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Integrated Application of ACC-Deaminase Containing Plant Growth Promoting Rhizobacteria and Biogas Slurry Improves the Growth and Productivity of Wheat under Drought Stress

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Abstract

Drought is one of the major environmental threats to global food security. The application of ACC-deaminase containing plant growth promoting rhizobacteria (PGPR) is effective to ameliorate the adverse effects of drought stress. Biogas slurry (BGS) improves water holding capacity of soil and enhances the soil fertility status thus promoting productivity of crops. The purpose of the study is to evaluate the integrated use of ACC-deaminase containing PGPR and biogas slurry to improve growth and yield of wheat crop under drought stress. Wheat seeds were inoculated with strains of *Alcaligenes faecalis*, *Bacillus amyloliquefaciens* and *Pseudomonas moraviensis* alone and in combination with BGS @ 600 kg ha^{-1}. The crop was subjected to water deficit stress at various water holding capacity (WHC) levels *i.e.*, 100, 70 and 50% WHC. The results revealed that the drought stress adversely effects on growth, biochemical and yield parameters of wheat. However, application of PGPR with biogas slurry enhanced wheat growth under drought stressed conditions. The *P. moraviensis* inoculation amended with BGS resulted significantly better in increasing the grain and biological yield (up to 46.7 and 40.5%, respectively) at 50% WHC level than the respective uninoculated control. Similarly, PGPR inoculation amended with BGS enhanced the mineral nutrients *i.e.*, nitrogen, potassium and phosphorus contents in straw and grains of wheat. It was concluded that the PGPR application amended with BGS could be used as an efficient strategy to improve wheat growth and yield under water deficit stressed conditions.

Keywords: ACC-deaminase; Biogas slurry; Drought; Rhizobacteria

Introduction

Drought stress is considered as a devastating environmental constraint to sustainable agriculture (Farooq *et al*., 2009; Lesk *et al*., 2016). The variation in rainfall pattern and rising temperature are among the main reasons of drought and cause a considerable reduction in crop productivity (Obidiegwu *et al*., 2015). Drought reduces the crop productivity by changing physiological and biochemical processes (Debaeke and Abdellah, 2004; Huang *et al*., 2015) and hampered the expansion of cell (Bartels and Sunkar, 2005). It disrupts membrane integrity along with closure of stomata, diminished osmotic stress and protein denaturation (Yang *et al*., 2010; Alcazar *et al*., 2011). In particular, there is a need to find solutions that fulfill the food requirements under deficit water conditions (Mancosu *et al*., 2015).

Under drought stress, the PGPR have been well documented to be involved in the rescue of plants and improve agricultural productivity in semiarid and arid regions (Kaushal and Wani, 2016). These drought tolerant rhizobacteria promote plant growth and provide systemic resistance (Kasim *et al*., 2013; Hussain *et al*., 2014). The PGPR promote plant growth and increase plant stress tolerance by modifying and manipulating the synthesis of phytohormones (Bresson *et al*., 2014) like gibberellic acid, auxins, cytokinins (Figueiredo *et al*., 2008; Cohen *et al*., 2009). The importance of PGPR in drought tolerance includes their capability to assist to host plants to tolerate drought stress (Coleman-Derr and Tringe, 2014). The rhizobacteria improve the physiological attributes linked with drought resistance (Ngumbi and Kloepper, 2016) by modifying the root architecture to increase the water uptake (Timmusk *et al*., 2014).

Ethylene is regarded as plant stress hormone which involves inducing plant physiological responses when exposed to drought stress (Kang *et al*., 2012). Under drought conditions, the ethylene concentration increases which inhibits the root growth leading to reduction in nutrients and water uptake (Kulkarni and Phalke, 2009). The

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1-aminocyclopropane-1-carboxylic acid (ACC) is a direct precursor of ethylene (Shaharoona *et al*., 2006). Drought can increase the ACC level which ultimately increase the rate of ethylene biosynthesis (Saleem *et al*., 2007). The ACC-deaminase containing PGPR transform ACC into ammonia and α-ketobutyrate (Shaharoona *et al*., 2006; Zafar-ul-Hye *et al*., 2014). Such PGPR increase plant growth by suppressing the accelerated endogenous ethylene synthesis (Lim and Kim, 2013; Nadeem *et al*., 2013).

Biogas slurry (BGS) is a soil amendment it is a rich source of nutrients for increasing crop growth (Garg *et al*., 2005; Liu *et al*., 2008). The digested biogas slurry contains amino acids, readily available nutrients and bioactive substances *viz.* vitamins, hormones and humic acids (Liu *et al*., 2008). It is considered that application of bio-slurry increases the quality and yield of crops (Gurung, 1997).

Application of rhizobacteria with organic amendment increased growth, quality and yield of cucumber and mung bean (Ahamd *et al*., 2015; Iqbal *et al*., 2016). Application of PGPR and BGS enhanced the crop growth and yield (Ahmad *et al*., 2014) and also improved soil health (Mehdi *et al*., 2011). The integrated application of PGPR and organic amendments are helpful in increasing crop production through improving nitrogen, phosphorus, potassium and organic carbon contents in soil and by reducing soil pH (Soomro *et al*., 2013).

