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RHIZOBACTERIA HAVING ACC-DEAMINASE AND BIOGAS SLURRY CAN MITIGATE SALINITY ADVERSE EFFECTS IN WHEAT

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Abstract

Salinity is a major hurdle to the sustainable production of crops at the world scale. It elevates the level of ethylene in plants that causes poor root growth and development thus resulting in low yield. Inoculation of rhizobacteria having ACC deaminase (ACCD) is an effective technique to regularize the endogenous ethylene generated via salinity induced stress. On the other hand, biogas slurry (BGS) is also well known for its plant-growth-promoting role, even in stressful conditions. Therefore, the present field trials were conducted to explore the individual and combined treatment effects of rhizobacteria and BGS on wheat under salinity stress. There were three ACC deminase rhizobacteria i.e., *Lysinibacillus fusiformis*, *Bacillus cereus* and *Alcaligenes faecalis* inoculated with and without BGS (as 600 kg ha^{-1}) on physiological, growth and yield indices of wheat under salinity stress. The results showed that the *B. cereus*+BGS significantly improved plant height (17 and 35%), number of tillers $plant^{-1}$ (46.2 and 57.9%), 1000-grain weight (34 and 33%), grain (46 and 50%) and straw (37 and 37%) yield over control under salinity stress in study 1 and 2, respectively. A significant decrease (24 and 29%) in electrolyte leakage also validated the effectiveness of *B. cereus* and *B. cereus+BGS* over control in the experiments 1 and 2, respectively, under salinity stress. Relative water content were also enhanced (36 and 27%) with *B. cereus+BGS* over control in the study 1 and 2, respectively. It is conclusively stated that the combined use of *B. cereus*+BGS is an efficacious treatment to alleviate the adverse effects of salinity in wheat.

Key words: Ethylene, Growth attributes, Electrolyte leakage, Salt stress, Organic amendment, Yield attributes.

Introduction

Triticum aestivum L. is a widely grown cereal crop. It covers 17% of the world arable land (Anon., 2012). With the passage of time, demand for wheat is continuously increasing with exponentially increasing population and social changes (Ivic, 2020). At the same time, in dry areas of the world, the yield of wheat is decreasing (20–43%) owing to salinity stress (Farooq *et al*., 2015; Singh & Jha, 2016a,b). According to FAO, approximately, 7 million ha of cultivatable land in Pakistan is salt affected (Anon., 2017). Higher evaporation and low rainfall are leading factors in dry areas for the development of salt affected soils (Hussain *et al*., 2018). When crops are cultivated on such salt affected soils, the higher concentration of water soluble salts induces oxidative damage, reducing the stomatal conductance, photosynthetic efficiency and water absorption by roots in plants (Kravchik & Bernstein, 2013). Presence of excessive soluble salts in the soil also exerts harmful effects on metabolism, germination and growth of plants as well as microbial life (Liu *et al*., 2015; Prakash *et al*., 2019; Metoui Ben Mahmoud *et al*., 2020).

Prolonged salinity stress results in the limited uptake of Ca^{2+} and K^+ simultaneously with an increased uptake of Na^+, therefore, inducing ionic toxicity and nutritional imbalance as well (Ahmad *et al*., 2016; Akbari *et al*., 2020). Most of the scientists suggest to use organic and environment friendly amendments conjointly to alleviate the salinity stress in plants. Among these amendments, plant-growth-promoting rhizobacteria (PGPR) have gained the center of attention due to its potential benefits. The PGPR colonize roots of plants and promote their growth through a variety of mechanisms (Zafar-ul-Hye *et al*., 2019, 2020a; Danish & Zafar-ul-Hye, 2020; Danish *et al*., 2020). The PGPR include diverse bacterial taxa that can promote plant growth through nutrient solubilization, production of phytohormones and sequestration of iron by bacterial siderophores (Nazli *et al*., 2020). In short, rhizobacteria improve the plant growth and yield of crops (Barnawal *et al*., 2014; Danish *et al*., 2019a). They modify plant physiological indices via release of growth regulators, ACC-deaminase and osmolytes etc., (Ahmad *et al*., 2013). Such PGPR seems to be involved in a significant increase in K^+ uptake and chlorophyll content, reduction in toxic Na^+ ionic content and leaf proline (Prakash *et al*., 2019).

