

Effect of ACC-deaminase Containing Rhizobacteria on Growth Promotion of Maize under Salinity Stress

RIZWANA KAUSAR¹ AND SHER MUHAMMAD SHAHZAD

Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan

¹Corresponding author's e-mail: crystalboy_uaf@yahoo.com

ABSTRACT

Experiment was conducted under axenic conditions to evaluate the effect of rhizobacteria containing ACC-deaminase enzyme for growth promotion of maize under salt stress. Minimal salt medium containing ACC as sole nitrogen source was used for growth of bacteria. Surface sterilized seeds of maize were germinated in petri plates. Uniformly germinated seeds were selected, inoculated with selected strains and placed between two filter paper sheets. Filter paper sheets containing seedling were placed in the jar and incubated at $27^{\circ}\text{C} \pm 1$ in a growth room. Different salinity levels for maize were maintained by using NaCl. Hoagland solution was used to provide nutrients to growing seedlings. Results showed that there were 3.3 folds increased in root length by inoculation with *Pseudomonas fluorescens* biotype A (N₃) in maize at EC 9 dS m⁻¹, whereas at 12 dS m⁻¹ shoot lengths increased by 2.3 folds by inoculation with *P. putida* biotype A (Q₇) over control. At 6 dS m⁻¹ N₃ increased fresh weight by 1.13 times over control.

Key Words: PGPR; Co-inoculation; Ethylene; ACC deaminase; Salinity; Maize

INTRODUCTION

Ethylene is also known as a stress hormone, because of its involvement in evoking physiological responses in plants exposed to a variety of different stresses including salt stress (Wang *et al.*, 1990; Abeles, 1993; Morgan & Drew, 1997). It is believed that stress stimulates 1-aminocyclo-propane 1-carboxylic acid (ACC) synthesis, an immediate precursor of ethylene (Wang & Adam, 1982). Thus salinity can increase rates of ethylene biosynthesis via elevated levels of ACC (El Beltagy *et al.*, 1997), which may lead to physiological changes in plant tissues. So any check on this accelerated ethylene production in plants can improve growth under salt stress.

Increase production of ethylene in plants is directly related with the concentration of ACC in plant tissue (Machackov *et al.*, 1997). Recently, it is found that some bacteria containing ACC-deaminase enzyme can alter the endogenous levels of ACC and hence ethylene. ACC-deaminase hydrolyzes ACC into ammonia and α -ketobutyrate, resulting in reduced production of C₂H₄ (Glick *et al.*, 2001). So bacterial strains having ACC-deaminase activity and abiding the roots can significantly decrease ACC levels in plants. Thereby the amount of stress ethylene production decreases and subsequent damage to the plant, which might occur as a consequence of that ethylene could also be minimum (Grichko & Glick, 2001).

Salinity is one of the major constraints, which hamper agriculture production. However, profitable utilization of salt affected soils for agricultural production is also dispensable. In addition to the use of traditional breeding and plant genetic engineering approaches of developing salt

tolerance of transgenic plants, the use of PGPR may prove useful in developing strategies to facilitate plant growth in saline lands. PGPR inoculants are inexpensive, simple to use and have no adverse effects. However, the degree of efficacy of the PGPR to enhance growth may vary with crops, varieties or species, cultural conditions and inoculant strains. It is predicted that ACC-deaminase can play an important role in the process of plant growth and resistance to stress. Objective of the present study was to screen the ACC-deaminase containing rhizobacteria and evaluate their potential for promoting growth of maize under salinity stress conditions.

MATERIALS AND METHODS

The experiment consisted of following treatments with three replications in completely randomized designed.

1. Control
2. Maize seedling + Q₇ (*Pseudomonas putida* biotype A) and N₃ (*P. fluorescens* biotype A) + NaCl salinity levels 1, 3, 6, 9 and 12 dS m⁻¹.

Pre-isolated rhizobacteria containing ACC-deaminase from Soil Microbiology and Biochemistry Laboratory were used for inoculation. Inoculum was prepared in flasks by using DF salt minimal medium as substrate (N source) without agar (Dworkin & Foster, 1958). Each flask containing broth was inoculated with selected strains of bacteria and incubated for 72 h under shaking (100 rpm) conditions. After incubation, optical density was measured and uniform population (10^7 - 10^8 colony forming unit per mL) of different strains was maintained prior to seedling inoculation.

Seeds of maize were surface sterilized by dipping them in 95% ethanol solution, for 5 min and 0.2% HgCl₂ solution for 3 min and subsequently washed 5 times in distilled water. Petri dishes with filter paper sheets were autoclaved and 30 seeds sown and incubated at 25°C for 3 - 4 days. Distilled water was used for maintaining optimum moisture for germination and seedling establishment. Then these seedlings were inoculated and used in jar experiments. Hoagland solution was used to supply nutrients to the seedling (Hoagland, 1950). The EC of Hoagland solution was 1.2 dS m⁻¹ (control). The rest of salinity levels 3, 5, 7 were maintained by using NaCl. The pH was adjusted at 6.8 - 7.0 by using NaOH.

Two sterilized filter paper sheets were soaked and saturated in suspension containing desired inoculum. Uniformly germinated seedlings after 4 days were selected and sandwiched between two soaked filter paper sheets, which were rolled and placed in sterilized glass jars. In case of un-inoculated control, sterilized broth was used. Sterilized Hoagland solution (half strength) of different salinity levels was applied in the jars, which were placed in growth chamber at 27°C for two weeks. Data regarding shoot and root elongation and fresh weight of seedlings was recorded and statistically analyzed using two factorial completely randomized designs.