Wheat is a widely cultivated cereal crop of the world especially developing countries of Asia. World wheat production in 2018 is forecast at 722.4 million tonnes (FAO, 2018). The wheat demand is increasing at the rate of 2% per year around the world (Rosegrant and Cline, 2003). Drought stress strongly effect the growth and yield formation in wheat (Farooq *et al*., 2014). The plant development and yield are adversely affected due to drought, which leads to the reduction in crop yield and serious threat to food security. The survivability ACC-deaminase containing PGPR under drought stress condition might be utilized as an opportunity for improving the association time between the plant and rhizobacteria. As the ACC-deaminase decreases the ethylene level, so inoculation with ACC-deaminase containing rhizobacterial strains could be an effective tool for promotion of wheat growth and yield under drought stress. Hence, the current experiment was conducted to examine the effect of ACC-deaminase containing rhizobacterial strains and biogas slurry in ameliorating the effect of drought stress to enhance the productivity of the wheat crop.

Materials and Methods

Site Description

A pot experiment was conducted by using three rhizobacterial isolates and air dried BGS to mitigate the effect of drought stress on wheat crop in greenhouse at the experimental farm of the Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan.

Preparation of Rhizobacterial Inoculum and Seed Dressing

Three drought tolerant ACC-deaminase containing rhizobacterial strains *i.e*., S4 (*Alcaligenes faecalis*), S17 (*Bacillus amyloliquefaciens*) and S27 (*Pseudomonas moraviensis*) which showed the prominent results during screening experimentation, were obtained from Soil Microbiology and Biochemistry Laboratory, Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan. The inoculum was prepared in 50 mL Erlenmeyer flasks containing DF salt minimal medium (Dworkin and Foster, 1958). A loopful of respective strain was inoculated in a flask containing DF salt minimal medium and incubated for 24 h in shaking incubator at 25 ± 2°C and 100 rpm. Wheat (*Triticum aestivum* L. cv. Galaxy 2013) seed dressing was done by mixing bacterial inoculum with sterilized clay, 10% sugar solution and peat.

Biogas Slurry Preparation

The BGS was obtained through biogas plant installed at Chah Kangan Wala Mouza Ferozpur, Multan and was air dried on a plastic sheet. By following the standard protocol as described by Ryan *et al*. (2001) the concentration of organic matter (38.5%), total nitrogen (1.45%), phosphorus (1.75%) and potassium (1.04%) contents in the BGS was analyzed. The pH of BGS was calculated as 7.5 and EC was 2.95 dS m^{-1} observed. BGS was applied (at the rate of 0 and 600 kg ha^{-1} BGS) according to treatments before sowing in pots.

Soil Characteristics

Air dried sieved soil was obtained from research area of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan. A composite soil sample was taken and characterized for various physico and chemical attributes, *i.e*., textural class, sandy clay loam; EC_e, 2.14 dS m^{-1}; pH, 7.8; saturation percentage, 34.5%; CEC, 5.18 $cmol_c$ kg^{-1}; organic matter, 0.89%; total nitrogen, 0.045%; extractable potassium, 113 mg kg^{-1} and available phosphorus, 8.1 mg kg^{-1} soil.

Pot Experimental Set-up

Twelve kg soil was filled in each pot according to treatment plan. Air dried BGS was mixed with the soil of respective pots at the rate of 0 and 600 kg ha^{-1}. Recommended NPK fertilizers as N, P_2O_5 and K_2O (120, 90, 60 kg ha^{-1}) was applied as urea, triple super phosphate and sulfate of potash, respectively. The nitrogen was applied in three doses (before sowing, tillering and booting stage), whereas, potassium and phosphate fertilizers were applied as basal. The dried BGS was mixed with soil and then filled the pots according to treatment plan. Before adding soil, the pots were lined with polythene sheets to provision leaching through the pots. The pots were randomly organized at ambient light and temperature with three repeats.

Description of Drought Levels

After seven days of sowing the three seedlings of wheat were maintained to get uniform population per pot. After 15 days of seed sowing three levels of irrigation were maintained *i.e*., 100%, 70% and 50% water holding capacity (WHC). To maintain the desired irrigation levels, firstly, the soil moisture contents were measured at WHC. In this context, the pot were weighted with soil and water was added to saturate and pot were left for 48 h to drain out the gravimetric water. The pots were reweighed and the difference in weight before saturation and 48 h after saturation was measured as moisture content at WHC. The measured water was applied for 100% water holding capacity, whereas, for 70 and 50% WHC levels the amount of 70 and 50% of water of water holding capacity was added, respectively (Erdogan *et al*., 2016). During the whole experimental duration, maintain irrigation levels *i.e*., 100, 70 and 50% WHC were maintained. The pots were randomly organized at ambient light and temperature with three repeats.

Data Collection

Growth and yield parameters: Data regarding the plant height (cm) was measured starting from the base to the tip and root length (cm) was measured starting from the end to the base of the plant and averaged at the time of harvesting (at crop at full maturity) with the help of a measuring tape. Parameters *i.e*., number of tillers plant^{-1}, root dry weight (g plant^{-1}), biological yield (g pot^{-1}), grain yield (g pot^{-1}), straw yield (g pot^{-1}) and hundred–grain weight (g) were determined at the time of crop harvesting.

Physiological parameters measurement: The plant physiological attributes of fully expanded wheat flag leaves were measured at 10:00 A.M. to 2:00 P.M. of drought-stressed and control pots. To determine the stomatal conductance (gs), transpiration rate (E), sub-stomatal CO_2 concentration (C_i) and CO_2 assimilation rate (A) the portable infrared gas analyzer [IRGA (LCA-4) Germany] was used (at 1,200–1,400 lmol m^{-2} s^{-1} photosynthetic photon flux density).