Ethylene is, though, essential for normal plant growth and ripening of fruits, but seed germination and root growth decrease due to an excessive amount of ethylene accumulation (Saravanakumar & Samiyappan, 2007; Metoui Ben Mahmoud *et al*., 2020). The ethylene production increased under abiotic or biotic stress conditions, is referred as stress ethylene. It exerts inhibitory effects on elongation of roots and suppresses the leaves expansion, leaves abscission and chlorosis (Guo *et al*., 2009). When excessive 1-aminocyclopropane-1-carboxylic acid (ACC) moves out from the plant root, while ACC deaminase containing PGPR can convert ACC to ammonia and α-ketobutyrate (Danish & Zafar-ul-Hye, 2019; Danish *et al*., 2019b). These PGPR can invade rhizoplane, penetrate into roots and induce salinity tolerance in plants (Tank & Saraf, 2010).

Biogas slurry (BGS) is a very effective organic amendment, that can act as a soil conditioner and improve crop production. The BGS is an environmentally safe material which is, otherwise, wasted unsafely (Maqbool *et al*., 2014). Rhizobacteria along with organic amendments improve yield and growth of mung bean (Iqbal *et al*., 2016), cucumber (Ahmad *et al*., 2015) and peanut (Zheng *et al*., 2016) by lowering the salt and sodium stress (Jabeen & Ahmad, 2017). Rhizobacteria in conjunction BGS may result in improved quality and yield of plants (Tan *et al*., 2016) as well as soil physical indices (Chen *et al*., 2017). The conjoint use of organic amendments and rhizobacteria is helpful in the promotion of crop production, modification of soil pH as well as improvement in the availability of nitrogen (N), phosphorus (P) and potassium (K) (Du *et al*., 2019).

Thus, the rhizobacteria having ACC deaminase activity reduce the effects of salinity (Dodd & Pérez-Alfocea, 2012). Moreover, the use of biofertilizers is cheap, easily available, environmentally safe and more effective in comparison with the chemical fertilizers. As, a little information is documented regarding combined use of ACC deaminase producing PGPR and BGS, so the current experiment was conducted to explore its efficacy to alleviate salinity stress and to promote wheat growth.

Materials and Methods

Collection of the strains: Three rhizobacterial i.e., *Bacillus cereus, Alcaligenes faecalis* and *Lysinibacillus fusiformis* (ACC–deaminase positive) were obtained from the Laboratory of Soil and Environmental Microbiology, Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan.

Inocula preparation: The fresh inocula were prepared in 500 mL flasks with DF minimal salt medium (Dworkin and Foster, 1958) that was kept at 100 rpm shaking and 25°C tenperature for three hours. With sterilized distilled water dilution of broth was accomplished at 0.45 nm on spectrophotometer to achieve10^7 to 10^8 CFU mL^{-1} population. The seed coating was carried out with inoculum, sterilized sugar solution (10%) and peat and clay. In control, the same but sterilized material was used.

Experimental site: Two field trials were conducted at the experimental farm of the Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University. Before carrying out the experiments, soil sampling was done for pre sowing analyses of physio-chemical attributes (Table 1).

Treatments and experimental design: The experiments consisted of two factors. There were four levels of factor 1 i.e., uninoculted control, *Lysinibacillus fusiformis, Bacillus cereus* and *Alcaligenes faecalis*. Factor 2 consisted of two levels i.e., control (no BGS) and BGS (600 kg ha^{-1}). The BGS was taken from a biogas plant. The necessary attributes of the BGS were determined following prevailing protocols (Ryan *et al*., 2001). The treatments were applied with three replicates following randomized complete block design (RCBD).

Table 1. Pre sowing physical and chemical attributes of soil and BGS.

