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Ameliorating the Drought Stress for Wheat Growth through Application of ACC-Deaminase Containing Rhizobacteria along with Biogas Slurry

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Abstract: The temperature increase around the world is leading to generation of drought, which is a big threat to the productivity of crops. Abiotic stresses like drought increase the ethylene level in plants. In higher plants, 1-aminocyclopropane-1-carboxylate (ACC) is considered as the immediate precursor of ethylene biosynthesis. The application of ACC-deaminase (ACCD) possessing rhizobacteria could ameliorate the harmful results of drought stress by transforming ACC into non-harmful products. Biogas slurry (BGS) improves the water-holding capacity and structure of the soil. Thus, we speculated that the integrated application of ACCD possessing rhizobacteria and BGS might be an efficient approach to mitigate the drought stress for better wheat productivity. A field experiment was conducted under skipped irrigation situations. On the tillering stage (SIT) and flowering stage (SIF), the irrigations were skipped, whereas the recommended four irrigations were maintained in the control treatment. The results of this field experiment exposed that the ACCD possessing rhizobacterial inoculations with BGS considerably improved the stomatal and sub-stomatal conductance, transpiration and photosynthetic rates up to 98%, 46%, 38%, and 73%, respectively, compared to the respective uninoculated controls. The *Pseudomonas moraviensis* with BGS application improved the grain yield and plant height up to 30.3% and 24.3%, respectively, where irrigation was skipped at the tillering stage, as compared to the uninoculated controls. The data obtained revealed that the *P. moraviensis* inoculation + BGS treatment significantly increased the relative water

content (RWC), catalase (CAT) activity, ascorbate peroxidase (APX) activity, as well as grain and shoot phosphorus contents, up to 37%, 40%, 75%, 19%, and 84%, respectively, at SIF situation. The results depicted that the *P. moraviensis* with BGS application under drought stress could be applied for enhancing the physiological, yield, and growth attributes of wheat.

Keywords: organic matter; PGPR; antioxidant activity; skipped irrigation

1. Introduction

Climate change is posing serious threats to worldwide food security. Sustainable crop production has become a big challenge. Owing to rapid changes in rainfall patterns and temperature, crop productivity and yields are going to decline [1–3]. Worldwide, drought stress is considered as the most imperative crop production limiting factor. Drought stress induces limited supply of water [4], reduces photosynthesis [5], causes hormonal imbalances [6], and hampers mineral uptake [7], which eventually lead to a reduction in plant yield [8,9]. Moreover, drought stress accelerates ethylene biosynthesis, which reduces root development and elongation [10].

The plant growth-promoting rhizobacteria (PGPR) provides tolerance to plants against drought and enhance crop productivity [11,12]. The drought-tolerant PGPR provide systemic resistance and endorse plant growth [13,14]. Under drought stress, ethylene biosynthesis regularization has been observed by using PGPR producing ACC-deaminase (ACCD) [15]. The ACCD producing PGPR cleave ACC into α-ketobutyrate and ammonia [16,17]. Besides ethylene regularization, the PGPR also endorse growth of plants by manipulating and modifying the synthesis of phytohormones (cytokinins, auxins, or gibberellic acid) [14], development of better root architecture for increasing the water uptake [16], increasing the nutrients uptake by roots [4], and exopolysaccharides production [18].

However, to mitigate the drought-stress effects, the imperative role of organic amendments to improve the availability of nutrients and the soil's water-holding capacity cannot be denied. Biogas slurry (BGS) is derived from the anaerobic decomposition of plant biomass and organic waste in a biogas digester, which is converted into biofuels [19]. BGS is one of the organic fertilizers that could enhance the fertility of soil and thus crop production [20,21]. BGS also contains abundant nutrients, amino acids, and bioactive substances [22]. The dried form of BGS could increase soil fertility and organic matter, which resultantly increase crop productivity [23]. The PGPR application with organic amendments increased the yield, quality, and growth of peanut [24,25], cucumber [26], and mung bean [27] through reducing the abiotic stress hazards [28]. The combined application of PGPR with BGS improve the quality and yield of the crops and soil health [29,30]. The integrated application of organic wastes and rhizobacteria is helpful in increasing the available potassium, phosphorus, nitrogen, sequestered organic carbon, soil pH, and also crop production [11,27,31].

Wheat (*Triticum aestivum* L.) is widely cultivated, spanning 17% of the world's arable land [32]. Throughout the world, the demand for wheat has increased up to 510 million tons, increasing with the population growth rate [33,34]. Wheat cultivation under limited supply of water considerably reduces its productivity. However, wheat demand is increasing with population growth. Therefore, it is time to increase the productivity of wheat in drought-stressed areas.

To date, there is no report demonstrating the role of BGS in ameliorating water-deficit stress. In the recent past, the scientists concentrated on the application of ACCD producing PGPR for mitigating the water-deficit stress. The aim and novelty of the current experiment are to observe the integrated effects of BGS and PGPR producing ACCD for the mitigation of drought stress. The current study was conducted with the supposition that the combined effect of BGS and drought-tolerant PGPR producing ACCD could be very effective in alleviating the drought-stress effect for enhancing wheat productivity.

2. Materials and Methods

A field experiment was conducted on an experimental farm of the Department of Soil Science, Bahauddin Zakariya University, Multan, Pakistan, to evaluate the effectiveness of ACCD producing PGPR and BGS for improving the antioxidant activities, physiology, growth, and agronomic yield of wheat.

