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Amelioration of drought stress in wheat by using plant growth-promoting rhizobacteria and biogas slurry

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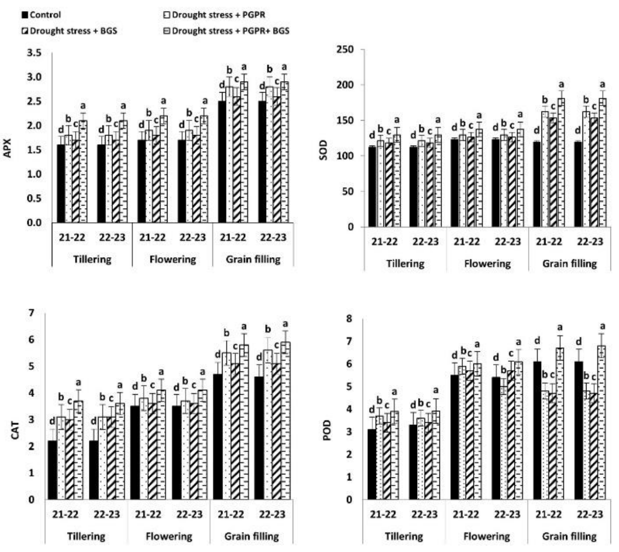
Graphical abstract



Abstract

Drought stress has a significant impact on cereal-based staple food production, particularly in developing countries like Pakistan. To ensure a sustainable and reliable food supply, it is essential to develop comprehensive production plans that incorporate various approaches to mitigate the effects of drought. In a study conducted using a randomized complete block design, we investigated the potential of plant growth-promoting rhizobacteria (PGPR) and biogas Slurry (BGs) either individually or in combination to alleviate drought stress at different stages of wheat growth. The two-year field research demonstrated that the application of *Azosprillium lipoferum* with biogas slurry resulted in improved water relations, chlorophyll content, grain quality, yield, and related characteristics in wheat plants compared to the stressed treatments. Particularly, the combined treatment of PGPR and BGs exhibited the most favorable outcomes. Notably, the combined treatment effectively mitigated drought stress by significantly increasing antioxidant levels (17% APX, 29% POD, 34% CAT, and 41% SOD) during the grain-filling stage (GFS) compared to the controls. The combined treatment resulted in a remarkable 40% improvement in the respective controls at the GFS stage. Overall, the combined use of PGPR and BGs was identified as an effective strategy to enhance the resilience of wheat plants to drought, particularly in arid and semi-arid regions.

Keywords: PGPR, Drought, Wheat, Antioxidants, Yield, Azospirillum lipoferum

1. Introduction

Wheat, the principal staple food crop in Pakistan, was grown over an enormous land area of 8,825 thousand hectares, yielding an astounding 24,946 million tons in 2017. (The Pakistani Statistical Office, 2020). Wheat contributes 1.7% to GDP and 8.7% to agricultural value added. On average, a Pakistani guy eats roughly 60% wheat per day. In Pakistan, a person eats about 125 kilograms of wheat annually. The need to feed a rapidly expanding global population compounds the already serious threats posed to global food security by climate change and water scarcity (Lesk *et al.* 2016).

The ubiquitous presence of drought as an unavoidable abiotic stress has been observed in various regions around the world, disregarding boundaries, and oftentimes without adequate warning. Accordingly, these causative factors, such as increasing temperature, light intensity, and reduced precipitation, may have an intense damaging effect on plant production, protection, and produce quality (Seleiman *et al.* 2021; Shahzad *et al.* 2021). Environmental stresses are the premier factor influencing the entirety of the process from cereal production to post-harvest consumption regarding safeguarding food security. It has been suggested that drought is a primary factor in the reduction of wheat productivity (Rashid *et al.* 2022) due to the detrimental effect it has on vital plant developmental phases (Sallam *et al.* 2019). In their study, Gull *et al.* (2019)

detailed the detrimental effects of drought on various phases of wheat development. The presence of this phenomenon at the tillering stage can lead to a decrease in plant height and the number of tillers per unit area, which subsequently results in reduced biomass, more spiked tillers, lower grains per spike, and finally, a decline in grain weight at the grain-filling stage, potentially leading to a 50% decrease in yield (Majid *et al.* 2007; Tiwari *et al.* 2014; Kulkarni *et al.* 2017).