Characteristics	Units	Soil	Units	BGS
Textural class	-	**Sandy clay loam**	-	-
Sand	%	53	-	-
Silt	%	20	-	-
Clay	%	27	-	-
pH_s	-	8.20	-	7.5
EC_e	dS m^{-1}	5.82	dS m^{-1}	2.95
Organic matter	%	0.41	%	38.50
Organic nitrogen	%	0.03	%	1.45
Available phosphorus	mg kg^{-1}	3.32	%	1.75
Extractable potassium	mg kg^{-1}	95	%	1.04

Seed rate and sowing: The inoculated and uninoculated seeds of wheat variety, Galaxy-2013 were sown (as 120 kg ha^{-1}) through a drill in 15 m^{-2} plots (with 3m width and 5m length).

Irrigation: Irrigation was done through canal water during experimentation. Total 4 irrigations (at Crown root Initiation, Tillering, Heading and Milking stages) were applied (Zafar-ul-Hye *et al*., 2019).

Fertilizer application: Nitrogen (as 120), phosphorus (as 90) and potassium (as 60) kg ha^{-1} were applied using urea, TSP and K_2SO_4 keeping in mind the concentration of the nutrient of BGS. As a basal dose, K and P fertilizers were applied once. Urea was added at sowing, tillering and booting stage. Standard crop management practices i.e., weeding (manual), hoeing and plant protection were adopted to raise the wheat crop till maturity and harvesting.

Harvesting: Plants were harvested at the time of maturity (approximately after 132 days of sowing). Data of growth and yield indices i.e., number of tillers, plant height, 1000-grain weight, biological, straw and grains yield were noted when the crop matured. For plant height, standard measuring tape was used. The number of tillers were counted manually. Analytical balance was used to get 1000-grain weight. For noting the straw and grain yield, top plate balance was used.

Sodium and potassium: Leaves were taken in centrifuge tubes with liquid N (Akhtar *et al*., 1998). The sap was collected after centrifuging for analysis of ions on flame photometer (Ryan *et al*., 2001).

Electrolyte leakage: For the determination of electrolyte leakage, equal size leaf discs were shaken on vortex for 120 min. and then electrical conductivity (EC0) was measured. After that the test tubes were autoclaved at a 121°C for 20 min. and final EC2 was measure at 25°C. Calculation was done with the formula given by Sunkar (2010);

$$\text{Electrolyte leakage (\%)} = \frac{EC_1 - EC_0}{EC_2 - EC_0} \times 100$$

Relative water content: The leaf samples were kept at 100% humidity in the absence of light for two days at 4°C. The weight was noted at full turgidity. Relative water content (RWC) in leaf samples were found out using the formula as described by Teulat *et al*., (2003);

$$\text{RWC (\%)} = \frac{\text{Fully turgid weight} - \text{Dry weight}}{\text{Fresh weight} - \text{Dry weight}} \times 100$$

Statistical analyses

Analysis of variance techniques (ANOVA) was carried out according to the randomized complete block design (RCBD) with two factorial arrangements (Steel *et al*., 1997) using Statistix 9.0®. The HSD test was used for the comparison of the significance of treatments at the 5% probability.

Results

Plant height and tillers: Effects of BGS and PGPR were significant in both years for plant height and no. of tillers plant^{-1} (Table 2). Results showed that the PGPR significantly increased plant height from control when inoculated with and without BGS in experiment 1. No significant change was observed where BGS was applied from control for plant height in experiment 1. However, BGS significantly enhanced plant height in experiment 2 without PGPR. In addition, *B. cereus* remained significantly better than *L. fusiformis* and *A. faecalis* with BGS for plant height in experiment 2. In case of number of tillers per plant, the PGPR differed significantly from control when inoculated with and without BGS in both the experiments. No significant change was observed where BGS was applied from control for number of tillers plant^{-1} in both trials (Table 2). It was noted that *B. cereus*+BGS got significantly better response from *Bacillus cereus* in trial 1 for no. of tiller plant^{-1}. Highest significant increase in plant height (17.0 and 35%) and number of tillers plant^{-1} (46.2 and 57.9%) were observed in *B. cereus*+BGS from control in the trial 1 and 2, respectively.