2.1. Pre-Sowing Soil Analysis

The physico-chemical properties of composite soil samples were noted before sowing of the crop (Table 1). The electrical conductivity (EC_e) and soil pH (pH_s) were determined [35]. The organic matter contents were found out through the Walkley [36] methodology. The available phosphorus was assessed through the Olsen and Sommers [37] methodology, whereas for extractable potassium the Chapman and Pratt [38] method was applied. The Moodie et al. [39] protocol was followed in the determination of soil texture. For soil free lime content ($CaCO_3$) determination, the procedure described by Allison and Moodie [39] was followed.

Table 1. Physical and chemical attributes of the soil for the wheat field trial.

Characteristics	Unit	Value
Textural Class		sandy clay loam
Sand	%	54
Silt	%	26
Clay	%	20
pH_s	-	7.9
EC_e	dS m^{-1}	2.42
Organic Matter	%	0.58
$CaCO_3$	%	0.59
Total Nitrogen	%	0.04
Available Phosphorus	mg kg^{-1}	7.1
Extractable Potassium	mg kg^{-1}	107

2.2. Experimental Setup

Three PGPR containing ACCD, i.e., *Pseudomonas moraviensis*, *Bacillus amyloliquefaciens*, and *Alcaligenes faecalis*, along with the BGS, were used in the experiment. The irrigation was skipped at the tillering (SIT) and flowering (SIF) stages of the crop while there were four irrigations applied during normal irrigation (NI). These treatments were arranged in three replications under a randomized complete block design (RCBD), with three factorial arrangements. Both inoculated and uninoculated seeds were drilled in plots having a size of 15 m^2 in a well-prepared field. The BGS was collected and air-dried and applied according to the treatment plan. Fertilization was done by applying a recommended dose of NPK as 120, 90, and 60 kg ha^{-1}, using urea, triple super phosphate, and a muriate of potash, respectively. At the time of field preparation, the total P and K fertilizers were applied, whereas the urea was broadcasted in three split doses (1/3 before sowing, 1/3 at tillering, and 1/3 at the flowering stages of the wheat crop).

2.3. Preparation of Rhizobacterial Inocula and Seed Inoculation and Biogas Slurry

The rhizobacterial inocula were prepared in Dworkin and Foster (DF) salt minimal medium in 500 mL Erlenmeyer flasks [40]. A loopful of the respective strains were inoculated in flasks containing DF salt minimal media. These flasks were left at 28 ± 2 °C temperature for 72 h at 100 rpm in a shaking incubator. A uniform population 10^7–10^8 CFU mL^{-1} of rhizobacteria were obtained by adding sterilized distilled water at a 540 nm wavelength by using a spectrophotometer (Biotechnology Medical Services, UV-1602, BMS, Canada). Wheat seeds were inoculated with the respective rhizobacterial inoculum. The sterilized clay, sugar solution (10%), and peat were used for seed dressing. The BGS

was acquired from a biogas plant working in Ferozpur, Multan, Pakistan. The BGS was analyzed [41] with a pH of 7.5 and EC of 2.95 dS m^{-1}, as well as a 1.04% total potassium, 1.75% total phosphorus, 1.45% total nitrogen, and 38.5% organic carbon concentration.

2.4. Characteristics of the PGPR Strains

The El-Tarabily [42] methodology was followed to measure the ACC-deaminase activity of the rhizobacteria. The indole acetic acid (IAA) production was measured with and without L-tryptophan in broth media on a spectrophotometer [43]. The Goldstein [44] methodology was followed for assessing the phosphorus solubilizing ability of the rhizobacterial strains. The exopolysaccharides production ability of the rhizobacterial strains were noted [45]. The MacFaddin [46] methodology was used to observe the catalase activity. The characteristics of the PGPR strains are provided in Table 2.

Table 2. Characterization of the rhizobacterial strains.

Characteristics		*Alcaligenes faecalis*	*Bacillus amyloliquefaciens*	*Pseudomonas moraviensis*
ACC-deaminase activity (nmol α-ketobutyrateg^{-1} protein h^{-1})		384	435	532
IAA production (mg L^{-1})	**Without L–Tryptophan**	2.21	6.12	5.63
	With L–Tryptophan (1 g L^{-1})	15.33	14.32	22.23
Phosphate solubilization		-	-	-
Exopolysaccharides production ability		-	-	-
Catalase activity		-	-	-

2.5. Plant Physiological Parameters

The physiological attributes of the fully expanded wheat flag leaves were observed at 10:30 a.m.–12:30 p.m. by using a Portable Infrared Gas Analyzer (LCA-4, Germany). The photosynthetic photon flux density of the IRGA was 1200–1400 lmol $m^{-2}s^{-1}$ for determining the stomatal conductance, substomatal conductance, transpiration, and photosynthetic rates. The SPAD chlorophyll meter (SPAD–502, Konika-Minolta, Japan) was used to measure the chlorophyll contents at the stage of flowering and tillering [47]. Water-use efficiency was measured by dividing the transpiration rate by the photosynthetic rate.

2.6. Relative Water Content, Electrolyte Leakage, Total Phenolics, and Proline in Plant Leaves

The relative water content (RWC) of the leaves was measured by putting them in 10 mL test tubes in the fridge for 48 h at 4 °C [48]. Then fully turgid and dry weights were calculated. The RWC was calculated by using formula as follows:

$$\text{RWC } (\%) = \frac{\text{Fully turgid weight } - \text{ Dry weight}}{\text{Fresh weight } - \text{ Dry weight}} \times 100 \quad (1)$$

The electrolyte leakage (EL) in the leaves was noted according to the methodology used by Jambunathan [49].