When it comes to plants, particularly cereal crops, there are a variety of strategies employed to counteract the damaging effects of drought (Gregory *et al.* 2017). The disastrous effects of drought can be mitigated through the use of drought-tolerant plant varieties, proper water management, and the priming of seeds (Abid *et al.* 2017). Inoculating plants with plant growth-promoting rhizobacteria (PGPR) is widely regarded as an efficient method for mitigating the negative effects of drought on crop production in the modern day. PGPR are known to colonize plant roots and promote plant development. Certain PGPB strains can enhance abiotic stress tolerance in certain plants, such as wheat, specifically in relation to salt and drought stress (Ashraf *et al.* 2004; Creus *et al.* 2004; Ullah *et al.* 2021; Ahmad *et al.* 2022a; Wasaya *et al.* 2024). The use of PGPR can alleviate pressure in situations of severe water scarcity. There is a need to adapt new formations and chances to increase production, as the performance of PGPR has been shown to vary among research, possibly as a result of the numerous environmental effects that affect their proliferation and growth in the plants (Bhardwaj *et al.* 2014). Halotolerant plant growth-promoting rhizobacteria (PGPR) have a role in enhancing the ability of different plants to tolerate high salt levels, enabling them to thrive in saline environments. This is accompanied by improvements in their morphological characteristics (Sezen *et al.* 2024). Many scientists have discovered that numerous species of phosphate-solubilizing bacteria secrete indole acetic acid (IAA), which is absorbed by the roots in the rhizosphere (Ahmed *et al.* 2011: Ahmed *et al.* 2012; Misra *et al.* 2012). This leads to an increase in the plant's existing pool of IAA. According to reports, an optimal level of IAA has a good effect on root growth, whereas an excessive concentration of IAA has a detrimental effect (Glick, 2012). The advantageous effects of PGPR have been seen in several crops, such as cereals (Cakmakci, 2001, Leigh, 2002; Ozturk *et al.* 2003), legumes, and oilseed crops (Hussain *et al.* 2013; Kiani *et al.* 2016; Parmar *et al.* 2000). It has also been reported that sunflower plants, when inoculated with PGPR that possess ACC-deaminase activity, exhibit improved growth in saline conditions compared to those that are not inoculated.

Biogas slurry is a nutrient-rich byproduct of the anaerobic digestion of organic matter such as animal manure, crop residues, and food waste to produce biogas. It is a dark liquid that contains a high concentration of nitrogen, phosphorus, and other essential plant nutrients, making it a valuable organic fertilizer for crop production. Biogas slurry (BGS) is not only the most eco-friendly organic fertilizer among all other kinds of organic and synthetic fertilizers, but it also effectively utilizes waste materials in many Asian nations (Haque *et al.* 2020; Ahmad *et al.* 2022b; Jan *et al.* 2022).

The implementation of appropriate agronomic or chemical techniques is imperative for sustaining crop growth in the presence of drought-induced stress. The confirmation of the effect of PGPR and biogas Slurry remains uncertain, and there is a lack of research on their combined application.

In a prior investigation, it was discovered that the simultaneous introduction of five rhizobacteria strains, Azosprillium Lipoferum, Bacillus megaterium, Agrobacterium fabrum, Pseudomonas moraviensis, Alcaligenes faecalis, to wheat seeds resulted in a notable enhancement in crop performance and grain yield when compared to plants that were not subjected to inoculation (Saleem *et al.* 2023). However, it was imperative to possess knowledge regarding the impact of these strains on crop performance in unfavorable conditions, such as drought. This knowledge was crucial due to its significance in understanding the effects of these strains on crop productivity.

It is imperative to implement an environmentally sustainable biological approach to sustain crop growth during periods of drought stress. Extensive research has been conducted on the individual effects of PGPR and biogas slurry; however, there remains a dearth of studies investigating their combined impact on mitigating drought-induced stress. Hence, the objective of this study was to investigate the impact of externally administered biogas slurry and the simultaneous introduction of PGPR on the growth and physiological attributes of wheat plants subjected to drought stress at various stages of development, namely tillering, flowering, or grain filling, in the years 2021-22 and 2022-23. We propose the hypothesis that the concurrent utilization of PGPR and biogas slurry will result in the preservation of plant growth and yield, surpassing the individual effects of each treatment.

The primary objective of this research study is to examine the impact of PGPR and biogas slurry on the performance of wheat (*Triticum aestivum*) plants subjected to drought stress conditions.

2. Material and Method

2.1. Location

A field experiment was conducted at the experimental farm of the Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, to assess the efficacy of rhizobacteria and air-dried BGS in mitigating the adverse effects of drought stress on wheat crops in a greenhouse setting.

2.2. PGPR preparation and seed coating

The prominent results of a drought-tolerant PGPR strain (*Azospirillum lipoferum*) obtained from the Soil Microbiology Laboratory of the Department of Soil Science at the Faculty of Agriculture and Environment at the Islamia University Bahawalpur were obtained through screening experimentation. An inoculum was generated in 50 mL Erlenmeyer flasks with DF salt minimal medium, as described by Dworkin and Foster in 1958. A sample of a specific strain was inoculated into a flask filled with DF salt minimal medium, and incubated for 24 hours in a shaking incubator set at a temperature of 25 ± 2 °C and a rotational speed of 100 rpm. The application of Ujalla-2016 wheat seed dressing was completed by blending a bacterial inoculum with sterilized clay, a 10% sugar solution, and peas.