Assessment of SPAD chlorophyll contents: SPAD chlorophyll meter (SPAD-OSK) was used to determine total chlorophyll contents (Hussain *et al*., 2000).

Straw and grain mineral nutrients measurement: Oven dried straw and grains samples were digested with H_2O_2 and H_2SO_4 (1:2 ratio) and made final volume with deionized water up to 50 mL (Wolf, 1982). Kjeldhal method was followed to measure the nitrogen concentration in straw and grains (Jackson, 1962). For phosphorus determination, in 10 mL of Barton reagent the extracted plant material was added (Ashraf *et al*., 1992) and made final volume with distilled water up to 50 mL. The phosphorus contents were determined by a spectrophotometer (Biotechnology Medical Services, UV-1602, BMS, Canada) and flame photometer was used to measure the plant potassium concentration (Ryan *et al*., 2001).

Statistical Analysis

Three-way analysis of variance (ANOVA) was used to analyze the results, based on completely randomized design (CRD) (Steel *et al*., 1997). However, Tukey's (HSD) test was applied to compare various significance of treatments at probability of 5% ($p < 0.05$). The "Statistix 8.1®" computer-based statistical software was used for analysis (Analytical Software, USA).

Results

Effect on Growth Parameters of Plant

The data regarding the effect of ACC-deaminase containing rhizobacteria and BGS on the plant height and number of tillers of wheat plants exposed to drought stress revealed that drought stress significantly reduced ($P \leq 0.05$) the plant height and no. of tillers of wheat (Table 1). The application of PGPR amended with BGS considerably enhanced the plant height and number of tillers under water deficit (50 and 70% WHC) as well as normal conditions (100% WHC). At 100% WHC, the strain S27 was found to be more effective to promote plant height as it caused up to 27.1 and 28.4% increase over uninoculated and un-amended control without and with BGS amendment, respectively. At 70% WHC, the strain S27 showed an increase in plant height up to 30.1% with BGS amendment. Similarly, at high drought stress (50% WHC), strain S27 showed the maximum increase in plant height 31.2 and 37.8% without and with BGS over control. Data regarding the number of tillers, the strain S27 with BGS amendment showed the maximum increase up to 22.2 at 70% WHC, and 61.9 at 50% WHC, with respect to control.

Drought stress significantly affected ($P \leq 0.05$) the root length and root dry weight of wheat plants (Table 1). The increase in drought (70 to 50% WHC) resulted in a significant increase in root length, whereas, dry weight of wheat plants roots was reduced. At 70% WHC, the strain S27 gave the highest increase (30.4%) in root length as compared to uninoculated and un-amended control in the presence of BGS. At high drought stress (50% WHC), in the presence of BGS the strain S4 gave maximum increment (45.7%) in root length, followed by S17 and S4 (30.4 and 39.2%, respectively) over drought stressed uninoculated control in the presence of BGS. Similarly, at 50% WHC with BGS amendment, the strain S27 enhanced the dry root weight up to 88.3%, which was at par with S17 but significantly ($P \leq 0.05$) better than uninoculated control.

Table 1: Effect of biogas slurry (BGS) and PGPR strains on plant height, number of tillers, root length and root dry weight of wheat plants under drought stress conditions. Where, S4 (*A. faecalis*), S17 (*B. amyloliquefaciens*) and S27 (*P. moraviensis*) are PGPR strains

WHC %	BGS (kg ha^{-1})	PGPR	Plant height (cm)	Number of tillers ($plant^{-1}$)	Root length (cm)	Root dry weight (g $plant^{-1}$)
100	No BGS	No PGPR	62.69 f-h	3.33 b-e	19.80 i	4.67 d-i
		S4	69.42 c-f	4.67 a-c	22.07 hi	7.09 ab
		S17	72.02 bc	4.67 a-c	23.33 g-i	5.47 b-g
		S27	79.71 a	5.00 ab	23.83 g-i	6.94 a-c
	600	No PGPR	70.90 b-d	3.67 a-d	23.80 g-i	5.41 b-g
		S4	77.10 ab	5.33 a	25.73 e-h	7.10 ab
		S17	79.47 a	4.67 a-c	25.13 f-i	7.32 a
		S27	80.50 a	5.33 a	26.27 d-h	7.09 ab
70	No BGS	No PGPR	53.86 i	3.33 b-e	24.20 g-i	4.31 f-j
		S4	64.26 d-g	4.00 a-d	28.10 d-g	4.39 e-j
		S17	64.08 d-g	3.67 a-d	26.60 d-h	4.69 d-i
		S27	68.05 c-f	4.00 a-d	28.47 d-g	6.11 a-f
	600	No PGPR	63.63 e-h	3.00 c-e	27.23 d-h	4.33 f-j
		S4	68.83 c-f	4.00 a-d	30.37 c-f	5.22 c-h
		S17	67.83 c-f	3.33 b-e	31.33 b-d	6.18 a-e
		S27	70.00 b-e	4.00 a-d	31.57 b-d	6.43 a-d
50%	No BGS	No PGPR	45.70 j	1.67 e	26.80 d-h	2.60 j
		S4	56.67 hi	2.33 de	30.43 c-f	3.20 ij
		S17	55.20 i	3.00 c-e	29.97 c-f	3.41 h-j
		S27	59.97 g-i	3.00 c-e	36.03 ab	3.99 g-j
	600	No PGPR	52.90 ij	2.33 de	30.97 b-e	2.79 j
		S4	59.57 g-i	2.67 de	37.27 a	3.07 ij
		S17	58.17 g-i	3.00 c-e	34.97 a-c	3.88 g-j
		S27	62.97 e-h	3.33 b-e	39.07 a	4.78 d-i
$HSD_{0.05}$			*1.8673*	*0.4473*	*1.4276*	*0.4757*
PGPR			*	*	*	*
BGS			*	NS	*	*
Drought			*	*	*	*
PGPR*BGS			*	NS	NS	*
PGPR*Drought			NS	NS	*	*
BGS*Drought			NS	NS	NS	NS
PGPR*BGS*Drought			NS	NS	NS	NS