The uniform leaves discs were placed in 10 mL test tubes with distilled (DI) water and vortexed for 2 h. Then the electrical conductivity (EC_0) was measured. These test tubes were put in a fridge overnight and another electrical conductivity (EC_1) reading was measured. At a 121 °C temperature, the test tubes were autoclaved for 20 min. After cooling the medium, the final electrical conductivity reading (EC_2) was taken. For determining the EL of the wheat leaves, the following formula was used:

$$\text{EL } (\%) = \frac{EC_1 - EC_0}{EC_2 - EC_0} \times 100 \quad (2)$$

The Giannakoula et al. [50] protocol was followed in the determination of total phenolics, by mixing the 100 µL Folin-Ciocalteu reagent (0.25 N) and 20 µL crude leaf extract and adding 0.2 M Na_2CO_3 solution (100 µL). The sample was incubated at room temperature for two hours and the optical density was noted at 760 nm. Total phenolics were calculated according to a standard curve of Gallic acid.

The proline contents in the fresh tissues were determined by grounding them with 3% sulfosalicylic acid. After centrifugation, the 2 mL supernatant was mixed in 10 mL of ninhydrin acid and acetic acid and boiled for 60 min. Toluene was added after cooling, and at 520 nm the absorbance of the mixture was noted by a spectrophotometer [51].

2.7. Enzymatic Antioxidant Activity Assay

The catalase (CAT) activity was measured in a reaction mixture containing the crude leaf extract, H_2O_2 solution (10 mM), and KH_2PO_4 buffer (50 m M). The H_2O_2 reduction was measured at 240 nm absorbance for three minutes at 25 ± 2 °C [52].

The ascorbate peroxidase (APX) enzyme activity was measured in a reaction mixture containing the crude leaf extract, H_2O_2 solution (10 mM), ascorbic acid solution (660 µL), and KH_2PO_4 buffer (50 mM). The H_2O_2 reduction was measured at 290 nm absorbance for three minutes at 25±2 °C by a spectrophotometer [53].

2.8. Measurement of Growth and Yield Parameters and Mineral Nutrients of Plant

The plant height (cm) and number of tillers (m^{-1}), as well as the straw, grain, biological yield, and 1000-grain weight (g) were collected at crop harvest. The Kjeldahl method was used to determine the nitrogen concentration. The phosphorus contents were measured by using the standard method used by Chapman and Prat [38]. A flame photometer (Jenway PFP-7, UK) was used for determining the potassium contents [41].

2.9. Statistical Analysis

Three-way analysis of variance (ANOVA) was used to analyze the data and the RCBD design was followed in the experiment [54]. "Statistix 9.0®", a computer-based statistical software, was used for analyzing the data. For comparing the significance of the treatments, the HSD test was applied at a 5% probability ($p < 0.05$).

3. Results

3.1. Growth Physiology

The results showed that the combined use of rhizobacteria with BGS significantly increased the photosynthetic rate compared to the uninoculated control (no BGS) at SIT and SIF (Figure 1a). At SIT and SIF, the photosynthetic rate was increased up to 73.8% and 72.9%, respectively, with the *P. moraviensis* + BGS treatment compared to the uninoculated control. The transpiration rate was significantly increased up to 38% and 36%, respectively, due to the effect of the ACCD rhizobacteria + BGS at SIT and SIF with respect to their corresponding uninoculated controls (Figure 1b). The *P. moraviensis* + BGS gave a significant improvement in stomatal conductance of the wheat leaves during NI and under the SIT and SIF situations, i.e., up to 68%, 58%, and 73%, respectively, compared to their respective uninoculated controls (Figure 2a). *P. moraviensis*, *B. amyloliquefaciens*, and *A. faecalis* showed that the substomatal conductance was significantly improved at SIT and SIF (Figure 2b).

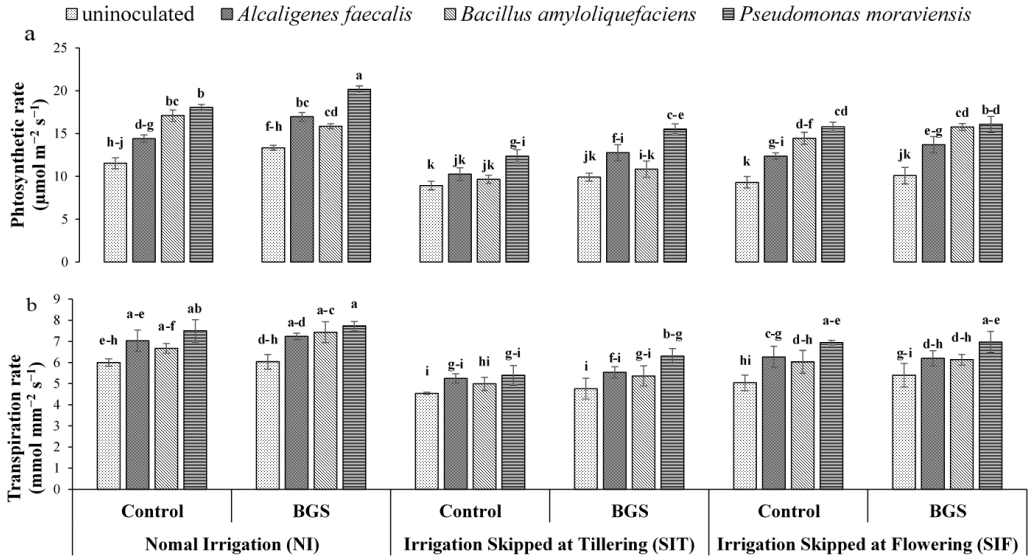

Figure 1. Effect of plant growth-promoting rhizobacteria (PGPR) and biogas slurry (BGS) on the photosynthetic (**a**) and transpiration (**b**) rates (µmol m^{-2} s^{-1}) of wheat plants under drought-stress conditions. Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (±standard deviation; n = 3).