2.3. BGS Preparation

The biogas slurry (BGS) was obtained from a biogas plant installed at Langriyal farm in Khair Pur Tamewali and was dehydrated by open-air drying on a plastic sheet. The content of organic matter (37.6%), total nitrogen (1.37%), phosphorus (1.62%), and potassium (1.09%) in the BGS was analyzed following the standard protocol delineated by Ryan *et al.* (2001). The pH of BGS was determined to be 7.5, with an electrical conductivity of 2.82 dS m^{-1} being recorded. The application of BGS (at the rate of 450, 550, and 650 kg ha^{-1} BGS) was carried out as per the treatments before sowing in the field.

2.4. Field experiment preparation

Each year, the wheat crop was sowed on October 20 after 2-3 plowings and planking. Under Bahawalpur's agroecological circumstances, the Ujalla-2016 wheat variety was the ideal genotype. Wheat seedlings were inoculated with PGPR through the preparation of a sugary solution. With the requisite experiments, biogas slurry was applied at @450, 550, and 650 kg per hectare. The fertilizer application rate was 120-80-60 kg Nitrogen-phosphorus-potassium per hectare. Wheat was given the recommended amount of irrigation. Drought stress is applied by ceasing irrigation at specific stages (tillering, flowering, and grain-filling stages). RCBD was used for field testing. The experiment consisted of three replications.

2.5. Recorded Parameters

The growth and yield-related parameters, including plant height (cm), spike length (cm), number of grains per spike, 1000-grain weight (g), biological yield per plant (g), and grain yield per plant (g), Nutrient (N, P, and k) contents and Protein contents were measured following established procedures and protocols (Bremner, 1965; Tkachuk, 1966; Ullah *et al.* 2018) at the time of crop maturity. The leaf area index (LAI), which represents the green leaf area per unit ground surface area (m^2 m^{-2}), along with leaf chlorophyll contents, relative water contents (RWCs), photosynthetic rate, and transpiration rate, were assessed at various growth stages.The LAI and leaf chlorophyll contents were quantified using a meter rod, a portable laser leaf area meter (model CI-2002 L, CID Bio-Science, USA), and a chlorophyll meter (model CL-01, Hansatech Instruments Ltd., UK), respectively. The LI 6250 gas analyzer, manufactured by Li-COR, was employed for the quantification of photosynthetic rate. The leaf relative water contents (RWCs) were determined using the formula provided by Barrs and Weatherley (1962).

RWC (%) = (Fresh weight- dry weight)/(turgid weight – dry weight) × 100

The leaves that were gathered from the wheat plants were carefully placed inside plastic bags and promptly transported to the laboratory to ascertain their fresh weight. The measurement of the weight of leaves under turgid conditions involved immersing them in distilled water for a period of 16 to 18 hours at ambient temperature. Subsequently, the wet leaves were carefully dried by gently blotting them with tissue paper. To ascertain the dry weight, the leaves were subjected to a drying process in an oven for 72 hours at a temperature of 70°C.

To analyze the nutrient composition of wheat leaves, the plant materials were subjected to a process of oven-drying, followed by grinding using a Wiley micro mill. The resulting ground material was then sieved to achieve a particle size of less than 2 mm. The dry material, weighing 0.5 g, was subjected to digestion using a mixture of hydrogen peroxide and sulfuric acid, as described by Wolf (1982). The phosphorus content in the digest was determined using spectrophotometry, while flame photometry was employed to determine the potassium content. The nitrogen content was estimated using the micro-Kjeldhal's method, as described by Bremner in 1965. The protein content in the digest was assessed by multiplying the nitrogen content by a factor of 5.70, as described by Tkachuk in 1966.

2.6. Enzymatic Activity

The activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) were measured using the procedure outlined by Kar and Mishra (1976). The enzymatic activity of Ascorbate peroxidase (APX) was quantified following the methodology described by Cakmak (1994).

2.7. Statistical Analysis

Fisher's analysis of variance (ANOVA) was used to statistically assess the collected data, and the means of significant treatments will be compared using the least significant difference (LSD) test at a 5% probability level (Steel *et al.* 1997).

3. Result

3.1. Growth and yield characters

The results in Tables 1 and 2 show that drought has some interesting effects on growth characters. The highest plant height (89.59 cm and 90.20 cm) was obtained under control treatment (T_0) followed by treatment T_{12} (89.33 cm and 89.55 cm) in both years and the lowest plant height (83.16 cm and 84.12 cm) was observed in treatment (T_1) when drought was occur at tillering stage. PGPR and biogas slurry application significantly affect the plant height under drought conditions at the tillering stage. Maximum spike length (12.06 cm and 13.21 cm) was observed under control treatment (T_0) followed by T_{12} (11.96 cm and 17.41 cm) in both years and lowest (10.07 cm and 10.55 cm) was observed in treatment (T_1) when drought occurs at tillering stage. The highest number of spikelets per spike (23.22 and 23.57) was observed under control treatment (T_0) followed by treatments T_4 (20.92, 21.55), and the lowest (14.18 and

14.22) was observed in T_5 treatment when drought occurred at flowering stage.