Straw and grain yields: Effects of PGPR and BGS differed significantly for straw and grains yield of wheat (Table 3). Inoculation of PGPR with and without BGS significantly enhanced straw yield in both the trials. Sole application of BGS significantly increased straw yield in trial1 but did not differ significantly in the trial 2 from control. Maximum increase of 36.8 and 37.4% in straw yield was observed in *B. cereus*+BGS from control in the trial 1 and 2 respectively. For grain yield, the PGPR with and without BGS remained significantly better from control in the trial 1. Inoculation of *B. cereus* and *L. fusiformis* with and without BGS showed a positive change in grain yield enhancement from control. However, *A. faecalis* differed significantly without BGS from control but remained non-significant from BGS in the trail 2 for grain yield (Table 3). Highest significant increase of 46.2 and 50.0% in grain yield was observed in *B. cereus*+BGS from control in the trail 1 and 2, respectively.

1000-grain weight and biological yield: For biological yield and 1000-grain weight of wheat, effects of PGPRs and BGS were significant (Table 4). For 1000-grain weight, *B. cereus* and *L. fusiformis* with and without BGS got positive response regarding an improvement from control in trial 1 and 2. However, *A. faecalis* differed significantly without BGS from control but was non-significant from BGS in the trial 2 for 1000-grain weight. Highest significant increase of 34 and 33% in 1000-grain weight was observed in *B. cereus*+BGS from control in the trial 1 and 2, respectively. For biological yield, inoculation of *B. cereus* without BGS remained significantly better from control at site 1 (Table 4).

Table 2. Effect of ACC deaminase containing rhizobacteria and BGS on plant height and number of tillers of wheat under saline conditions.

Rhizobacteria	Study 1		Study 2		Study 1		Study 2	
	Plant height (cm)				Number of tillers (plant^{-1})			
	Control	BGS	Control	BGS	Control	BGS	Control	BGS
Uninoculated	77.3 c	82.2 bc	69.4 e	81.7 d	9.1 d	9.7 cd	9.5 c	10.0 c
Alcaligenes faecalis	85.4 ab	90.3 a	83.1 cd	87.9 b	11.0 bc	12.3 ab	12.9 b	13.7 ab
Bacillus cereus	88.1 ab	90.5 a	88.9 b	94.0 a	11.7 b	13.3 a	14.2 ab	15.0 a
Lysinibacillus fusiformis	85.6 ab	88.9 a	84.1 c	89.0 b	11.0 bc	12.3 ab	13.5 ab	14.3 ab

The treatments, sharing the similar letters do not have Honest Significant Difference with each other at $p \leq 0.05$ (n=3)

Table 3. Effect of ACC deaminase containing rhizobacteria and BGS on straw and grains yields of wheat under saline field conditions.

Rhizobacteria	Study 1		Study 2		Study 1		Study 2	
	Straw yield (Mg ha^{-1})				Grain yield (Mg ha^{-1})			
	Control	BGS	Control	BGS	Control	BGS	Control	BGS
Uninoculated	3.02 d	3.55 c	2.97 e	3.30 de	2.26 e	2.66 d	2.63 e	3.09 cd
Alcaligenes faecalis	3.57 c	4.07 ab	3.48 cd	3.68 bc	2.77 bcd	2.93 abc	3.10 cd	3.28 c
Bacillus cereus	3.84 abc	4.13 a	3.86 ab	4.08 a	2.95 ab	3.02 a	3.23 cd	3.84 a
Lysinibacillus fusiformis	3.69 bc	3.90 ac	3.59 bcd	3.80 abc	2.70 cd	2.99 ab	3.05 d	3.63 b

The treatments, sharing the similar letters do not have Honest Significant Difference with each other at $p \leq 0.05$ (n=3)

Table 4. Effect of ACC deaminase containing rhizobacteria and BGS on 1000 grains weight and biological yield of wheat under saline conditions.

