

## **Efficacy of Plant Growth Promoting Rhizo Bacteria Containing Acc-Deaminase Activity for Enhancing Growth of Maize (*Zea Mays* L) under Salt-Stressed Conditions**

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**Abstract:** Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it is increasing day by day. Salinity is one of the major anthropogenic as well as environmental stresses that reduce plant growth. Maize is a plant known for food, feed, and energy value, but being a greater biomass, it may also be utilized to extract pollutants from soil. Plant growth-promoting rhizobacteria (PGPR) may act as bio fertilizer to improve plant health. Some rhizobacteria are beneficial to plants and affect plant growth positively through different mechanisms of action. Ethylene, a plant hormone, also known as a stress hormone, is released by the plant as a physiological response when exposed to a variety of different stresses including both edaphic and adaphic. Growth of maize plants behaves better under saline environment as inoculated with different rhizobial strain showing ACC Deaminase activity due to the production of ethylene under stressed conditions. Reduction in sodium uptake by the utilization of different rhizobial strains under saline environment is a positive sign to induce salt tolerance biologically.

**Keywords:** Maize growth, Salinity, Ethylene, Rhizobial strains, Salt tolerance, PGPR ACC Deaminase.

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### **1. INTRODUCTION**

Salinity is one of the most decisive problems and retards agriculture productions in many areas around the world as well as Pakistan (Hasegawa *et al.*, 2000). The beginning of 21st century is discernible by global shortage of water resources, environmental pollution and increased salinization of soil and water. Mounting human population and reduction in land available for cultivation are two intimidations for agricultural sustainability (Shahbaz and Ashraf, 2013). Salt stress causes many aspects of plant metabolism and accordingly, growth and yield are reduced. Surfeit of salt in soil solution may harmfully affect plant growth either through osmotic reticence of water uptake by roots or specific ion effects. Salinity increases the uptake of Na<sup>+</sup>, which finally outcome into decrease in uptake of Ca<sup>+2</sup> and K<sup>+1</sup> (Yildirim *et al.* 2006). Uptake and accumulation of Cl<sup>-1</sup> may interrupt photosynthetic function through the inhibition of nitrate reeducate activity (Xu *et al.* 2000). Once the capacity of cells to store salts is pooped salts build up in the intercellular space leading to cell dryness and death (Sheldon *et al.* 2004).

Diverse environmental stresses i.e. high winds, severe temperatures, soil salinity, drought and flood have affected the production and cultivation of agricultural crops, among these soil salinity is one of the most demoralizing environmental stresses, which causes major reductions in cultivated land area, crop productivity and quality (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013). A saline soil is categorized in which the electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeds 4 dS m<sup>-1</sup> at 25<sup>0</sup>C and 15% exchangeable sodium. The yield of most crop plants is reduced at this ECe, however, many crops reveal yield reduction at lower ECes (Munns, 2005; Jamil *et al.*, 2011). It has been estimated that globally 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity. Moreover, the salinized areas are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices. It has been estimated that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011).

Salt stressed soils are known to repress the growth of plants (Paul, 2012). Plants in their natural environment are colonized both by endo cellular and intracellular microorganisms (Gray and Smith, 2005). Rhizosphere microorganisms, particularly beneficial bacteria and fungi, can improve plant growth under stress environments and, consequently, enhance yield both directly and indirectly (Dimkpa *et al.*, 2009). Some plant growth-promoting rhizobacteria (PGPR) may exert a direct stimulation on plant growth and development by providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate (Hayat *et al.*, 2010). Others do this indirectly by protecting the plant against soil-borne diseases, most of which are caused by pathogenic fungi (Lutgtenberg and Kamilova, 2009). The problem of soil salinization is a curse for agricultural productivity worldwide. Crops grown on saline soils endure due to high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions and reduced crop productivity. The present review stresses on the enrichment of productivity under stressed conditions and increased resistance of plants against salinity stress by use of plant growth promoting microorganisms. Plant growth-promoting rhizobacteria (PGPR) may well play a significant role in the development of sustainable agriculture. These are rhizobacteria that are beneficial to plants and affect plant growth directly or indirectly through various mechanisms of action (Mantelin and Touraine, 2004). All those bacteria inhabiting plant roots and influencing the plant growth positively by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR) (Asghar *et al.*, 2002).

Amid the plant hormones whose concentrations are most likely to be tainted by the PGPR include ethylene, auxin, gibberellins and cytokinin (Zahir *et al.*, 2005). The toxic penalty of high salt concentrations on plants includes increase of root ethylene synthesis, osmotic shock, and ionic imbalance (Mayak *et al.* 2004b).

Ethylene, a plant hormone, also known as a stress hormone, is unconfinned by the plant as a physiological response when showing to a variety of different stresses including both edaphic and adaphic. Salinity can increase the rate of ethylene biosynthesis via superior levels of 1-aminocyclopropane-1-carboxylic acid (ACC), which may lead to physiological changes in plant tissues. Any check on this accelerated ethylene production in plants can improve growth of plants under salt stress. Recently it has been found that bacteria containing ACC deaminase enzyme can hydrolyze the endogenous levels of ACC, an instant precursor of ethylene, into ammonia and acetobutyrate resulting in reduced production of C<sub>2</sub>H<sub>4</sub> (Hontzeas *et al.* 2004).