Table 1. Effect of PGPR and BGS on wheat resilience to drought at different growth stages.

Treatments	Plant height (cm)		Spike length (cm)		Number of spikelets spike-1		Number of grains spike-1	
	2021-22	**2022-23**	**2021-22**	**2022-23**	**2021-22**	**2022-23**	**2021-23**	**2022-23**
T_0	89.59 a	90.20 a	12.06 a	13.21 a	23.22 a	23.57 a	46.72 a	47.33 k
T_1	83.16 i	84.12 f	10.07 m	10.55 g	18.36 f	19.21 c	42.55 e	43.41 j
T_2	84.72 h	85.33 e	10.50 k	10.74fg	19.87 c	20.41 b	44.16 c	45.26 i
T_3	85.13 g	85.79 d	10.41 l	10.87 a	19.52 d	19.87 bc	43.57 d	44.44 i
T_4	86.22 f	87.21 c	10.56 j	10.65 f	20.92 b	21.55 a	44.75 b	45.54 h
T_5	87.26 e	88.34 c	11.01 i	11.21de	14.18 l	14.22 f	34.61m	35.37 g
T_6	87.27 de	87.87c	11.09 g	11.31 de	15.32 j	15.45 f	35.57k	36.48 e
T_7	87.27 de	88.54 c	11.04 h	11.17 e	15.25 k	15.31ef	34.91 l	35.62 f
T_8	87.63 d	88.47 b	11.26 f	11.55 d	16.52 i	16.74 e	37.18 i	38.18 d
T_9	88.52 c	89.24 b	11.51 e	15.21 c	17.38 h	17.45 d	36.64 j	37.33 c
T_{10}	88.92 b	89.55 b	11.62 d	16.55 bc	18.42 e	19.52 c	38.71 g	39.38 b
T_{11}	88.97 b	89.18 b	11.65 c	16.55 ab	18.26 g	18.37 c	37.64 h	38.45 c
T_{12}	89.33 a	89.87 b	11.96 b	17.41 ab	19.87 c	20.54 b	40.37 f	41.52 a
LSD (0.05)	0.282	0.391	0.012	0.014	0.016	0.017	0.020	0.014
PGPR	*	*	*	*	*	*	*	*
BGS	*	*	*	*	*	*	*	*
Drought	*	*	*	*	*	*	*	*
PGPR*BGS	*	*	*	*	*	*	*	*
PGPR*Drought	NS	NS	*	*	*	*	*	*
BGS*Drought	NS	NS	*	*	*	*	*	*
PGPR*BGS*Drought	NS	NS	*	*	*	*	*	*

Where T_0 = Control, T_1 = DrT, T_2 = DrT + P, T_3 = DrT + BGS, T_4 = DrT + P + BGS, T_5 = DrF, T_6 = DrF + P, T_7 = DrF + BGs, T_8 = DrF + P + BGS, T_9 = DrGF, T_{10} = DrGF + P, T_{11} = DrGF + BGS, and T_{12} = DrGF + P + BG, DrT = Drought at tillering, P = PGPR, BGS = Biogas slurry, DrF = Drought at flowering, and DrGF = Drought at grain filling

Table 2. Effect of PGPR and BGS on wheat yield characters under drought.

Treatments	1000-grain weight (g)		Grain yield (t ha^{-1})		Biological yield (t ha^{-1})	
	2021-22	**2022-23**	**2021-22**	**2022-23**	**2021-22**	**2022-23**
T_0	35.90 a	37.85 a	5.23 a	5.28 a	13.22 a	13.28 a
T_1	30.32 e	32.41 e	4.27 g	4.36 g	11.32 h	11.38 g
T_2	33.51 c	35.37 c	4.43 c	4.53 d	12.34 d	12.40 d
T_3	33.14 d	35.18 d	4.35 e	4.39 e	12.24 e	12.30 e
T_4	34.13 b	36.44 b	4.55 c	4.62 b	12.72 b	12.78 b
T_5	27.31 i	29.12 i	4.26 g	4.14 h	11.30 i	11.12 h
T_6	28.67 g	30.47 g	4.32 f	4.36 f	11.73 f	11.79 f
T_7	28.31 h	30.22 h	4.28 g	4.32 g	11.66 g	11.72 g
T_8	29.14 f	31.20 f	4.39 d	4.42 c	12.45 c	12.52 e
T_9	25.33 m	27.44 m	3.78 j	3.80 l	9.92 m	9.98 l
T_{10}	26.79 k	28.81 k	3.82 i	3.85 j	10.22 k	10.29 j
T_{11}	25.78 l	27.78 l	3.84 i	3.86 k	10.10 l	10.16 k
T_{12}	27.21 j	29.37 j	3.94 h	3.95 i	10.43 j	10.49 i
LSD (0.05)	0.012	0.021	0.016	0.019	0.020	0.020
PGPR	*	*	*	*	*	*
BGS	*	*	*	*	*	*
Drought	*	*	*	*	*	*
PGPR*BGS	*	*	*	*	*	*
PGPR*Drought	N.S	N.S	*	*	*	*
BGS*Drought	*	*	*	*	*	*
PGPR*BGS*Drought	N.S	N.S	*	*	*	*