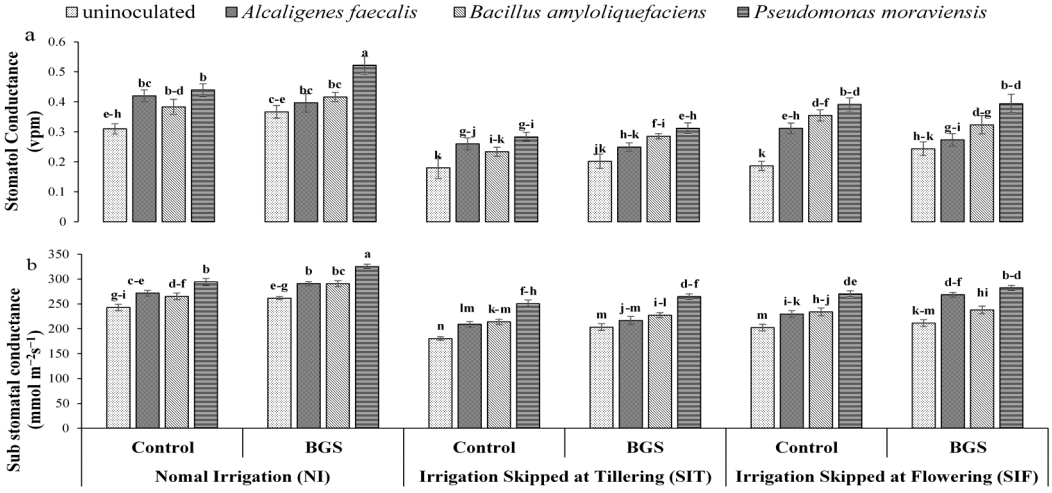

Figure 2. Effect of PGPR and BGS on the stomatal conductance (vpm) (**a**) and sub-stomatal conductance (mmol m^{-2} s^{-1}) (**b**) of wheat plants under drought-stress conditions. Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (±standard deviation; n = 3).

The ACCD containing rhizobacterial strains showed high chlorophyll contents with the BGS application in wheat flag leaves (Figure 3a). However, drought stress significantly reduced the chlorophyll contents both in the uninoculated and inoculated plants. The chlorophyll contents were maximally increased up to 15% and 26% at SIF and SIT, respectively, due to the *P. moraviensis* inoculation. The water-use efficiency was increased up to 39% in *B. amyloliquefaciens* + BGS compared to the uninoculated control (Figure 3b), which significantly mitigated the effect of the drought stress.

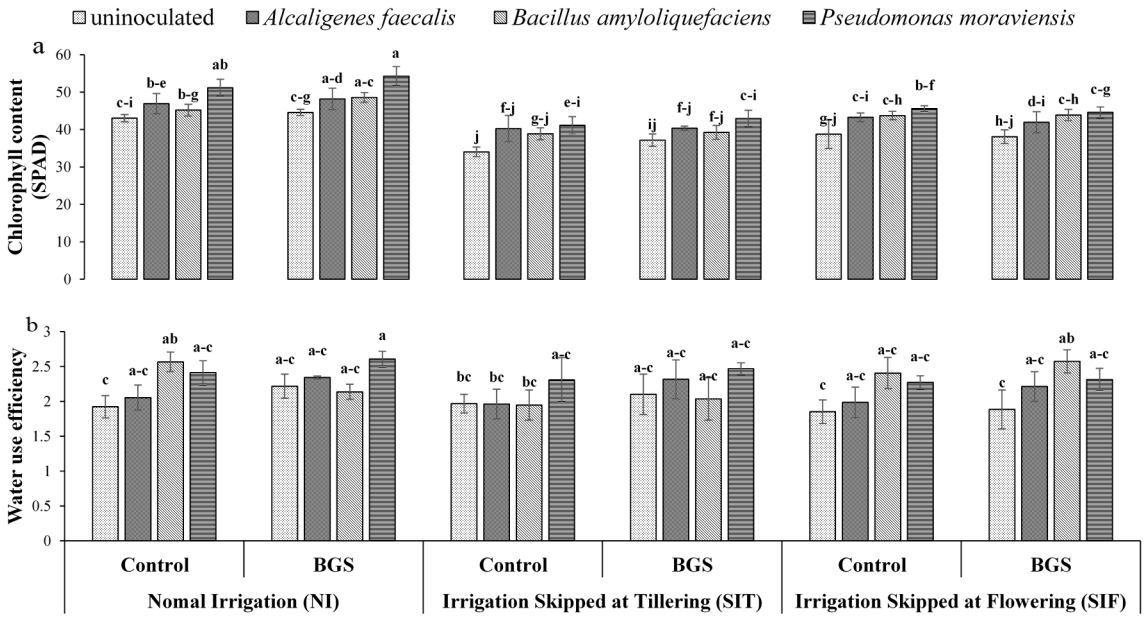

Figure 3. Effect of PGPR and BGS on the chlorophyll content (**a**) and water-use efficiency (**b**) of wheat plants under drought-stress conditions. Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (±standard deviation; n = 3).

