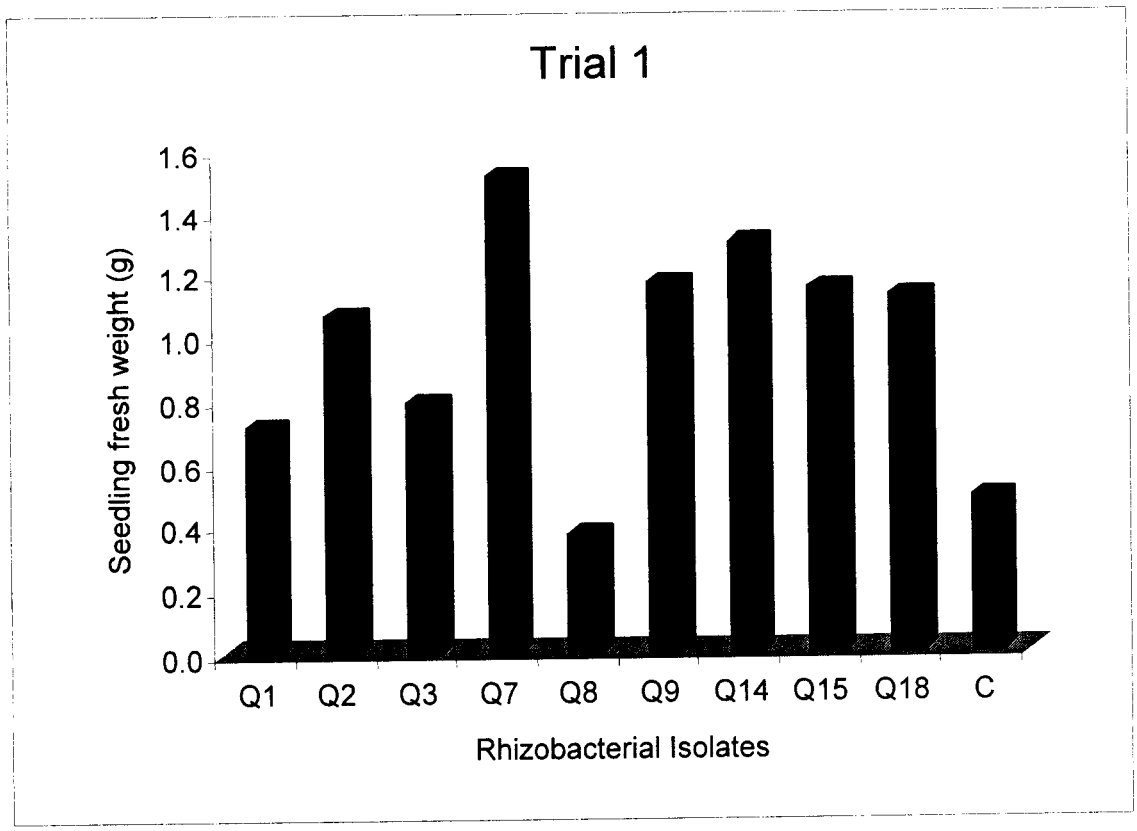


Fig 2. Effect of ACC-enriched rhizobacteria on shoot length of maize seedlings under gnotobiotic conditions. (Average of 3 replicates)