Where T_0 = Control, T_1 = DrT, T_2 = DrT + P, T_3 = DrT + BGS, T_4 = DrT + P + BGS, T_5 = DrF, T_6 = DrF + P, T_7 = DrF + BGs, T_8 = DrF + P + BGS, T_9 = DrGF, T_{10} = DrGF + P, T_{11} = DrGF + BGS, and T_{12} = DrGF + P + BG, DrT = Drought at tillering, P = PGPR, BGS = Biogas slurry, DrF = Drought at flowering, and DrGF = Drought at grain filling

The maximum number of grains per spike (46.72 and 47.33) was obtained in the control treatment (T_0) followed by T_4 (44.75 and 45.54) and the lowest (34.61 35.37) was obtained in treatment (T_5) when drought occurs at flowering stage. PGPR and biogas slurry application significantly affect the spike length under drought conditions.

The control treatment (T_0) had the highest 1000-grain weight, grain yield, and biological yield (Table 2) in both growing seasons, which indicates that the application of drought stress negatively affected the wheat crop's growth and yield. The lowest values were recorded for the treatment that experienced drought stress during grain filling (T_9-T_{12}), indicating that this stage is the most sensitive to drought stress. The results also suggest that the application of PGPR and BGS had a positive impact on the wheat crop's growth and yield under drought-stress conditions. The treatments that received both PGPR and BGS (T_4 and T_{12}) had higher 1000-grain weight, grain yield, and biological yield than the treatments that received either PGPR or BGS alone.

4. Nutrient uptake

NPK and proline content uptake is significantly affected by the PGPRs and BGs (Table 3). Maximum nitrogen uptake (0.64 and 0.66 mg g^{-1}) is observed in treatment T_{12} followed by treatment T_9 (0.62 and 0.065 mg g^{-1}) when drought occurs at grain filling stage and lowest uptake (0.027 and 0.029 mg g^{-1}) was observed in control treatment T_0 during both years.

Table 3. Effect of PGPR and biogas slurry on wheat under drought.

Treatments	N-uptake (mg g^{-1})		P-uptake (mg g^{-1})		K-uptake (mg g^{-1})		Protein content (%)	
	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
T_0	0.027 j	0.029 k	1.87 a	1.89 a	5.90 ab	5.92 i	9.93 l	9.95 k
T_1	0.049 f	0.051 g	0.64 m	0.64 m	5.92 ab	5.93 h	10.76k	10.77 j
T_2	0.045 g	0.047 h	0.73 k	0.73 k	5.93 ab	5.94 gh	10.81 i	10.82 i
T_3	0.033 i	0.035 j	0.69 l	0.69 l	5.93 ab	5.94 g	10.80 j	10.82 i
T_4	0.048 e	0.050 f	0.78 j	0.78 j	5.94 ab	5.95 f	10.83 h	10.84 h
T_5	0.050 d	0.052 d	0.86 i	0.86 l	5.96 ab	5.97 e	11.21 g	11.22 g
T_6	0.047 e	0.049 f	0.92 g	0.92 g	5.97 ab	5.98 d	11.60 e	11.61 e
T_7	0.039 n	0.041 i	0.88 h	0.88 h	5.63 b	5.66 j	11.50 f	11.51 f
T_8	0.053 d	0.054 e	0.99 f	0.99 f	5.65 f	5.67 j	11.82 d	11.83 d
T_9	0.062 b	0.065 b	1.05 e	1.06 e	6.16 a	6.17	11.96 c	11.97 c
T_{10}	0.060 c	0.062 c	1.44 c	1.44 c	6.19 a	6.21 b	11.98 b	11.99 b
T_{11}	0.050 e	0.051 f	1.24 d	1.24 d	6.19 a	6.21 b	11.97 b	11.97 c
T_{12}	0.064 a	0.066 a	1.53 b	1.53 b	6.23 a	6.24 a	12.14 a	12.16 a
LSD (0.05)	0.121	0.023	0.011	0.012	0.246	0.0023	0.020	0.022
PGPR	*	*	*	*	*	*	*	*
BGS	*	*	*	*	*	*	*	*
Drought	*	*	*	*	*	*	*	*
PGPR*BGS	*	*	N.S	N.S	*	*	*	*
PGPR*Drought	*	*	*	*	*	*	*	*
BGS*Drought	*	*	N.S	N.S	*	*	*	*
PGPR*BGS*Drought	*	*	N.S	N.S	*	*	*	*