Rhizobacteria	Study 1		Study 2		Study 1		Study 2	
	1000-grain weight (g)				Biological yield (Mg ha^{-1})			
	Control	BGS	Control	BGS	Control	BGS	Control	BGS
Uninoculated	27.93 d	32.86 c	30.82 d	36.26 c	7.01 c	8.25 b	7.63 f	8.11 C
Alcaligenes faecalis	33.87 bc	35.84 ab	35.50 c	37.57 bc	8.71 ab	9.22 ab	8.16 ef	8.40 C
Bacillus cereus	35.46 abc	37.52 a	37.11 c	40.94 a	8.73 ab	9.27 a	9.66 b	9.94 A
Lysinibacillus fusiformis	34.25 bc	36.24 ab	37.39 c	40.63 ab	8.44 ab	8.90 ab	8.80 cd	9.06 B

The treatments, sharing the similar letters do not have Honest Significant Difference with each other at $p \leq 0.05$ (n=3)

Table 5. Effect of ACC deaminase containing rhizobacteria and BGS on electrolyte leakage and relative water content of wheat under saline conditions.

Rhizobacteria	Study 1		Study 2		Study 1		Study 2	
	Electrolyte leakage (%)				Relative water content (%)			
	Control	BGS	Control	BGS	Control	BGS	Control	BGS
Uninoculated	64.2 a	64.5 a	69.4 b	75.0 a	50.9 d	56.6 cd	50.6 c	51.9 C
Alcaligenes faecalis	60.9 ab	57.9 abc	62.9 c	68.0 b	65.5 ab	63.7 abc	57.6 abc	59.3 AB
Bacillus cereus	48.8 d	51.6 cd	55.7 de	53.1 e	66.5 a	69.3 a	60.8 ab	62.6 A
Lysinibacillus fusiformis	53.3 bcd	59.1 abc	58.1 d	63.0 c	58.5 bcd	61.9 abc	52.9 c	54.5 BC

The treatments, sharing the similar letters do not have Honest Significant Difference with each other at $p \leq 0.05$ (n=3)

Table 6. Effect of ACC deaminase containing rhizobacteria and BGS on potassium and sodium content of wheat under saline conditions.

Rhizobacteria	Study 1		Study 2		Study 1		Study 2	
	Electrolyte leakage (%)				Relative water content (%)			
	Control	BGS	Control	BGS	Control	BGS	Control	BGS
Uninoculated	1.31 d	1.33 cd	1.35 c	1.37 c	0.75 a	0.66 ab	0.80 a	0.74 a
Alcaligenes faecalis	1.42 bc	1.44 b	1.47 b	1.52 ab	0.56 cd	0.53 cd	0.61 b	0.58 bc
Bacillus cereus	1.57 a	1.59 a	1.60 a	1.62 a	0.49 cd	0.47 d	0.51 c	0.51 c
Lysinibacillus fusiformis	1.54 a	1.58 a	1.54 ab	1.55 ab	0.58 bc	0.55 cd	0.53 bc	0.51 c

The treatments, sharing the similar letters do not have Honest Significant Difference with each other at $p \leq 0.05$ (n=3)

Electrolyte leakage and relative water content: Effects of PGPR and BGS remained significant for electrolyte leakage and relative water content in wheat (Table 5). The results showed that *B. cereus and L. fusiformis* without BGS differed significantly for a decrease in electrolyte leakage in the trial 1. In the case of trial 2, the PGPR inoculation with and without BGS remained significant from control for the reduction in electrolyte leakage. Electrolyte leakage was significantly decreased (24%) in *B. cereus* and *B. cereus*+BGS (29%) compared to BGS in the experiment 1 and 2, respectively. For relative water content, inoculation of *B. cereus* with and without BGS differed significantly better from BGS and the control. It was noted that *A. faecalis* also significantly increased relative water content from control when applied without BGS in the experiment 1 but remained significant with BGS in the experiment 2. The highest increase of 36 and 27% in relative water content were observed in *B. cereus* +BGS from control in the experiment 1 and 2 respectively.