Many plant growth promoting rhizobacteria (PGPR) make possible plant growth indirectly by reducing plant pathogens or directly by facilitating the uptake of nutrients from environment. It has also been observed that plants inoculated with PGPR having different PGPR traits are more resistant to the toxic effect of stress ethylene synthesized as a result of stress conditions (Zahir *et al.* 2008). Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it vibrant for nutrient turn over and sustainable for crop production (Ahemad *et al.*, 2009; Chandler *et al.*, 2008). In reality, the bacteria living around/in the plant roots (rhizobacteria) are more flexible in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils (Hayat *et al.*, 2010). Therefore, the rhizobacteria are the dominant driving forces in recycling the soil nutrients and consequently, they are critical for soil fertility (Glick, 2012).

Newly, it has been exposed that certain plant growth promoting rhizobacteria having ACC-deaminase enzyme activity that changes ACC in to  $\alpha$ - ketobutyrate and ammonia (Tahir *et al.*, 2006; Arshad *et al.*, 2007) and reduce the amount of ACC in addition to ethylene outside the germinating seeds. Ethylene is a plant hormone that is concerned in the regulation of many physiological responses. Ethylene is the most important growth hormone produced by almost all the plants, which mediates a wide range of plant responses (Arshad and Frankenberger 2002). It is produced naturally and is involved in several developmental processes ranging from seed germination to senescence. Its synthesis is fastened in response to various environmental stresses (Mayak *et al.* 2004a, b), particularly in plants subjected to salinity stress (Zapata *et al.* 2004). A sharp increase in ACC levels under stress conditions, which subsequently results in accelerated C<sub>2</sub>H<sub>4</sub> synthesis, has been frequently reported (Mayak *et al.* 2004b; Arshad *et al.* 2007). Keeping in view the fact, this study was conducted to screen and evaluate rhizobacteria containing ACC deaminase for inducing salt tolerance and thus improving the growth of maize under salt-stressed conditions.

## 2. MATERIALS AND METHODS

The study was carried at National Agriculture Research Centre Islamabad to screen and evaluate rhizobacteria containing ACC deaminase for inducing salt tolerance and thus improving the growth of maize under salt-stressed conditions. ( $EC_e=5.58 \text{ dS m}^{-1}$ ) as indicated in table-1. The salinity was developed by adding salts. The soil for this purpose was taken from NARC (National Agriculture Research Center). The design was completely randomized with three repeats. Wheat seeds were inoculated with rhizo bacterial strains which were:  $M_1$   $M_4$   $M_5$   $M_8$  and  $M_9$ . Salinity ( $5.58 \text{ dS m}^{-1}$ ) was artificially developed using salts. A soil sample (0-20 cm depth) was collected from experimental soil before sowing of crop and fertilizers application. Plant samples were collected to investigate the effect of different rhizobial strains on the availability of nutrients to plants. Soil samples were analyzed for various physicochemical properties using standard methods (Ryan *et al.*, 2001 and Sparks *et al.*, 1996) and soil texture by Bouyoucous Hydrometer method Practical Agri. Chemistry Kanwar and Chopra (1959). The data obtained were subjected to statistical analysis using the STATISTIX statistical software (Version 8.1) and the mean values were compared using Least significant difference (LSD) multiple range test  $P: 0.5\%$ . (Steel and Torrie, 1997).

**Table1.** *Physiochemical analysis of soil used in the experiment*

Characteristics	Unit	Values
pH	-	7.50
Electrical conductivity	( $\text{dS m}^{-1}$ )	5.58
Organic Matter	(%)	0.72
Na (AB-DTPA)	ppm	150
K (AB-DTPA)	ppm	204
P (AB-DTPA)	ppm	1.63
Ca+Mg	(meq/L)	20
Carbonate	(meq/L)	0.6
Bicarbonate	(meq/L)	0.19
SAR	meq/L)	9.98
Soil texture	-	Sandy Loam

## 3. RESULTS AND DISCUSSION

Growth of maize plants was significantly affected by different rhizobial strains containing ACC Deaminase activity under artificially developed saline conditions ( $EC_e= 5.58 \text{ dS m}^{-1}$ ) mentioned in table-2. Plant height significantly affected by the inoculation maize seeds with different rhizobial strains under artificially saline soil i.e.  $EC_e= 5.58 \text{ dS m}^{-1}$  (Table-2). The highest plant height (13cm) was gained by inoculating  $M_9$  and lowest height in plant (9.0 cm) was observed in control i.e. without inoculation. This depicted that inoculation of maize seed with rhizobial strains showed better behaviour in plant height mitigating the toxic impacts of saline conditions ( $EC_e=5.58 \text{ dS m}^{-1}$ ). Plant fresh weight showed statistically significant results in data of wheat plants inoculated with strains under saline environment as indicated in table-2. Maximum fresh weight ( $8.6 \text{ g plant}^{-1}$ ) was attained by  $M_9$ . Remaining strains showed better fresh weight comparing with control saline conditions at  $EC_e=5.58 \text{ dS m}^{-1}$ . Plant dry weight of maize plants also depicted similar findings as indicated in plant fresh weight. Chlorophyll content is a criterion for the citadel in plant photosynthesis. Significant findings were noted as depicted in table-2.  $M_9$  attained the highest value (49%) which was statistically at par with  $M_8$  and  $M_5$ . The lowest value (33%) attained in control treatment. Yildirim *et al.* (2006) reported an enhancement of squash plant when applied directly or as a transplant under salinity stress. Inoculation of plant with ACC deaminase containing PGPR resulted in enhanced chlorophyll contents of maize as well as lettuce (Han and Lee 2005).