Where T_0 = Control, T_1 = DrT, T_2 = DrT + P, T_3 = DrT + BGS, T_4 = DrT + P + BGS, T_5 = DrF, T_6 = DrF + P, T_7 = DrF + BGs, T_8 = DrF + P + BGS, T_9 = DrGF, T_{10} = DrGF + P, T_{11} = DrGF + BGS, and T_{12} = DrGF + P + BG, DrT = Drought at tillering, P = PGPR, BGS = Biogas slurry, DrF = Drought at flowering, and DrGF = Drought at grain filling

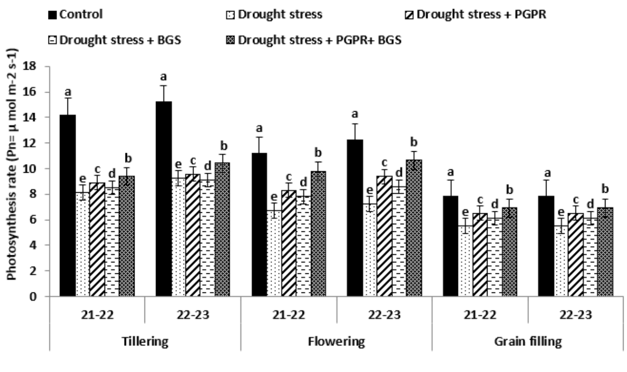


Figure 1. Effect of PGPR and BGs on Photosynthesis of wheat under drought

Maximum phosphorus uptake (1.87 and 1.89 mg g^{-1}) was observed in the control treatment followed by T_{12} (1.53 mg g^{-1}) and minimum P-uptake (0.64 mg g^{-1}) was noticed in treatment T_1 in both years 2021-22 and 2022-23. Maximum K-uptake (5.92 and 5.93 mg g^{-1}) was observed in treatment T_1 followed by treatment T_{12} (1.53 mg g^{-1}) and minimum (5.90 and 5.92 mg g^{-1}) was observed in control treatment in both years. Maximum protein content was observed in treatment T_{12} (12.14 and 12.16%) followed by treatment T_{10} (11.98 and 11.99%) and the lowest (9.93 and 9.95%) was observed in treatment T_0 during both years 2021-22 and 2022-23.

The statistics on the rate of photosynthesis are shown in Figure 1. All therapies were shown to be adversely impacted. During all drought phases, the maximum photosynthetic rate was seen in the control treatments, followed by the combination of BGs and PGPR. The least amount of dryness was seen during the grain-filling stage of growth. Also, it was discovered that PGPR inoculation yields better results than BGs application. The photosynthetic rate is lowest after being exposed to drought during all stages of plant development.

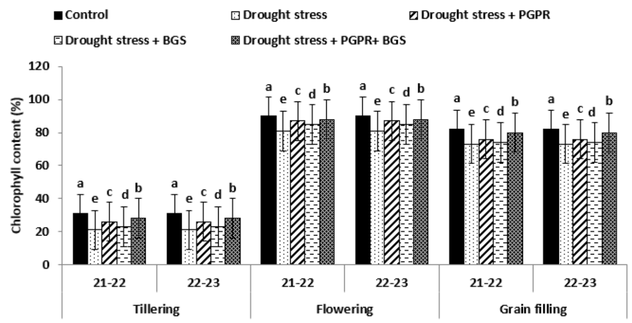


Figure 2. Effect of PGPR and BGs on Chlorophyll content of wheat

Figure 2 represents the data regarding chlorophyll content. It was observed that all treatments are significantly affected. Maximum chlorophyll content was observed in control treatment followed by PGPR + BGs application under drought stress conditions. In terms of drought at the growth stage minimum was noticed at tillering stage.

PGPR or BGs application significantly affects LAI in all treatments (Figure 3). It was noticed that the highest LAI was noticed in the control treatment followed by the PGPR + BGs application.

The lowest was noticed at drought stress treatments in all growth stages. Non-significant difference was noticed between only applied PGPR treatment and only BGs applied treatment under drought.

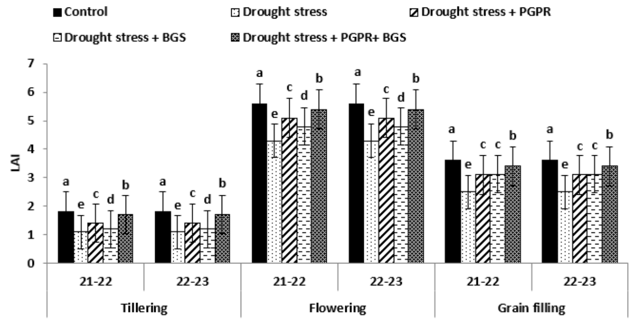


Figure 3. Effect of PGPR and BGs on leaf area index of wheat

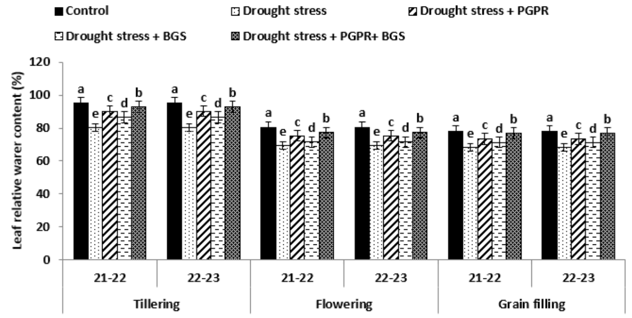


Figure 4. Effect of PGPR and BGs on leaf relative water content of wheat under drought