Tukey-HSD test ($P \leq 0.05$) was used for differentiation among treatments mean of replicated thrice (n=3). Where ∗ were significant differences at $P \leq 0.05$ levels, respectively

Effect on Yield Parameters

There was a significant ($P \leq 0.05$) effect of ACC-deaminase containing PGPR and BGS on the biological yield of wheat at various drought levels. Under un-stressed conditions (100% WHC), a significant ($P \leq 0.05$) increase in biological yield was recorded with and without BGS treatments, due to rhizobacterial inoculation, over respective uninoculated and un-amended control (Table 2). However, the drought stress (70 and 50% WHC) significantly reduced the biological yield of uninoculated wheat plants. Rhizobacterial strains ameliorated the effect of water deficit stress with respect to their respective control treatments. The S27 strain inoculation amended with BGS was observed the most effective for increasing biological yield (41 and 40%) when drought stress was applied (70 and 50% WHC, respectively) over respective control treatments. Drought stress applied to wheat plants significantly ($P \leq 0.05$) reduced grain yield. Whereas rhizobacterial inoculation, caused by significant increase ($P \leq 0.05$) in grain yield over the respective control treatments, under drought conditions in the presence or absence of amended BGS. In general, the S27 isolate was observed the most effective strain in enhancing the grain yield (70 and 50% WHC) under drought stressed conditions.

The data regarding the effect of ACC-deaminase containing PGPR and BGS on straw yield and 100-grains weight of wheat exposed to drought stress is presented in Table 2. It is revealed that water deficit stress significantly reduced ($P \leq 0.05$) the straw yield and 100-grains weight of wheat. The application of PGPR significantly enhanced ($P \leq 0.05$) the straw yield and 100-grains weight under drought (50 and 70% WHC) as well as normal conditions (100% WHC). At 100% WHC, the strain S27 was found to be more effective to increase straw yield as it caused up to 11.6 and 31.3% increase (over uninoculated and un-amended control) under without and with BGS amendment, respectively. Similarly, at high drought stress (50% WHC), the strain S27 showed the maximum increase in straw yield (36.8%) with BGS over control. The S27 with BGS amendment showed the maximum increase (up to 28%) at 50% WHC and (up to 41%) at 70% WHC, with respect to control.

Effect on Physiological Parameters

It is revealed from the data that drought stress was significantly increased ($P \leq 0.05$) the SPAD chlorophyll content of wheat flag leaves (Fig. 1). At 50 and 70% drought level, the leaves of uninoculated wheat plants had substantial lower chlorophyll contents (11.4 and 9.5%) as compared to their corresponding non-stressed (100% WHC)

Table 2: Effect of biogas slurry (BGS) and PGPR strains on grain yield, biological yield, straw yield and 100-grain weight of wheat plants under drought stress conditions. Where, S4 (*A. faecalis*), S17 (*B. amyloliquefaciens*) and S27 (*P. moraviensis*) are PGPR strains

WHC %	BGS (kg ha^{-1})	PGPR	Biological yield (g pot^{-1})	Grain yield (g pot^{-1})	Straw yield (g pot^{-1})	Hundred-grain weight (g)
100	No BGS	No PGPR	29.70 fg	10.69 e-g	18.79 de	2.93 f-i
		S4	32.27 e	11.62 de	20.27 cd	3.45 a-c
		S17	34.45 d	12.93 cd	21.02 bc	3.47 a-c
		S27	34.93 cd	13.70 bc	20.98 bc	3.37 b-d
	600	No PGPR	31.52 ef	11.85 de	19.33 cd	3.08 d-h
		S4	37.30 b	14.54 ab	22.36 b	3.70 ab
		S17	36.97 bc	14.11 a-c	22.39 b	3.25 c-f
		S27	40.53 a	15.44 a	24.67 a	3.72 a
70	No BGS	No PGPR	21.76 op	8.03 i-k	13.58 kl	2.62 ij
		S4	26.38 h-j	9.99 f-h	15.96 g-j	3.28 c-e
		S17	25.87 i-k	9.93 f-h	15.70 g-j	3.23 c-g
		S27	28.40 gh	10.67 e-g	17.27 e-g	3.25 c-f
	600	No PGPR	24.56 j-m	9.28 g-i	14.99 i-k	3.00 e-h
		S4	27.30 hi	10.91 ef	16.10 g-i	3.42 a-c
		S17	26.69 hi	9.50 f-i	16.85 f-h	3.24 c-g
		S27	30.90 ef	11.79 de	18.67 d-f	3.69 ab
50	No BGS	No PGPR	18.06 q	6.53 k	11.20 m	2.40 j
		S4	22.24 no	7.55 jk	14.26 jk	2.83 hi
		S17	19.81 pq	7.27 jk	12.17 lm	2.84 hi
		S27	22.68 m-o	8.57 h-j	13.66 kl	2.94 e-i
	600	No PGPR	19.28 q	7.10 jk	11.90 lm	2.46 j
		S4	23.64 l-o	8.50 h-j	14.71 i-k	2.90 g-i
		S17	24.21 k-n	8.33 ij	15.64 g-j	2.88 hi
		S27	25.36 i-l	9.58 f-i	15.33 h-k	3.08 d-h
$HSD_{0.05}$			*0.5438*	*0.4012*	*0.477*	*0.0884*
PGPR			*	*	*	*
BGS			*	*	*	*
Drought			*	*	*	*
PGPR*BGS			*	*	*	*
PGPR*Drought			*	*	*	NS
BGS*Drought			*	*	*	*
PGPR*BGS*Drought			*	*	*	NS

