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Exploitation of Plant Growth Promoting Rhizobactria For ACC Deaminase Induced Salt Tolerance In Sugarcane (Saccharum Officinarum L.)

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Abstract: ***Sugarcane (Saccharum officinarum L.), is an important cash crop of Pakistan and plays an important role in the uplift of socioeconomic conditions of the growers. Salinity is one of the most critical constraints and hampers agriculture productions in many areas around the world, including Pakistan. Ethylene is the plant growth regulating hormone produced in response to salinity. PGPR under salt stressed environment exhibits 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity which reduces the level of ACC and endogenous ethylene, mitigating the deleterious effects of salt stress on plant growth. The plants inoculated with PGPR having ACC deaminase, are relatively more tolerant to salt stress. The study was carried at National Agriculture Research Centre, Islamabad to investigate the effect of PGPR (Plant Growth Promoting Rhizobacteria) on Sugarcane crop, in saline soil. Completely randomized design was applied with three repeats. Rhizobacterial isolates, i e., RS-17AT, ES-17 SOW, ERS-17 WATERY, RS-17 LY and ES-17 SW, were inoculated to dip with solution mixing distilled water the sugarcane sets of variety YT-55 and inoculated sets were sown in plastic tubs having saline soil (8.00 dS m-1) during spring, 2014. Results showed that plant height was significantly affected by different rhizobial isolates . Maximum plant height (23.66 cm) was attained by inoculating RS-17 AT and maximum plant fresh and dry weights 2.65 and 1.32 g plant-1 were attained respectively by inoculating RS-17 LY strain of bacteria under saline conditions. The results of this study revealed that two rhizobial isolates , i.e. RS-17 AT and RS-17 LY, showed better results than control; showing mitigating salt stress and producing more salt tolerance in sugarcane plants.***

Keywords: Salinity, Ethylene, Rhizobial isolates, Salt tolerance**.**

1. Introduction

Plant growth promoting rhizobacteria (PGPR) are considered as advantageous bacteria in the rhizosphere and helpful for sustainable agriculture by assisting plant growth and development, directly or indirectly (Muhammad *et al*., 2007). PGPR exert some of these functions by means of specific enzymes, which agitate certain physiological and biochemical changes in plants (Bashan *et al*., 2004). Hass and Keel (2003) classified PGPR) based on their activities as biofertilizers (increasing the availability of nutrients to plant), phyto stimulators (plant growth promoting, usually by the production of phytohormones), rhizoremediators (degrading organic pollutants) and bio pesticides (controlling diseases by the production of antibiotics and antifungal metabolites). Bhattacharyya and Jha (2012) reported that PGPR are the rhizosphere bacteria that can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, Interference with pathogen toxin production, etc.

Salinity is one of the most critical constraints and hampers agriculture productions in many areas around the world, including Pakistan (Hasegawa and Bresan, 2000). Sugarcane is the second largest cash crop of Pakistan and is being cultivated on 0.966 million hectares, contributing around 3.6 % of GDP. Sugarcane currently accounts 4.8% of cropped area and 11% value added of the total crops (Economic Survey of Pakistan, 2012). Salinity is a severe problem for temperate and tropical agriculture system, affecting 20% of global agriculture land. The harmful effects of presence of salts in soil result in increased level of ethylene in root, ionic imbalance and hyper-osmotic condition in plants. Physical removal of salts from the surface of soil or chemical treatment of soil is not only expensive but can't be applied to vast areas for soil reclamation purposes (Measham, 2009). These PGPR tolerate wide range of salt stress and enable plants to withstand salinity by hydraulic conductance, osmotic accumulation, sequestering toxic Na+ ions, maintaining the higher osmotic conductance and photosynthetic activities (Barashi *et al.*,2006).

Ethylene is the plant growth regulating hormone produced in response to water logging salinity and/or drought. PGPR from stressed environment exhibit 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity which reduces the level of ACC and endogenous ethylene, mitigating the deleterious effects of stress on overall plant growth. The plants inoculated with PGPR having ACC deaminase are relatively more tolerant to environmental stress (Naveed *et al*., 2008). Keeping in view

of these contraints in saline environment, a pot experiment was conducted to see the response of PGPR, for ACC-deaminase activity induced salt tolerance in sugarcane *(Saccharum officinarum L.)* crop and determine the extent and degree to which sugarcane plant growth is affected with the inoculation of different bacterial strains.

2. MATERIALS AND METHODS

The study was carried at National Agriculture Research Centre, Islamabad to see the effect of PGPR (Plant growth promoting bacteria rhizobacteria) on Sugarcane crop under saline soil (ECe=8.00 dS m^{-1}) as indicated in table-1. To see that bacterial strains having ACC deaminase had significant effect on sugarcane growth and ionic concentration. The salinity was developed by adding salts. The soil for this purpose was taken from NARC (National Agriculture Research Center). The design was completely randominzed with three repeats. Sugarcane sets were inoculated with rhizobacterial strains which were: RS-17AT, ES-17 SOW, ERS-17 WATERY. RS-17 LY, and ES-17 SW. These strains were isolated from the sugarcane salt-affected fields.The Sugarcane sets of variety YT-55 were sown on 27th March, 2014 in plastic tubs , each tub contained average 13kg soil and 65 gram of NaCl . A soil sample (0-20 cm depth) was collected from experimental area before sowing of crop and fertilizers application. Plant samples were collected at maturity stage to study different fertilizers effect on the availability of nutrients to plants. Soil samples were analyzed for various physicochemical properties using standard methods (Ryan *et al.,* 2001;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).

