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Mitigation of salt stress in wheat plant (*Triticum aestivum*) by plant growth promoting rhizobacteria for ACC deaminase

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Abstract

Wheat (*Triticum aestivum*) is the essential diet of population as it constitutes 60% of the daily diet of common man in Pakistan Salinity is a barrier towards growing a sustainable food production system and environment management. PGPR (Plant growth promoting bacteria Rhizobacteria) under salt stressed conditions causes 1-aminocyclopropane-1-carboxylate (ACC) deaminase action which minimizes the intensity of ACC and endogenous ethylene justifying the toxic 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 conducted at National Agriculture Research Centre Islamabad to examine the consequence of PGPR on wheat crop under saline environment to see that bacterial strains having ACC deaminase had significant effect on wheat growth and ionic concentration. The design was completely randominzed with three repeats.Wheat seeds were inoculated with rhizobacterial strains which were: WPR-51, WPR-61,WM-4, WM- and WPS-8. Salinity (9.68 dS m^{-1}**)** was artificially developed using salts.. Growth of wheat plants performs better under saline environment as inoculated with different rhizobial strains 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 mitigate salt stress biologically

Keywords: Wheat growth, Salinity, Ethylene, Rhizobial strains, Salt tolerance

Introduction

Wheat is the essential diet of population as it constitutes 60% of the daily diet of common man in Pakistan and average per capita consumption is about 125 kg and occupies a central position in agricultural policies ofthe government (Gop, 2013). Salinity is a barrier towards growing a sustainable food production system and environment management throughout the world. Such dilemma has its origin from aquatic, ecological and anthropogenic actions. Soil salinity severely deteriorates soil health (socio-economic well being) which in turn declines crop productivity.

Arid and semi-arid lands worldwide have been intensily affecting the problem of soil. Approximately the increase in saline soils is at the rate of 7% in the world (Tester and. Davenport,2003). At a global level, the total amount of saline soils is around 15% in arid and semi-arid regions and approximately40% in irrigated lands (Zahran,1997). Elevated soil salinity negatively affects the physical and chemical properties of soil, thereby openly affecting the growth and assortment of organisms that live in or on soil such as plants, microbes, protozoa and nematodes. In plants, continuing high soil salinity situation causes ionic and osmotic stress that harmfully affects the functioning of various biochemical processes (Parida an.Das,2005). Under high salinity conditions, plants survive with stress which ultimately restricts expansion of leaves. This shows that cell division and expansion processes are sternly affected firstly besides the closure of stoma (Munns.,2002 and Flowers,2004). Premature ageing of leaves during ionic stress affects photosynthesis process and end result in stunted growth (Cramer and. Nowak,1992). More, excessive concentrations of sodium and chloride negatively affect the energy production and physiology of the plants by snooping with various enzymes activities (Larcher,1980).Significant decrease in productivity of salt-sensitive and salt-tolerant crops was resulted in salt stress conditions. Mostly the salt stress thresholds of the cereal crops ise low e.g. wheat can tolerate salinity up to 6 dSm^{-1}, while the salinity threshold for maize is three times less (approximately 2 dSm^{-1}) (Saeed *et al*.,2001). Kotuby-Amazher *et al*.,(2000) exposed that, useful microorganisms can lessen salt stress in maize and wheat crops around 50%. In addition, it has been established that positive microorganism play a significant role in alleviating salt stress in plants, causing in better crop produce. Plant-growth-promoting (PGP) bacteria are a set of microorganisms that colonise the root of plants or free-living organisms that directly or indirectly the growth of plants (Lugtenberg and F. Kamilova,2009 and Ahmadet al., 2013). In direct growth promotion, they produce some compounds (indole acetic acid, siderophore, HCN, etc.), solubilise minerals and break organic matters for easy uptake by plants and for their own use. They also fix atmospheric nitrogen and produce siderophores that increase the bioavailability of iron and synthesise phytohormones such as cytokinins, auxins and gibberellins which have beneficial roles in various stages of plant growth (Lucy *et al*.,2004, Gray and Smith,2005 and Richardson and Simpson,2011). Ultimately, they help in lessening or inhibiting the injurious effects of pathogenic organisms by increasing the host resistance to pathogenic organisms (Bloemberg and Lugtenberg, 2001 and Van,2007).

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, mainly 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

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 contrints in saline environment a pot experiment was conducted to see the response of Plant Growth Promoting Rhizobacteria for ACC-Deaminase activity iInduced 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.