3.2. Relative Water Content, Electrolyte Leakage, Proline Content, and Total Phenolic Content

The data showed that the RWC in the wheat flag leaves was significantly increased with PGPR inoculations and BGS (Table 3). The *P. moraviensis* inoculation + BGS treatment showed that, at the SIT situation, the RWC increased up to 37.6%, and at the SIF situation, increased up to 35.9% compared to their corresponding controls. In contrast to RWC, the EL was significantly reduced with the application of PGPR with BGS at the SIF and SIT situations (Table 3). A significant reduction in EL was seen with the *P. moraviensis* + BGS, i.e., up to 34% at SIT and up to 25% at SIF compared to their respective controls.

The proline and total phenolic contents were reduced significantly compared to their respective uninoculated and unamended controls in the inoculated wheat plant's leaves (Table 3). The treatment *P. moraviensis* + BGS decreased the total phenolic contents up to 45% and 33% at SIT and SIF, respectively. Similarly, the *P. moraviensis* inoculation with BGS decreased the proline content about 43.7% at the SIF stage and up to 47.4% at the SIT stage compared to the corresponding uninoculated controls.

Table 3. Effect of ACC-deaminase containing PGPR and BGS on the relative water content, electrolyte leakage, proline content, and total phenolic content of wheat plants under drought-stress conditions.

Treatments	Electrolyte Leakage (%)						Relative Water Content (%)					
	NI		SIT		SIF		NI		SIT		SIF	
	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS
No PGPR	64 c–f	57 f–k	80 a	73 ab	69 bc	68 b–d	60 e–j	67 b–g	45 k	52 i–k	52 i–k	57 g–j
A. faecalis	50 k–n	55 h–m	73 ab	61 c–h	63 c–f	58 f–k	69 a–f	71 a–c	50 jk	59 f–j	61 d–j	59 f–j
B. amyloliquefaciens	55 g–m	49 l–n	62 c–g	66 b–e	61 d–i	59 e–j	63 c–h	76 ab	57 g–j	56 h–j	60 d–j	70 a–e
P. moraviensis	47 mn	45 n	56 f–l	53 i–m	51 k–n	52 j–n	71 a–d	78 a	59 f–j	61 c–i	65 c–h	71 a–d
	Proline Content (μg g^{-1})						**Total Phenolic (μg g^{-1})**					
No PGPR	0.47 c–f	0.45 c–g	0.63 ab	0.71 a	0.52 cd	0.63 a	99 g–j	90 h–j	165 b	192 a	183 a	160 b
A. faecalis	0.43 c–h	0.37 f–i	0.50 c–e	0.65 a	0.41 e–i	0.42 d–i	72 kl	88 jk	132 c	112 fg	122 c–f	113 e–g
B. amyloliquefaciens	0.36 g–i	0.41 e–i	0.53 bc	0.48 c–e	0.44 c–g	0.44 c–h	90 ij	70 l	135 c	132 c	131 cd	130 c–e
P. moraviensis	0.33 i	0.39 e–i	0.40 e–i	0.38 f–i	0.33 hi	0.41 e–i	67 l	58 l	106 f–i	114 d–g	107 f–h	91 h–j

Note: Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (n = 3). NI is normal irrigation; SIT is skipped irrigation at tillering; SIF is skipped irrigation at flowering; and BGS is biogas slurry.

3.3. The Enzymatic Antioxidant Activity

The rhizobacterial inoculation alone and with BGS interaction significantly increased the CAT and APX contents in wheat (Figure 4a,b). The CAT activity was observed as at its maximum at the SIT situation. The CAT activity was maximally increased up to 72.4% and 40.5% at the SIF and SIT stages due to the *P. moraviensis* with BGS amendment compared to their respective controls. The APX was also significantly increased with the *P. moraviensis* inoculation with BGS amendment at normal and skipped irrigation situations (SIT and SIF). At the SIT stage, a significant increase (42.4%) was noted in APX activity with a *P. moraviensis* inoculation with BGS amendment compared to the unamended control. The APX contents were significantly increased at the SIF stage up to 75.1%, 47.1%, and 43.8% with the *P. moraviensis*, *A. faecalis*, and *B. amyloliquefaciens* application, respectively, compared to the uninoculated controls.