Tukey-HSD test ($P \leq 0.05$) was used for differentiation among treatments mean of replicated thrice (n=3). Where ∗ were significant differences at $P \leq 0.05$ levels, respectively

control. The rhizobacterial inoculation ameliorated the influence of drought stress with respect to their corresponding control. The S27 Inoculation was resulted most efficient in increasing chlorophyll contents (22.1 and 44.7%, without BGS) and (42.1 and 45.7%, amended with BGS) over respective noninoculated and un-amended control, when drought stress was applied (70 and 50% WHC, respectively).

There was a significant ($P \leq 0.05$) effect of ACC-deaminase containing PGPRs and BGS on photosynthetic rate of wheat flag leaves at different drought levels (Fig. 2). At 100% WHC there was a significant increase in the photosynthetic rate up to 56 and 60% were recorded due to S17 strain inoculation with respect to uninoculated control (without and with BGS, respectively). Rhizobacterial isolates reduced (up to 58 and 53%, respectively) the influence of drought stress (70 and 50% WHC) on photosynthetic rate with respect to their corresponding control. Drought stress applied to wheat plants significantly ($P \leq 0.05$) reduced photosynthetic rate. Similarly, under drought-stressed conditions the rhizobacterial strains inoculation observed in significant ($P \leq 0.05$) improvement in transpiration rate with respect to the uninoculated control (Fig. 3). S27 was more effective in improving (34 and 29%) the transpiration rate at drought stressed (70 and 50% WHC, respectively) conditions. Similarly, under drought stress the inoculation with S27 strain was found significant ($P \leq 0.05$) in promoting transpiration rate (over control) up to 35% amended with BGS.

Drought stress applied to wheat plants had significant ($P \leq 0.05$) negative effects on stomatal conductance and sub-stomatal conductance (Fig. 4 and 5) in wheat flag leaves. The inoculation of rhizobacterial strains significantly ($P \leq 0.05$) improved the stomatal conductance and sub-stomatal conductance over respective control, under unstressed and drought-stressed conditions. The S27 was the most effective strain in increasing the stomatal conductance at 70% WHC and 50% WHC drought levels. Under normal conditions, with S27 strain inoculation, the stomatal conductance and sub-stomatal conductance was increased up to 45 and 42%, respectively, over uninoculated unstressed control. At 50% drought stress, the S27 strain inoculation caused the maximum increase in the stomatal conductance (69%) and sub-stomatal conductance (33%) with BGS amendment.

Effect on Mineral Nutrients uptake by Plant

The data regarding the effects of ACC-deaminase

Table 3: Effect of biogas slurry (BGS) and PGPR strains on shoot and grain nitrogen, phosphorus and potassium of wheat plants under drought stress conditions. Where, S4 (*A. faecalis*), S17 (*B. amyloliquefaciens*) and S27 (*P. moraviensis*) are PGPR strains