**Table 2.** *Effect of ACC deaminase on maize growth under saline conditions*

Treatments	Plant Height (cm $\text{plant}^{-1}$ )	Plant fresh weight (g $\text{plant}^{-1}$ )	Plant dry weight (g $\text{plant}^{-1}$ )	Chlorophyll contents (%)
Control	9.0c	2.4d	0.9d	33c
$M_1$	10.0bc	3.8c	1.2 c	36c
$M_4$	11.0 b	5. 6b	1.7b	42b
$M_5$	9. 7bc	3.8c	1.3c	48a
$M_8$	10.3bc	3.9c	1.2c	49a
$M_9$	13. 7a	8.6a	3.1a	49a
LSD (0.5%)	1.9	0.4	0.2	10

Values followed by same letter(s) are statistically similar at P=0.05 level of significance

Salinity is one of the solemn environmental evils that cause osmotic stress and decline in plant growth and crop productivity in irrigated areas of arid and semiarid regions (Cicek and Cakirlar 2002). Plants that are treated with plant growth-promoting rhizobacteria (PGPR) containing ACC deaminase are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of drought and salinity stresses (Mayak *et al.* 2004a, b). When ACC deaminase containing PGPR are bound to the developing seedling, they may act as a sink for ACC ensuring that the ethylene level does not become grand to the point where root growth is impaired (Grichko *et al.*, 2000). Soil microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote plant growth by sequestering and cleaving plant-produced ACC, and thereby lowering the level of ethylene in the plant (Penrose *et al.*, 2001). A decreased ethylene level permits the plant to be extra resistant to a wide variety of environmental stresses such as salinity, drought and metal toxicity (Glick, 2005).

Maize seed inoculated with different strains of bacteria having ACC deaminase effect on plant growth under saline conditions (EC<sub>e</sub>= 5.58 dS m<sup>-1</sup>). Ionic concentration of P (%) in wheat plants showed significant differences among treatments (Table-3). Uptake of P (%) was more (0.2%) by M<sub>4</sub> and control showed the lowest (0.097%). Uptake of K (%) was the highest (3.0 %) by M<sub>1</sub> and lowest was determined in control (Table-3). Sodium ionic concentration showed significant results among treatments (Table-3). However Na (%) was the highest in control and lowest by the maize plant tissues inoculation by M<sub>4</sub>. This means that reduction in sodium ions in maize plants using inoculation with rhizobial strains mitigates the salinity and grows maize plants in better conditions to induce salt tolerance with the reduction of ethylene production under saline environment. Ahemad, 2012; Hayat *et al.*, 2010; Rajkumar *et al.*, 2010; Braud *et al.*, 2009 investigated in their experiments that PGPR containing ACC Deaminase excite plant growth through mobilizing nutrients in soils. Yuhashi *et al.*, (2000) reported that reduction in levels of ACC upshot in lowering the synthesis of endogenous ethylene, which lessen the inhibitory effects of higher ethylene levels 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 i.e. at high salt concentration (Kausar and Shahzad, 2006; Nadeem *et al.*, 2007).

**Table 3.** Effect of ACC deaminase on the uptake of nutrients of maize plants

Treatments	P%	K%	Na%	Mn (ppm)	Fe (ppm)	Zn (ppm)
Control	0.097c	2.00d	3.01a	69.67bc	0.04 <sup>NS</sup>	0.038c
M <sub>1</sub>	0.147b	3.00a	1.97c	70.67b	0.05	0.043b
M <sub>4</sub>	0.200a	2.00d	1.94 c	68.00c	0.04	0.042b
M <sub>5</sub>	0.143b	2.00d	1.91 c	76.33a	0.05	0.051a
M <sub>8</sub>	0.140b	2.50b	2.08 b	71.33b	0.04	0.052a
M <sub>9</sub>	0.087d	2.37c	2.13 b	69.67bc	0.04	0.044b
LSD (0.5%)	0.004	0.01	0.09	2.22	-----	0.008

Values followed by same letter(s) are statistically similar at P=0.05 level of significance

#### 4. CONCLUSION

Growth of maize plants behaves better under saline environment as inoculated with different rhizobial strain showing ACC Deraminaze activity due to the production of ethylene under stressed conditions. Reduction in sodium uptake by the utilization of different rhizobial strains under saline environment is a positive sign to induce salt tolerance biologically.

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