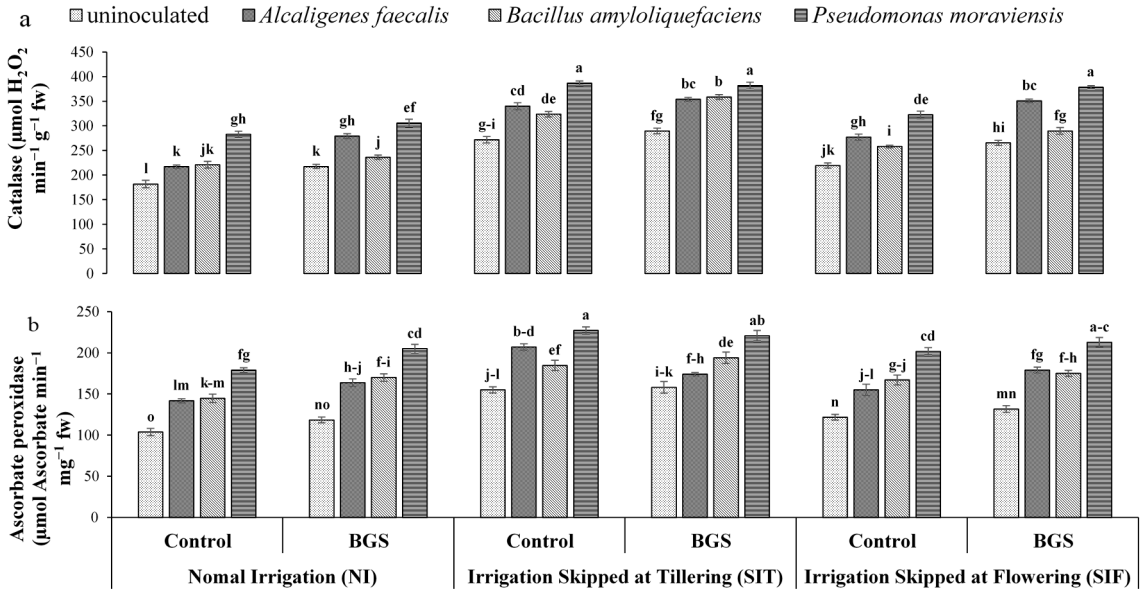

Figure 4. Effect of PGPR and BGS on the catalase (µmol H_2O_2 min^{-1} g^{-1} fw) (**a**) and ascorbate peroxidase (µmol Ascorbate min^{-1} mg^{-1} fw) (**b**) of wheat plants under drought-stress conditions. Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (±standard deviation; n = 3).

3.4. Growth and Agronomic Yield

The data of the field experiment presented in Table 4 showed that the ACC-deaminase possessing PGPR inoculation with BGS application improved the plant height under drought conditions. Under drought conditions created by SIT, the *P. moraviensis* + BGS treatment revealed up to a 24.3% increase compared to the uninoculated control in plant height. However, at SIF, the maximum plant height was increased (10.6%) with the *B. amyloliquefaciens* + BGS treatment compared to the control. At the NI situation, the number of tillers was increased up to 64% with the *A. faecalis* + BGS treatment compared to the uninoculated control (Table 4). As the SIT stage, *B. amyloliquefaciens* + BGS significantly increased (up to 22.9%) the number of tillers and mitigated the drought effects. Table 4 shows that the PGPR strains with BGS significantly enhanced the biological, grain, and straw yield, as well as the 1000-grain weight. At the NI, SIT and SIF stages, the straw yield was increased up to 13.8%, 24.3%, and 11.8% with *P. moraviensis* + BGS, respectively, compared to their respective uninoculated controls.

Table 4. Effect of ACC-deaminase containing PGPR and BGS on the plant height (cm), number of tillers (m^{-1}), 1000-grain weight (g), grain yield (Mg ha^{-1}), straw yield (Mg ha^{-1}), and biological yield (Mg ha^{-1}) of wheat plants under drought-stress conditions.

Treatments	Plant Height (cm)						Number of Tillers (m^{-1})					
	NI		SIT		SIF		NI		SIT		SIF	
	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS
No PGPR	82 de	82 c–e	63 ij	62 j	76 d–h	75 e–h	214 jk	284 b–e	215 jk	216 i–k	213 k	222 h–k
A. faecalis	90 a–c	92 a	70 g–i	70 g–i	83 b–e	84 b–d	302 bc	353 a	232 g–k	243 f–k	280 b–f	295 bc
B. amyloliquefaciens	91 ab	93 a	69 h–j	74 f–h	79 d–f	84 b–d	317 ab	345 a	251 e–j	265 c–g	253 e–i	269 c–g
P. moraviensis	93 a	93 a	72 f–h	78 d–g	84 b–d	84b–d	307 b	347 a	243 f–k	256 d–h	268 c–g	294 b–d
	1000-Grain Weight (g)						Grain Yield (Mg ha^{-1})					
No PGPR	36 c–g	37 c–g	29 i	31 i	31 hi	36 e–g	3.16 d–g	3.34 b–f	2.29 j	2.40 ij	3.05 e–h	2.91 gh
A. faecalis	40 a–c	41 ab	30 i	32 hi	35 f–h	38 b–f	3.47 b–d	3.72 ab	2.84 gh	2.82 gh	3.34 b–f	3.32 c–f
B. amyloliquefaciens	40 a–d	39 a–d	31 hi	32 hi	36 c–g	36 d–g	3.39 b–e	3.63 a–c	2.74 hi	2.98 f–h	3.30 c–f	3.31 c–f
P. moraviensis	41 ab	42 a	32 hi	34 gh	38 b–g	39 a–e	3.62 a–c	3.90 a	2.98 f–h	3.05 e–h	3.19 dg	3.19 d–g
	Straw Yield (Mg ha^{-1})						Biological Yield (Mg ha^{-1})					
No PGPR	5.05 b–f	5.28 a–e	4.05 i	4.12 i	4.80 c–h	4.80 c–h	10.9 d–f	11.7 c–f	8.4 g	10.0 fg	9.9 fg	11.0 d–f
A. faecalis	5.27 a–e	5.54 ab	4.35 g–i	4.71 d–i	4.92 b–h	5.29 a–e	12.7 b–d	14.1 ab	10.5 ef	11.3 c–f	11.3 d–f	11.6 c–f
B. amyloliquefaciens	5.18 a–e	5.46 a–c	4.26 hi	4.64 e–i	5.08 b–f	5.00 b–g	12.1 c–e	12.8 a–d	10.6 ef	10.8 d–f	11.2 d–f	11.6 c–f
P. moraviensis	5.36 a–d	5.75 a	4.49 f–i	5.03 b–f	5.33 a–d	5.36 a–d	13.2 a–c	14.7 a	10.6 ef	11.4 c–f	11.7 c–f	12.2 b–e