WHC (%)	BGS (kg ha^{-1})	PGPR	Nitrogen content in shoot (%)	Nitrogen content in grain (%)	Phosphorus content in shoot (%)	Phosphorus content in grain (%)	Potassium content in shoot (%)	Potassium content in grain (%)
100	No BGS	No PGPR	0.81 hi	1.89 de	0.21 d-h	0.47 f-i	0.69 f-i	0.38 f-h
		S4	1.20 bc	2.07 bc	0.24 c-f	0.54 b-f	0.93 bc	0.47 b-f
		S17	1.10 c-e	1.99 c-e	0.23 c-f	0.56 b-d	0.81 c-g	0.46 b-f
		S27	1.27 ab	2.09 a-c	0.29 ab	0.60 b	0.92 bc	0.52 a-c
	600	No PGPR	0.90 gh	1.95 c-e	0.26 b-d	0.55 b-e	0.85 c-e	0.43 c-g
		S4	1.19 bc	2.19 ab	0.28 a-c	0.59 bc	1.07 a	0.51 a-d
		S17	1.19 bc	2.00 cd	0.23 c-f	0.59 bc	1.02 ab	0.56 ab
		S27	1.34 a	2.25 a	0.31 a	0.68 a	1.10 a	0.60 a
70	No BGS	No PGPR	0.70 i-k	1.25 lm	0.15 j-l	0.35 k-m	0.55 jk	0.35 g-i
		S4	0.95 fg	1.68 f-h	0.20 e-j	0.48 e-i	0.87 cd	0.41 e-g
		S17	0.88 gh	1.47 i-k	0.18 g-k	0.46 g-i	0.86 cd	0.40 e-g
		S27	1.10c-e	1.55 g-i	0.22 d-h	0.44 h-j	0.86 cd	0.41 d-g
	600	No PGPR	0.74 ij	1.53 h-j	0.17 h-l	0.42 i-k	0.72 e-i	0.35 g-i
		S4	1.03 d-f	1.72 f	0.23 d-g	0.50 d-h	0.86 cd	0.43 c-g
		S17	0.95 fg	1.83 ef	0.21 e-i	0.45 g-i	0.93 bc	0.45 c-f
		S27	1.13 cd	1.70 fg	0.24 b-e	0.52 c-g	0.88 cd	0.50 b-e
50	No BGS	No PGPR	0.68 jk	1.05 n	0.13 l	0.29 m	0.47 k	0.23 j
		S4	0.98 e-g	1.19 mn	0.16 i-l	0.34 lm	0.76 d-i	0.34 g-j
		S17	0.87 gh	1.29 lm	0.16j-l	0.32 lm	0.64 ij	0.29 h-j
		S27	0.92 f-h	1.37 kl	0.17 h-l	0.37 j-l	0.77 d-h	0.34 g-i
	600	No PGPR	0.62 k	1.30 lm	0.13 kl	0.30 lm	0.68 h-j	0.27 ij
		S4	0.88 gh	1.40 i-l	0.19 e-j	0.42 i-k	0.79 d-h	0.35 g-i
		S17	0.92 f-h	1.38 j-l	0.19 f-j	0.37 j-l	0.68 g-j	0.39 fg
		S27	0.96 fg	1.55 g-i	0.21 d-h	0.43 ij	0.82 c-f	0.41 e-g
$HSD_{0.05}$			*0.0313*	*0.0416*	*0.0133*	*0.0187*	*0.0337*	*0.0264*
PGPR			*	*	*	*	*	*
BGS			*	*	*	*	*	*
Drought			*	*	*	*	*	*
PGPR*BGS			*	NS	NS	*	*	*
PGPR*Drought			*	*	*	*	*	NS
BGS*Drought			*	*	NS	NS	*	NS
PGPR*BGS*Drought			*	*	NS	*	*	NS

Tukey-HSD test ($P \leq 0.05$) was used for differentiation among treatments mean of replicated thrice (n=3). Where * were significant differences at $P \leq 0.05$ levels, respectively

containing PGPRs on the shoot and grain nitrogen contents in wheat revealed that drought stress considerably reduced ($P \leq 0.05$) the shoot and grain nitrogen contents (Table 3). The application of PGPR and BGS significantly enhanced the nitrogen concentration in shoot and grains under water deficit (50 and 70% WHC) as well as normal conditions (100% WHC). At 70 and 50% WHC, the strain S27 was found to be the most effective strain in increasing the shoot nitrogen concentration as it caused up to 60 and 40% increase, respectively (over uninoculated control) with BGS amendment. Whereas, at drought stress (50% WHC), the strain S27 showed the maximum increase in shoot nitrogen concentration (55%) without BGS over control. Data regarding the grain nitrogen content, the strain S27 with BGS amendment showed the maximum increase up to 36% at 70% WHC, with respect to control. At 50% WHC drought level, the strain S27 resulted in the significant increase (up to 48%) in grain nitrogen with BGS amendment over corresponding control.

Improvement in phosphorus and potassium concentration in grains and straw of wheat was noted due to rhizobacterial inoculation under normal, and drought stressed conditions (Table 3). However, with BGS amendment, the maximum grain and shoot phosphorus concentration of 64 and 47%, respectively, was found in plants inoculated with S27 at 50% WHC with respect to uninoculated control. Data regarding the grain and shoot phosphorus contents showed that the strain S77 with BGS amendment caused a significant ($P \leq 0.05$) increase (up to 47%) and (60%) in grain and shoot phosphorus, respectively, at 70% WHC, with respect to control. Under normal conditions, the S27 strain proved as the most effective and significantly better strain ($P \leq 0.05$) in increasing shoot and grain potassium concentration which was at par with S17 and S4 with respect to control both with and without BGS amendment (Table 3). The S27 inoculation was found to be the most prominent strain for enhancing shoot and grain potassium contents (65 and 73%, respectively) at 50% WHC over corresponding control, with and without BGS amendment.

Discussion

In the present investigation, the effect of three ACC-deaminase containing PGPR (*A. faecalis*, *B. amyloliquefaciens* and *P. moraviensis*) and BGS was evaluated for wheat growth and yield under drought stress at

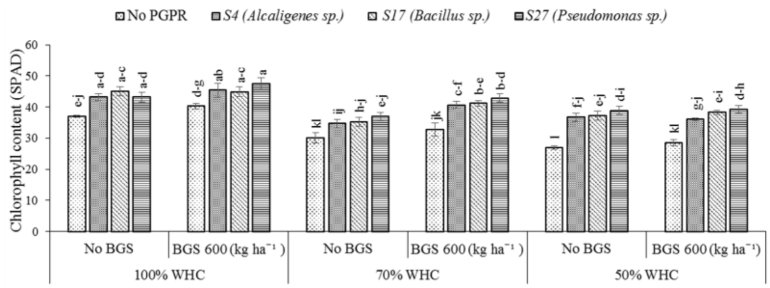

Fig. 1: Effect PGPR and BGS on chlorophyll content (SPAD) of wheat under drought stress conditions where vertical bars different letters on bars are showing statistical differences at $P \leq 0.05$ represent the standard deviation of means each treatment (n=3)