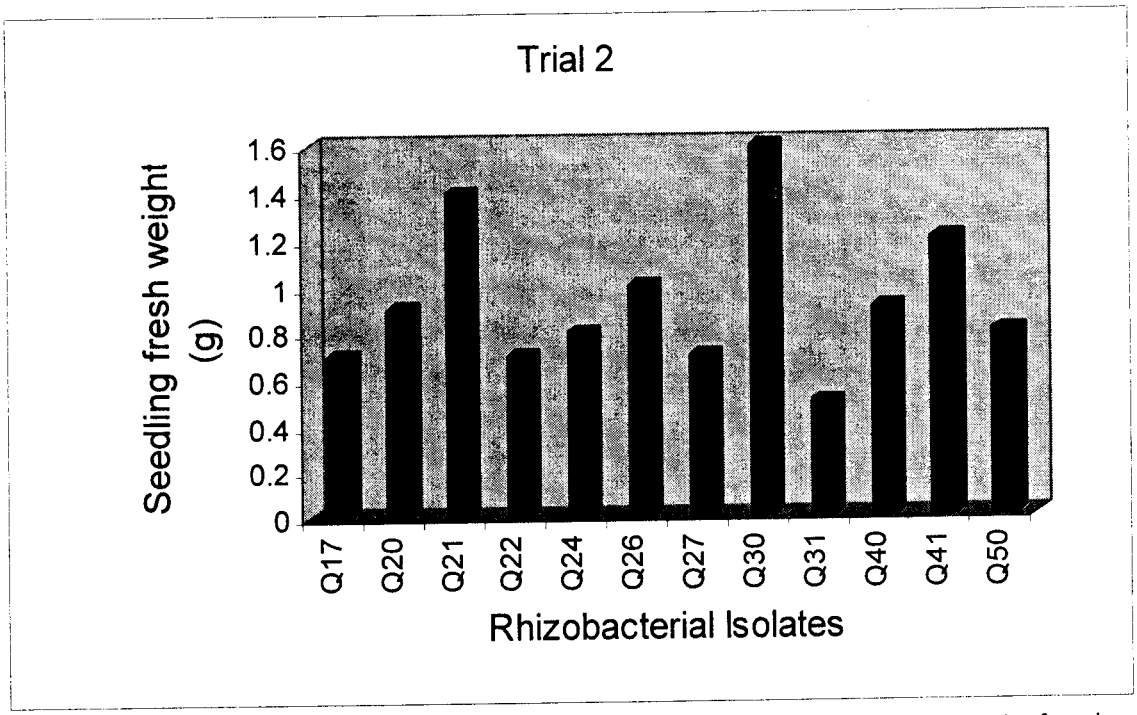


Fig 3. Effect of ACC-enriched rhizobacteria on seedling fresh weight (root+shoot) of maize under gnotobiotic conditions. (Average of 3 replicates)

isolates that resulted in 6.2-folds higher shoot length over uninoculated control. Once again isolate Q8 caused minimum increase in shoot length. In 2nd trial increase in shoot length ranged from –0.3 - to 2.0-folds over uninoculated control (Fig.2b). The most promising increase of 2.0-folds over uninoculated control was observed in case of inoculation with isolate Q30. The next effective isolate was Q41, which caused an increase of 1.5-folds over uninoculated control. Similar to root elongation, isolate Q31 was least effective in promoting shoot length (30% less) than uninoculated control.

Seedling fresh weight (root + shoot)

Seedlings inoculated with ACC-deaminase containing rhizobacterial isolates caused significant increase in fresh weight of seedlings (root + shoot) over uninoculated control (Fig. 3). In 1st trial 2.0- folds increase over uninoculated control was observed in isolate Q7. Isolate Q14, Q15 & Q18 resulted in 1.6-, 1.3-& 1.2- folds higher seedling fresh weight (shoot + root) over uninoculated control respectively (Fig.3a). In 2nd trial Q30 was found to be the best isolate that caused 1.7- folds higher seedling fresh weight as compared to uninoculated control (Fig.3b). Next to it was isolate Q21 that resulted in 1.3-folds more seedling fresh weight as compared to control. Once again isolates Q8 and Q31 caused minimum seedling fresh weight in 1st and 2nd trial respectively.

DISCUSSION

Almost all the rhizobacterial isolates obtained through enrichment on ACC as the sole N source showed growth-promoting activity in maize under axenic conditions but with variable efficacy. Since no efforts have been successful in isolating a bacterium capable of utilizing ACC as a precursor of ethylene (Arshad and Frankenberger, 1990; 2002), this implies that the rhizobacteria grown on ACC utilized it as a N source via deaminase trait i.e., ACC is converted into NH_3 and α-ketobutyric acid instead of ethylene (Arshad and Frankenberger, 2002). Thus it is highly likely that the ability of these ACC enriched rhizobacterial isolates to deaminate ACC was the responsible mechanism of action for promoted root and shoot growth because lowering of the ACC levels result in decreased endogenous ethylene production. This contention is strongly supported by the work reported by several other researchers (Glick et al., 1998; Hall et al., 1996; Mayak et al., 1999).

The variation in growth promotion by different isolates may be due to the differences in their efficiency to colonize the germinating roots and deaminating the ACC formed in roots. The isolates with greater efficiency of deaminating endogenous ACC might have caused more root growth promotion by eliminating the inhibitory effects of higher ethylene concentrations produced from endogenous ACC. Similar results have been reported by others (Shah et al., 1998; Li et al., 2000; Wang et al, 2000). Moreover, the rhizobacterial isolates might have produced other biologically active substances, which had affected the growth. Future studies on finding correlation between ACC deaminase vs. growth promotion caused by a strain will further resolve the issue. In 1st trial the better survival of maize seedlings inoculated with ACC-deaminase containing rhizobacteria than control upon exposure to temperature stress might also be due to less ethylene production in inoculated roots. It is supported by the established fact that any kind of stress results in sharp increase in ACC levels followed by out burst of endogenous ethylene synthesis by the plant tissues (Davies, 1995). The hormone ethylene has also been designated as "stress hormone" because of its involvement in physiological responses evoked by the stress. Grichko and Glick (2001) and Belimov et al (2001) also reported better stress tolerance (flooding and metal toxicity) in plant inoculated with ACC-deaminase containing rhizobacteria.

The differences in magnitude of inoculation effects observed in 1st vs. 2nd trial could be attributed to exposure of seedlings to high temperature stress in 1st trial. High temperature stress might have induced greater ACC synthesis, which suppressed the growth in control, while ACC-deaminase containing rhizobacteria hydrolyzed the stress induced ACC. Therefore, inoculated seedlings showed more increase in root elongation and shoot length over control compared to increases observed in 2nd trial. Increase in seedling fresh weight was almost same in both trials because high temperature stressed seedlings in 1st trial were although shorter but thicker which could most likely due to extra ethylene produced in response to high temperature stress. The observations made in 1st trial provide important information regarding the utility of ACC-deaminase containing rhizobacteria to ameliorate stress-induced effects.

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