Note: Treatments sharing similar letters do not have an Honest Significant Difference between each other at $p \leq 0.05$ (n = 3). NI is normal irrigation; SIT is skipped irrigation at tillering; SIF is skipped irrigation at flowering; and BGS is biogas slurry.

3.5. Mineral Nutrition

The data obtained from the field trial revealed that the nitrogen, phosphorus, and potassium contents were increased at all drought levels in the straw and grain of wheat acquired by PGPR inoculations with BGS application (Table 5). Nitrogen contents in the shoot and grain were improved significantly up to 32.3% and 31.7%, respectively, at NI with *P. moraviensis* + BGS compared to their respective uninoculated controls. The shoot and grain nitrogen were considerably raised up to 38.8% and 39.7%, respectively, with the *P. moraviensis* + BGS treatment compared to their corresponding uninoculated controls at the SIT situation.

The *P. moraviensis* with BGS application acquired an improvement at the SIF, SIT, and NI stages in grain phosphorus contents up to 19.4%, 24.3%, and 26.6%, respectively, compared to their respective uninoculated controls. The *A. faecalis* + BGS also acquired shoot phosphorus increments up to 84%, 80%, and 31% at the SIF, SIT and NI situations, respectively, compared to their respective uninoculated controls of wheat. The *P. moraviensis* + BGS revealed a significant increment in potassium content in wheat shoot (42.8%) and grains (48.8%) compared to their uninoculated controls at the SIT stage.

Table 5. Effect of ACC-deaminase containing PGPR and BGS on the nitrogen, phosphorus, and potassium contents (%) in the grain and straw of wheat under drought-stress conditions.

Treatments	Grain Nitrogen (%)						Shoot Nitrogen (%)					
	NI		SIT		SIF		NI		SIT		SIF	
	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS	No BGS	BGS
No PGPR	1.56 h–k	1.83 c–f	1.15 q	1.27 o–q	1.25 pq	1.30 n–q	1.21 d–f	1.44 bc	0.79 j	1.02 g–i	1.01 g–i	1.17 e–g
A. faecalis	1.88 b–e	2.02 a–c	1.32 m–q	1.46 k–o	1.49 j–n	1.53 h–l	1.36 cd	1.51 a–c	0.93 ij	1.03 g–i	1.22 d–f	1.24 de
B. amyloliquefaciens	1.79 d–g	1.95 a–d	1.35 l–p	1.50 i–m	1.69 e–i	1.70 e–h	1.44 bc	1.53 ab	1.05 f–i	0.95 h–j	1.17 e–g	1.26 de
P. moraviensis	2.08 ab	2.13 a	1.47 k–n	1.60 g–k	1.68 f–j	1.80 d–g	1.59 ab	1.61 a	1.14 e–g	1.10 e–h	1.23 de	1.35 cd
	Grain Phosphorus (%)						**Shoot Phosphorus (%)**					
No PGPR	0.54 e–i	0.54 e–i	0.44 k	0.45 jk	0.49 i–k	0.51 g–j	0.27 c–e	0.27 c–e	0.12 i	0.14 hi	0.15 g–i	0.21 d–f
A. faecalis	0.61 b–d	0.65 ab	0.50 g–k	0.52 f–i	0.57 d–g	0.54 d–i	0.31 a–c	0.36 a	0.13 i	0.21 e–g	0.19 f–h	0.29 bc
B. amyloliquefaciens	0.60 b–e	0.57 c–g	0.50 h–k	0.52 f–i	0.54 e–i	0.56 d–h	0.29 bc	0.30 bc	0.17 f–i	0.20 fg	0.14 hi	0.22 d–f
P. moraviensis	0.63 a–c	0.68 a	0.54 e–i	0.55 d–i	0.56 d–h	0.58 c–f	0.32 a–c	0.35 ab	0.22 d–f	0.22 d–f	0.22 d–f	0.27 cd
	Grain Potassium (%)						**Shoot Potassium (%)**					
No PGPR	0.47 f–i	0.54 d–f	0.36 jk	0.31 k	0.40 i–k	0.39 i–k	0.94 i–k	1.15 c–g	0.79 k	0.98 h–j	0.84 jk	0.97 h–j
A. faecalis	0.64 a–c	0.70 a	0.43 h–j	0.45 g–j	0.52 d–g	0.50 e–h	1.25 b–e	1.31 bc	1.16 c–g	1.12 e–h	1.03 g–i	1.17 c–g
B. amyloliquefaciens	0.54 d–f	0.67 ab	0.50 e–h	0.48 e–h	0.48 e–h	0.51 e–h	1.15 c–g	1.39 ab	1.06 g–i	1.07 f–i	1.09 e–i	1.12 e–h
P. moraviensis	0.65 ab	0.71 a	0.53 d–f	0.54 d–f	0.60 b–d	0.56 c–e	1.29 b–d	1.51 a	1.18 c–g	1.13 d–h	1.22 c–f	1.24 b–e

Note: Treatments sharing similar letters do not have an Honest Significant Difference with each other at $p \leq 0.05$ (n = 3). The NI is normal irrigation; SIT is skipped irrigation at tillering; SIF is skipped irrigation at flowering; and BGS is biogas slurry.