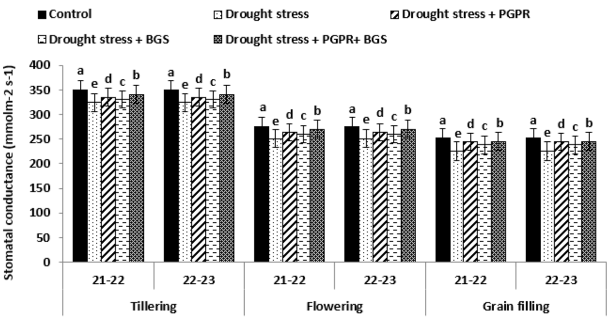


Figure 5. Effect of PGPR and BGs on stomatal conductance of wheat under drought

All treatments had a considerable impact on leaf relative water content (Figure 4) and stomatal conductance (Figure 5). Maximum relative leaf water content and stomatal conductance were observed in the control treatment, followed by the application of PGPR + BGs, and minimum values were observed in the drought treatment.

Data regarding antioxidant enzymes (Figure 6) shows that the activities of enzymes were significantly affected by the application of PGPR + BGs under drought stress. Maximum enzyme activities were noticed under drought conditions followed by when PGPR + BGs applied in the combined form under drought and the lowest was noticed under control treatment.

5. Discussion

Plant height is the utmost component that increases the biomass of plants. Shoot is the main part of the plant. Drought stress affects the plant height which ultimately decreases biomass production. Raza *et al.* (2017) reported that when drought occurs at the tillering stage it will affect plant height. 4.2, and 4.8% plant height was increased during 2021-22 and 2022-23 when both BGs and PGPR were applied. Spike length is an important component in the wheat plant as it contains grains in it. For measuring yield spike length is considered as an important component, increased spike length means more no of grains which results in increased yield. [24] reported that water stress decreased the spike length of wheat plants our findings conclude the same result 11, 9, and 6% lower spike lengths were observed during 2021-22- and 11.23, 9.5, and 5.5% were observed during 2022-23. But the combined application of PGPR + BGS under drought at all stages increases the spike length up to 15, 12, and 7% during 2021-22 and 14.5, 10.25, and 7.23% during 2022-23. The number of spikelets was affected by drought when occurring at the flowering stage (Dencic *et al.* 2000) but according to Raza *et al.* (2012), the number of spikelets was most affected when drought occurred at the grain filling stage. In our experiment minimum decrease of 17.25, 14.87, and 13.21% was observed during 2021-22 and 17.55, 14.74, and 13.33% was observed when drought occurred during 2022-23 at the tillering, flowering, and grain filling stage. The combined application of PGPR and BGs significantly increases the number of spikelets per spike.

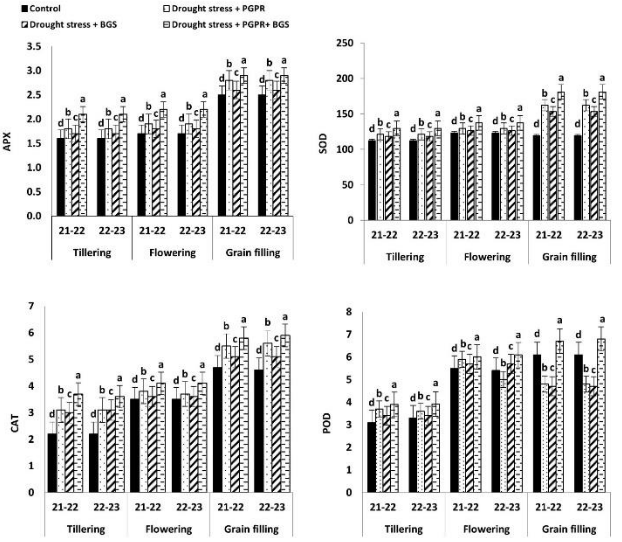


Figure 6. Effect of PGPR and BGs on antioxidant enzyme activities in wheat under drought