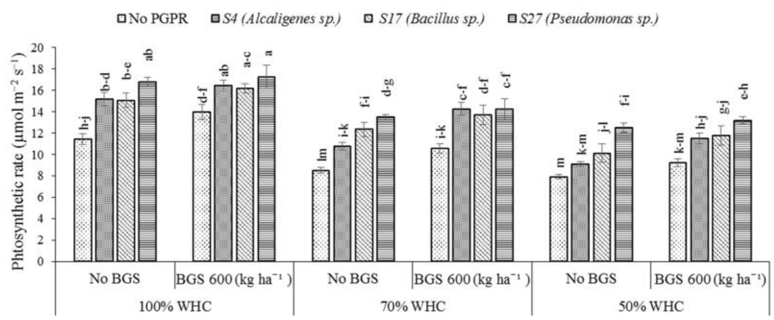

Fig. 2: Effect PGPR and BGS on photosynthetic rate (μmol m^{-2} s^{-1}) of wheat under drought stress conditions where vertical bars different letters on bars are showing statistical differences at $P \leq 0.05$ represent the standard deviation of means each treatment (n=3)

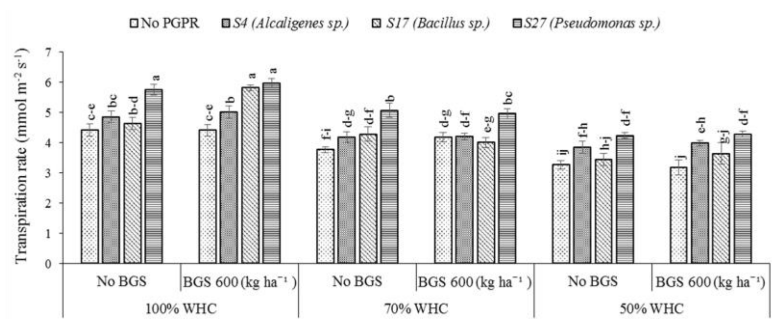

Fig. 3: Effect PGPR and BGS on transpiration rate (μmol m^{-2} s^{-1}) of wheat under drought stress conditions where vertical bars different letters on bars are showing statistical differences at $P \leq 0.05$ represent the standard deviation of means each treatment

70% and 50% WHC conditions. The results showed that the drought stress had a strong effect on the development of uninoculated wheat plants, whereas, the rhizobacterial inoculation improved the physiological and yield attributes of wheat plants, over uninoculated treatments.

In general, *Pseudomonas sp.* inoculations with or without the application of BGS resulted a significant ($P \leq 0.05$) increase in plant height, number of tillers per plant, root length and root dry weight over the respective uninoculated and no BGS control treatments. This could be evident owing to the reduction of increased production of stress ethylene under drought stress conditions through ACC-deaminase containing rhizobacterial strains. Under drought stress the synthesis of ethylene in plants have been reported (Zahir *et al*., 2008). In another study under water deficit stress, sharp and consequently increases in ACC level due to ethylene biosynthesis in plants have been revealed by Zahir *et al*. (2008). Also, as a potential root colonizer, these PGPRs evoked various metabolic and physiological procedures for assisting the growth of plants under drought stress (Fernandez *et al*., 2012). Moreover, ACC-deaminase containing rhizobacterial inoculations increased root length and root dry weight as the drought stress increased that could be supportive in relatively more water uptake under drought situation (Zahir *et al*., 2008).

Interestingly, increasing in drought stress revealed a significant increment in length of wheat plants roots (Table 1). Plant's root system architecture is very important to endure drought stress (Huang *et al*., 2014). Under water deficit situations to maintain the plant productivity include a deeper

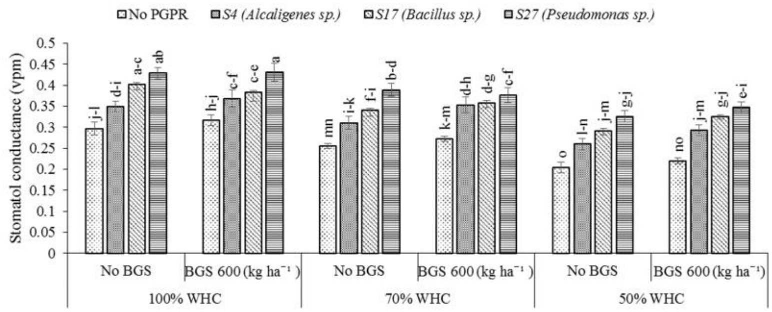

Fig. 4: Effect PGPR and BGS on stomatal conductance (vpm) of wheat under drought stress conditions where vertical bars different letters on bars are showing statistical differences at $P \leq 0.05$ represent the standard deviation of means each treatment