4. Discussion

Drought stress inhibits the production as well as growth of wheat. Under drought stress, the effect of a BGS application has remained unexplored up to date. The integrated application of ACCD containing rhizobacteria with BGS as an organic amendment for enhancing wheat productivity has been rarely exploited.

The data revealed that the growth of the uninoculated plants was severely influenced due to drought stress. Whereas, the growth, physiology, and productivity of the wheat plants was efficiently improved with the use of ACCD possessing rhizobacterial inoculation amended with BGS. Hussain et al. [4] reported that the ACCD possessing rhizobacteria had significantly improved the root length, proposing a positive influence of ACCD activity under stress conditions. Chandra et al. [55] described that the ACCD possessing rhizobacteria have specific capabilities to hydrolyze the ACC biosynthesis in roots. Under stress conditions, the ACCD containing rhizobacteria decreased the biosynthesis of stress ethylene, which enhances the induced systemic tolerance in plants [56–58].

The growth and yield parameters of wheat were improved, which might be due to better root growth, eventually increasing the water and nutrient uptake. The synergistic effects of the ACCD activity and auxin of rhizobacteria might be the crucial factors that contributed to root proliferation during drought stress [59]. Beside this, the rhizobacterial strains produce exopolysaccharides that create a biofilm on the root surface that could enhance the nutrients and water [18,60].

The valuable influence of a BGS amendment and inoculations of rhizobacteria were also obvious given the increased physiological parameters compared to the uninoculated controls. However, drought stress adversely affected these gaseous exchange attributes by decreasing the energy utilization capacity [61,62]. The results obtained from the experiment showed that the integrated application of BGS and ACCD possessing rhizobacteria increased the stomatal conductance and transpiration rate, which increased the photosynthetic rate as described by Naveed et al. [63] and Ahmad et al. [64].

Owing to a decrease in RWC, turgor pressure and cell expansion were reduced [65]. The EL in the leaves was increased under the stressed conditions [66–68]. Whereas, with the integrated application of the BGS and ACCD possessing rhizobacteria, the EL was reduced, showing an increment in relative tolerance [69]. This might be due to the application of the BGS that increased the hydraulic conductivity, water-holding capacity, structure, and volumetric moisture content of the soil [70–72]. The chlorophyll contents in the wheat flag leaves were increased due to ACCD possessing rhizobacteria that might suppress ethylene biosynthesis under drought stress [4,73,74].

The total phenolic and proline contents could maintain cellular turgor and reduce the water potential. The proline and total phenolic contents were significantly higher under drought stress in the leaves of wheat plants that were not inoculated [75,76]. Whereas, with the integrated application of the BGS and ACCD possessing rhizobacteria, the proline and total phenolic contents were reduced compared to the uninoculated controls. Naveed et al. [63] also described that under stress conditions the proline contents were increased, but reduced with ACCD possessing rhizobacterial treatments. The APX and CAT enzymes in wheat plants were increased in the rhizobacterial inoculated treatments. Our findings were correlated with the results of Sandhya et al. [77], who noted that the ACC level was decreased in the root zone due to the ACCD activity of the rhizobacterial strains.

Under water-deficit stress, the concentrations of N, P, and K were increased in the grains and straw due to the integrated application of the BGS and ACCD rhizobacteria. This could be due to root proliferation. That resulted in the efficient uptake of nutrients and the production of more root biomass, which exploited more soil volume. The rhizobacteria might have enhanced the solubility of the mineral nutrients due to the production of organic acids and siderophores and an increase in phosphorus solubilization activity in the rhizosphere of plants [74,78]. The BGS application also enhanced the nutrient uptake and biomass yield [70].

5. Conclusions

It can be inferred from the present study that the integrated application of BGS and ACCD possessing rhizobacteria gave better results as compared to their separate application. Our observations depicted that the combined application of BGS and rhizobacteria with ACCD activity could be an effective approach for increasing the productivity of wheat under drought conditions. The ACC-deaminase containing rhizobacteria has a better potential to be used as a biofertilizer and could be used to increase wheat productivity in arid regions. The *Pseudomonas moraviensis* strain showed more effectiveness in enhancing the yield as compared to other strains. The future perspectives include that these rhizobacteria with ACCD activity should be studied in other crop species. Consortia of these rhizobacteria should be explored to get their maximum benefits and roles in nutrient cycling.

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Abbreviations

ACC	1-Aminocyclopropane-1-carboxylate
ACCD	ACC-deaminase
ANOVA	Analysis of variance
BGS	Biogas slurry
CAT	Catalase
CFU	Colony forming unit
EC	Electrical conductivity
EL	Electrolyte leakage
HSD	Honest Significant Difference
IAA	Indole acetic acid
NI	Normal irrigation
PGPR	Plant growth promoting rhizobacteria
ppm	Parts per million
RCBD	Randomized complete block design
rpm	Resolution per minute
RWC	Relative water content
SIF	Skipped irrigation flowering stage
SIT	Skipped irrigation tillering stage
WUE	Water-use efficiency

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