3. RESULTS AND DISCUSSION

Plant height was significantly affected by different rhizobial strains. The maximum height (23.66 cm) was attained by inoculating RS-17 AT and lowest height in plant was noted by the control, i.e., without inoculation. However, rhizobial strins ES-17 SOW, RS-17 LY and ES-17SW results were very close to the control. RS-17 AT and RS-17 WATERY strains showed better performance in plant height than control under saline conditions at ECe=8.00 dS m^{-1}
(Table-2). Data regarding plant fresh weight showed significant results among treatments as indicated in table-2. Maximum fresh weight (2.65 g $plant^{-1}$) was attained by RS-17 AT which was at par with RS-17 WATERY(2.47 g $plant^{-1}$). Remainig three strains showed statistically similar as control under saline conditions at ECe=8.00 dS m^{-1}. Similar results were showed in plant dry weight as plant fresh weight, indicated in table 2. The positive effects of OSU 142 and M3 on the yield and growth of crops such as apricot, tomatoes, sugar beet, and barley were explained by N_2 fixation ability, phosphate solubilizing capacity and antimicrobial substance production (Sahin *et al.,* 2004; Abbasi *et al.,*2012; Cakmakci *et al.,*2001; Esitken *et al.,* 2003, 2006).
When sugarcane sets were inoculated with different strains of bacteria having ACC deaminase effect on sugarcane growth under saline conditions (ECe= 8.00 dS m^{-1}), ionic concentration of P (%) in sugarcane plants showed significant differences among treatments (Table-3). Uptake of P (%) was more (0.21%) by RS-17 WATERY that was very close to RS-17 AT(0.20%) and other inoculated sets and control showed similar results. Uptake of K (%) was the highest (2.16 %) by RS-17 AT that was very close to RS-17 WATERY (2.11%) and other inoculated sets and control showed statistically similar results (Table-3). Sodium ionic concentration showed significant results among treatments (Table-3). However, Na (%) was the highest in control and lowest by the sugarcane sets had inoculation of RS-17 WATERY. Fe, Zn and Cu showed non-significant behavior in all the treatments as indicated in table-3. Singh *et al* (2013) reported that judicious use of chemicals along with bio fertilizers and organic resources can be helpful in sustaining the crop productivity and soil health.

4. CONCLUSION

After the measuring of plants height, weight, chemical and statistical analysis, we conclude that the plants of sugarcane showed significant results in all parameters (height, weight, ionic concentration of nutrients) which were inoculated with the ACC deaminase containing bacteria, named RS-17 LY .These plants showed more salt tolerance and RS-17 AT also followed, it also gave better results but the last one was more efficient than all other applied strains than control.

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Table1; Physiochemical analysis of soil used in the experiment

Charachteristics	Unit	Values
Electrical conductivity	(dS m^{-1})	8
Organic Matter	(%)	0.631
Na	ppm	786
K	ppm	48
P (AB-DTPA)	ppm	26.92
Ca+Mg	(meq/L)	430
Carbonate	(meq/L)	2.1
NO_3-N	ppm	9.48
Bicarbonate	(meq/L)	1.8
SAR	meq/L)	53.61
Soil type	-	Sandy Loam

Table.2 Effect of AC C deaminase on growth of sugarcane under saline conditions

Treatments	Plant Hieght (cm $plant^{-1}$)	Plant freshweight (g $plant^{-1}$)	Plant dry weight (g $plant^{-1}$)
Control	15.66 c	1.55 b	0.74 b
RS-17 AT	23.66 a	2.65 a	1.37 a
ES-17 SOW	16.16 c	1.83 b	0.67 b
RS-17 WATERY	20.50 ab	2.47 a	1.28 a
RS-17 LY	16.83 b c	1.51 b	0.72 a
ES-17SW	17.06 c	1.62 b	0.82 b
LSD (0.5%)	3.72	0.41	0.29

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

Table.3 Effect of ACC deaminase on the ionic concentration of nutrietns in sugarcane plants

Treatments	P (%)	K (%)	Na (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)
Control	0.15 b	1.64 c	3.84 a	592.5	27.58	1.60
RS-17 AT	0.20 a	1.98 abc	2.64 b	602.00	29.68	1.80
ES-17 SOW	0.19 a	1.90 abc	1.83 c	586.67	29.73	1.66
RS-17 WATERY	0.21 a	2.11 ab	1.58c	610.73	26.86	1.63
RS – 17 LY	0.18 a	2.16 a	1.93c	694.32	23.46 a	1.76
ES- 17 SW	0.16 b	1.76 bc	2.93 b	674.56	23.60 d	1.80
LSD(0.5%)	0.03	0.38	0.54	NS	NS	NS

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