RESULTS AND DISCUSSION

The statistical analysis of results showed significant differences in root elongation depending upon different treatments i.e., inoculation with rhizobacterial strain, control and salinity level. At salinity level of 1 dS m⁻¹ both the rhizobacterial strains N₃ and Q₇ increased root elongation significantly that was 36 and 34% higher, respectively over control (Fig. 1). At salinity level of 3 dS m⁻¹ isolate Q₇ was found more effective and there was significant increase in root elongation (45% higher than control). At 6 dS m⁻¹, 171% increase in root elongation as compared to control was observed due to inoculation with N₃ of 9 dS m⁻¹. Q₇ and N₃ increased root length to 333 and 300%, respectively greater than un-inoculated control. At 12 dS m⁻¹ Q₇ significantly increased root elongation that was 17% higher than control. Q₇ at under control condition showed 3% increase in shoot length over control (Fig. 2). At 3 dS m⁻¹ both Q₇ and N₃ significantly increased the shoot length that was 41.8 and 48% higher than control, respectively. At 6 dS m⁻¹ both strains exhibited similar increase in shoot length (20.6%). At 9 dS m⁻¹ both Q₇ and N₃ increased shoot length 59.5 and 38% compared to control.

Inoculation with ACC-deaminase containing rhizobacterial strains significantly increased fresh weight of seedling (root + shoot) over control (Fig. 3). Under control, both Q₇ and N₃ increased fresh weight up to 25% and 6.94% over control. At 3 dS m⁻¹ both the rhizobacterial strains significantly increased fresh weight that was 65 and 80% greater than control. At 6 dS m⁻¹, 113.7% increase in fresh

Fig. 1. Effect of ACC- deaminase containing rhizobacteria on root elongation of Maize (cm) at different salinity levels under axenic conditions

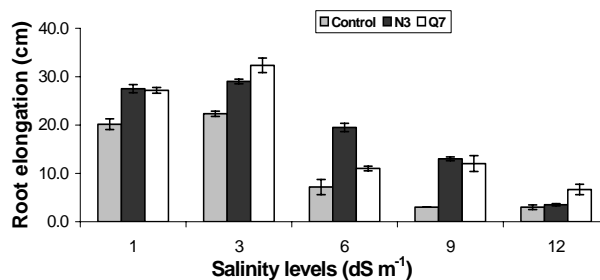


Fig. 2. Effect of ACC- deaminase containing rhizobacteria on shoot length of Maize (cm) at different salinity levels under axenic conditions

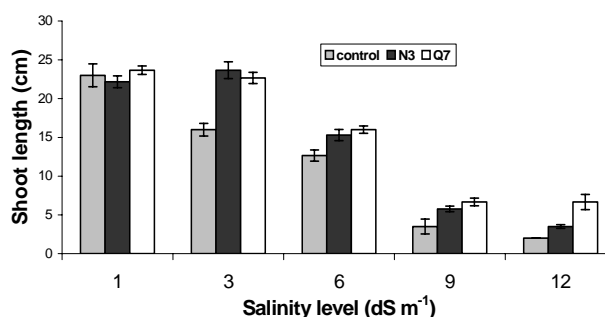
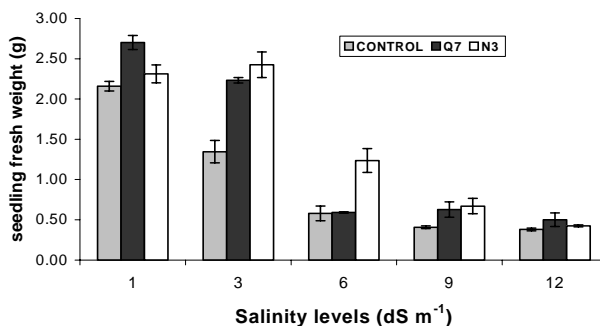


Fig. 3. Effect of ACC- deaminase containing rhizobacteria on fresh weight of Maize (g) at different salinity levels under axenic conditions



weight was noted due to inoculation with N₃. At 9 dS m⁻¹ both strains increased fresh weight (53.6 & 63% higher than control). At 12 dS m⁻¹ Q₇ and N₃ significantly increased fresh weight 31.5 and 10.5% more than control, respectively.

Inoculation with ACC-deaminase containing rhizobacterial strains boosted root elongation significantly under saline conditions. Glick (1997) reported the development of canola seedlings in presence of PGPR having ACC deaminase activity under stress conditions. Under all conditions the wild type bacterium promoted shoot and root elongation and seedlings fresh weight under non-saline and saline soil environment exposed to cold night

temperature. Mayak *et al.* (2004) evaluated the potential of rhizobacteria populating in dry salt environment to generate resistant in tomato against induced salt stress. The bacterium significantly increased the fresh and dry weight of seedlings and reduced the production of ethylene by roots.

It is concluded that the inoculation with rhizobacteria having ACC-deaminase activity is effective in promoting plant growth under salt stress by lowering the ethylene or ACC accumulation, whose higher levels have inhibitory effect on root and shoot growth.

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(Received 15 April 2006; Accepted 20 September 2006)