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 wheat crop under saline soil (ECe=9.68 dS m^{-1}) as indicated in table-1. To see that bacterial strains having ACC deaminase had significant effect on wheat 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. Wheat seeds were inoculated with rhizobacterial strains which were: WPR-51, WPR-61,WM-4, WM- and WPS-8. Salinity(9.68 dS m^{-1}**)** was artificially developed using salts. A soil sample (0-20 cm depth) was collected from experimental area before sowing of crop and fertilizers application. Plant samples were collected to see 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	-	7.41
Electrical conductivity	(dS m^{-1})	9.68
Organic Matter	(%)	0.523
Na	ppm	303
K	ppm	47
P (AB-DTPA)	ppm	0.68
Ca+Mg	(meq/L)	19
Carbonate	(meq/L)	0.5
Bicarbonate	(meq/L)	0.18
SAR	meq/L)	10.08
Soil type	-	Sandy Loam

Results and Discussion

Plant height significantly affected by the inoculation wheat seeds with different rhizobial strains under artificially developedsalin e soil i.e. ECe= 9.86 dS m^{-1} (Table-2). The highest plant height (13.97 cm) was gained by inoculating WPR-61 which was statistically at par with the results attained with WPS-8 and WM-9 and lowest height in plant (10.97 cm) was observed in control i.e. without inoculation.This indicated that inoculation of wheat seed with rhizobial strains showed better responses in plant height mitigating the adverse effects of saline conditions (ECe=8.00 dS m^{-1}). Statistically significant results were attained in plant fresh weight data of wheat plants inoculated with strains under saline environment as indicated in table-2.Maximum fresh weight (2.06 g $plant^{-1}$) was attained by WPR-51.

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

Treatments	Plant Height (cm $plant^{-1}$)	Plant freshweight (g $plant^{-1}$)	Plant dry weight (g $plant^{-1}$)	Chlorophyll contents(%)
Control	10.07 d	0.98d	0.43 d	38.37b
WPR-51	12.33 b	2.06 a	1.01 a	38.97a
WPR-61	13.97 a	1.75 b	0.89 b	37.97bc
WM-4	11.17 c	1.20 cd	0.70 c	29.43c
WM-9	13.68 a	1.31 c	0.80 bc	29.03cd
WPS-8	13.93a	1.09 d	0.48 d	28.77d
LSD(0.5%)	0.37	0.13	0.11	0.56

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

Remainig strains showed negative results comparing with control saline conditions at ECe=9.86 dS m^{-1}. Plant dry weight indicated same results as plant fresh weight in table 2. Cholophyll content is a criteria for the fastness in plant photosynthesis. Significant findings were obtained in this parameter as indicated in table-2. WPR-51 attained the highest value (38.97%). After that contol treatment i.e. without inoculation gained this parameter lowring the values of remaining strains.Many researchers have reported better growth in plants inoculated with bacteria containing ACC-deaminase (Mayak *et al.,* 2004; Shaharoona *et al.,* 2006). Ethylene is a stress hormone and is produced at higher concentration under any kind of stress including salinity. It is very likely that the rhizobacterial strins promoted root and shoot growth by lowering the endogenous inhibitory levels of ethylene in roots becauseof its high ACC metabolizing ability (Kang *et al*., 2010).Such promising rhizobial strains could possibly be used under field conditions in saline environment where agriculture is exclusively reliant on saline conditions (Hamayun *et al*., 2010).

When seeds were inoculated with different strains of bacteria having ACC deaminase effect on wheat plant growth under saline conditions (ECe= 9.86 dS m^{-1}). ionic concentration of P (%) in wheat plants showed significant differences among treatments(Table-3). Uptake of P (%) was more (0.18%) by WPS-8 and control showed the lowest (012%). Uptake of k (%)was the highest(3.38 %) by WPS-8 and lowest was determined in control as well as in WPR- (Table-3). Sodium ionic concentration showed significant results among treatments (Table-3). However Na (%) was the highest in control and lowest by the wheat seed inoculation by WPR-51. This means that reduction in sodium ions in wheat plants using inoculation with rhizobial strains mitigates the salinity and grows wheat plants in better conditions under saline environment. 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.

Table.3 Effect of ACC deaminse on the ionic concentration of nutrietns in wheat plants

Treatments	P (%)	K (%)	Na (%)
Control	0.13 d	2.87c	3.01 a
WPR-51	0.12 d	2.87 c	1.78d
WPR-61	0.17 ab	2.17 d	2.94 a
WM-4	0.15 c	2.93c	1.91 c
WM-9	0.16 b	3.10b	2.08 b
WPS-8	0.18 a	3.38a	2.13 b
LSD(0.5%)	0.01	0.16	0.09

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

Conclusion

Growth of wheat plants performs better under saline environment as inoculated with different rhizobial strains 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 mitigate salt stress biologically.

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