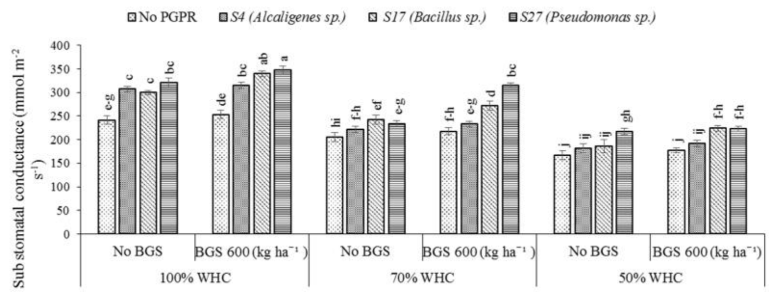

Fig. 5: Effect PGPR and BGS on sub stomatal conductance (mmol m^{-2} s^{-1}) of wheat under drought stress conditions where vertical bars different letters on bars are showing statistical differences at $P \leq 0.05$ represent the standard deviation of means each treatment

root system and by increasing the number of roots with a shorter diameter (Wasson *et al*., 2012; Comas *et al*., 2013). A significant increase in plant growth was might be due to increase in surface area to contact water in soil that could be explored more water (Gowda *et al*., 2011). Several PGPR can promote root growth and resultantly lead to better nutrients and water uptake with encouraging influence on growth of plants (Timmusk *et al*., 2014).

Under drought stress, the PGPR strains amended with BGS have better effects on wheat by improving the straw yield, hundred grains weight, grain yield and biological yield as documented in Table 2. In the current study, the *P. moraviensis* was resulted the most effective strain for improving yield parameters of wheat, out of three rhizobacterial strains. However, the other two strains, *i.e*., *A. faecalis* and *B. amyloliquefaciens* also showed significant over to uninoculated control through the integrated use of BGS as an organic amendment. The comparative effectiveness of *P. moraviensis* over other strains in enhancing the root length and subsequently straw and grain yield (Table 2) might be related to a decrease in ethylene production due to the high ACC-deaminase activity and also due to increase root colonization and phosphorus solubilizing ability (Nadeem *et al*., 2009). The growth and yield of lentil crop increased due to the integrated application of organic matter and PGPR (Iqbal *et al*., 2013). The drought stress inhibits the shoot growth due to reducing the leaf area to reserve the evaporative water loss (Neumann, 2008; Skirycz and Inzé, 2010) therefore, a reduction in yield potential occur (Ngumbi and Kloepper, 2016). The application of PGPR containing ACC-deaminase decreases the intensity of stress by several mechanisms (Vardharajula *et al*., 2011) and hence improve the dry biomass, shoot growth and plant productivity (Kasim *et al*., 2013; Grover *et al*., 2014).

The rhizobacterial strains inoculation increased the chlorophyll contents of flag leaves of wheat plants as documented in Fig. 1. Due to the ACC-deaminase activity, the PGPRs might have suppressed ethylene synthesis that resulted in slowing the decay of chlorophyll (Arshad and Frankenberger, 2002; Naveed *et al*., 2014). The chlorophyll contents might be increased due to the enhancement of leaf area by the application of rhizobacterial inoculation, over respective uninoculated control treatment. Drought stress reduces the leaf area of plants (Fatemi, 2014; Hussain *et al*., 2018).

The advantageous effects of rhizobacterial inoculations and BGS amendment were also apparent for enhanced physiological attributes over to uninoculated treatment. Under drought stress, the photosynthetic rate (Fig. 2) was reduced both in uninoculated as well as inoculated treatments devoid of disruption in stomatal conductance. Due drought stress, the growth was interrupted by reducing the energy utilization capacity, which may reduce the activity of photosynthesis (Wang *et al*., 2003). The rhizobacterial inoculation improved the stomatal conductance, transpiration rate and sub-stomatal conductance (Fig. 3, 4 and 5, respectively) under water deficit condition. The role of BGS was also helpful in relations of enhanced physiological attributes. This might be due to the role of organic matter in improving the soil conditions. Effects on photosynthetic attributes have been noticed in the studies having favorable interactions between microbes and plants (Yandigeri *et al*., 2012; Naveed *et al*., 2014).

The inoculations of rhizobacteria were also played an active part in improving the nitrogen, phosphorus and potassium contents of straw and grains (Table 3). Under drought conditions, the mineral nutrient nitrogen, phosphorus and potassium contents in straw and grains were improved due to inoculation and BGS. This might be due to root proliferation that exploited more volume of soil for nutrients uptake efficiently by wheat plants, subsequently the more production of root biomass. Plant growth promoting rhizobacteria enhance the production of siderophores, phosphorus solubilization and low molecular weight organic acids in the wheat plant's rhizosphere which might increase the solubility of mineral nutrients (Rodriguez *et al*., 2006; Glick *et al*., 2007). The PGPR application significantly increased the phosphorus and potassium ion concentrations as compared to uninoculated plants (Kang *et al*., 2012; Jay *et al*., 2013). The concentration of potassium in shoot and grain was increased by increasing the drought stress (Table 3). The application of BGS as organic amendment enhanced the biomass yield and nitrogen uptake (Möller *et al*., 2008; Arthurson, 2009) by improving the physical properties of soil by increasing the moisture retention and hydraulic conductivity and by reducing the bulk density of soil (Barbosa *et al*., 2014).

Conclusion

Integrated application of ACC-deaminase containing rhizobacteria and BGS as an organic amendment can be applied as a useful approach to improve the growth and yield of wheat under drought. The improved plant physiological parameters ultimately lead to improved wheat quality and yield. Therefore, to mitigate the water deficit stress the rhizobacterial inoculation and BGS could be proficiently applied to increase the growth and yield of wheat.

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