Potassium and sodium concentration: Interactive effects of PGPR and BGS remained significant for potassium and sodium concentration in wheat (Table 6). The results showed that the PGPR with and without BGS differed significantly for the increase of potassium and decrease of sodium concentration in the **study 1 and 2.** For potassium concentration, *B. cereus* performed significantly better with and without BGS as compared to *A. faecalis* in both the studies. Highest significant increase of 21 and 20% in potassium concentration was observed in *B. cereus* + BGS from control in both the trials, respectively. For decrease in sodium concentration, *B. cereus* proved significantly better without BGS from *A. faecalis* in the study 2. The PGPR remained equally effective towards sodium decrease in the study 1 with and without BGS. Highest significant decrease of 37 in sodium concentration was noted in *B. cereus*+BGS from control in the study 1.

Discussion

In the current experiments, salinity stress significantly declined the growth and yield of wheat (Tables 2 to 4). However, integrated use of *A. faecalis*, *B. cereus* and *L. fusiformis* and BGS significantly improved the wheat plant height and no. of tillers in wheat under salinity stress. The improvement in plant height and number of tillers were due to decrease in sodium concentration as a result of PGPR inoculation with and without BGS. Inoculation of PGPR can enhance the crop productivity in salt-affected soils under axenic and field conditions (Kang *et al*., 2014; Nadeem *et al*., 2014; Naseer *et al*., 2019). Bacterial synthesis of ACC-deaminase can dilute the salt generated stress by lowering the stress ethylene, that would have inhibited the root growth, otherwise (Akbari *et al*., 2020). Hydrolysis of ACC by ACC deaminase converts the ethylene into ammonia and α-ketobutyrate (Glick *et al*., 1998). In addition to above, exopolysaccharide binds the Na^+ due to its cementing ability, thus minimizing the bioavailability of Na^+ to plants (Metoui Ben Mahmoud *et al*., 2020). Low electrolyte leakage, especially in *B. cereus*+BGS, was another allied factors responsible for the improvement in straw, grain and biological yield of wheat under saline conditions (Table 5). Application of BGS significantly modified soil physio-chemical properties. Presence of organic amendment, BGS decrease the bulk density of soil, improve hydraulic conductivity and moisture, thus enhancing phyto-availibiulity of N. Improvement in nutrients ultimately resulted in enhancement of yield (Möller *et al*., 2008; Arthurson, 2009; Barbosa *et al*., 2014). However, improvement in 1000-grain weight was due to improvement in relative water content and improvement in potassium concentration of wheat under salinity stress. A higher level of salts in soil induces the osmotic stress that decreases the availability of water to the plants (Ahmad *et al*., 2013). Treatment *B. cereus*+BGS significantly enhanced the uptake of water that also played an imperative role in dilution of salinity stress in wheat. Our findings regarding RWC are in agreement with the results of Nadeem *et al*., (2010a). They reported improvement in RWC of mungbean (*Vigna radiata*) and wheat (*Triticum aestivum*) in salinity stress via bacterial inoculation. These bacteria promote the root growth by minimizing the adverse effects of salts, thus provide a chance to plant for better water and nutrients uptake (Marulanda *et al*., 2009). Higher Na^+ concentration usually distracts the ions balance in crops (Nadeem *et al*., 2010b). The disturbance in the ionic environment of the cell is regulated when the optimum level of K becomes part of the cell environment which decreases the toxic effects of Na (Kavitha *et al*., 2012). In our studies, we found that the rhizobacterial inoculation significantly improved K^+ that could be due to improvement in root length. The same was reported by Mahdi *et al*., (2012) and Zafar-ul-Hye *et al.*, (2020b).

Conclusion

The ACC deaminase containing rhizobacteria i.e., *Bacillus cereus*, *Lysinibacillus fusiformis* and *Alcaligenes faecalis* with BGS improved the physiological growth and yield indices of wheat under saline conditions. Application of *Bacillus cereus*+BGS was an efficacious combination for improvement in growth, yield, potassium concentration and RWC of wheat under saline stress. Similarly, *Bacillus cereus*+BGS proved efficacious technique to decrease electrolyte leakage and sodium concentration in wheat under salinity stress. In conclusion, *Bacillus cereus*+BGS is a better approach for mitigation of salinity stress in wheat as compared to the sole application of BGS and *B. cereus*.

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