The number of grains per spike was significantly affected by BGs and PGPRs. A decline of 8.50, 27.22, and 19.81% in 2021-22 and 9.65, 26.72, and 19.27% in 2022-23 was seen due to the application of drought at all growth stages. The number of grains increased to 3.28, 18.56, and 13.20% in 2022-21 and 3.14, 18.11, and 12.73% when PGPR and BGs were used in combination at the same phases. 80% of PGPR create distinct plant hormones in the rhizosphere of several crops as secondary metabolites that stimulate plant development directly, and other studies have observed the same thing (Ahmad *et al.* 2014; Khalid *et al.* 2017; Elahi *et al.* 2023; Raza *et al.* 2023). 1000-grain weight is the important yield-related parameter in the final yield of the crop. When the grain weight is increased it will increase the crop yield. Maximum 1000 grain weight is obtained in control treatment followed by treatment T_4 when PGPR and BGs are applied in combination at the tillering stage. It was observed that 22% of grain weight is lost when drought occurs at the grain-filling stage. 1000-grain weight decreased by 9.41, 16.18, and 27.70% in 2021-22 and 9.31, 15.42, and 26.33% in 2022-23 when drought applied at all growth stages. The application of biogas slurry along with chemical fertilizer @50% each significantly enhanced the 1000-grain weight (Hussain *et al.* 2019).

Researchers in the field of agriculture are primarily concerned with grain yield per plant. Under drought stress conditions grain yield is decreased significantly. It was observed that maximum grain yield was obtained in the control treatment followed by treatment T_4. Grain yield per hectare was decreased to 9.09, 7.22, and 22.71% in 2022-13 and 9.04, 11.26, and 22.43% in 2022-23 when drought applied at all growth stages. More grain yield was recorded at 5.19% in 2020-21 and 5.07% in 2022-23 in treatment (T_{12}) by the combined application of PGPR and BGs under drought.

Biological yield (t ha^{-1}) was significantly affected by the combined application of PGPR and BGs under drought. It was observed that under drought treatments biological yield was decreased up to 10.39, 10.35, and 26.31% in 2021-22 and 10.31, 15.22, and 26.10% in 2022-23. But this reduction remained at 4.35, 6.64, and 22.35% in 2021-22 and 3.21, 6.64, and 22.21% in 2022-23. PGPR induces morphological and physiological changes in the root that improve water and nutrient uptake to promote plant development, increasing biological yield (Zahir *et al.* 2020). PGPR also speeds up the movement of many nutrients in the soil, makes more growth regulators, and improves the soil's structure (Bashan *et al.* 2014).

The plant's ability to absorb nitrogen is a crucial physiological factor in determining plant development. Nitrogen uptake in wheat is significantly affected by the application of PGPR and BGs. NPK and proline content uptake is significantly affected by the PGPRs and BGs (Table 3). Maximum nitrogen uptake (0.64 and 0.66 mg g^{-1}) is observed in treatment T_{12} followed by treatment T_9 (0.62 and 0.065 mg g^{-1}) when drought occurs at grain filling stage and lowest uptake (0.027 and 0.029 mg g^{-1}) was observed in control treatment T_0 during both years. Rhizobacteria that promote plant development transform air nitrogen into a form that is used by plants. Because PGPR converts nitrogen to ammonia via the enzyme nitrogenase (Bashan *et al.* 2014), seed inoculation with PGPR increases nitrogen availability and absorption (Salehi Gharaviran *et al.* 2014).

Plants only take up monobasic ($H_2PO_4^-$) and a dibasic (HPO_4^{-2}) ion from the soil, which is insoluble in P (Yazdani *et al.* 2009). Phosphorus is found as an inorganic mineral like apatite or in many organic forms like phosphodiester and phosphomonesters. PGPR changes complex forms into simpler forms that plants can use. In this study, we found that applying BGs also made P-uptake better. It's not possible to draw a clear conclusion about the role of BGs in P-uptake, so more research needs to be done on how BGs are used in P-uptake. Maximum phosphorus uptake (1.87 and 1.89 mg g^{-1}) was observed in control treatment followed by T_{12} (1.53 mg g^{-1}) and minimum P-uptake (0.64 mg g^{-1}) was noticed in treatment T_1 in both years 2021-22 and 2022-23 (Table 3). PGPR inoculation and BGs application significantly enhanced the P-uptake in wheat under drought.

Maximum K-uptake (5.92 and 5.93 mg g^{-1}) was observed in treatment T_1 followed by treatment T_{12} (1.53 mg g^{-1}) and minimum (5.90 and 5.92 mg g^{-1}) was observed in control treatment in both years. Maximum protein content was observed in treatment T_{12} (12.14 and 12.16%) followed by treatment T_{10} (11.98 and 11.99%) and the lowest (9.93 and 9.95%) was observed in treatment T_0 during both years 2021-22 and 2022-23 (Table 3). Sheng *et al.* (2005) said that PGPR breaks down nutrients into simpler forms that the plant can use. It also encourages the growth of new roots so the plant has a better chance of taking in potassium. The effect of BGs on K-uptake was not very big.

Protein contents were increased to 10.30, 16.42, and 23.12% in 2021-22 and 10.21, 16.41, and 22.87% in 2022-23 when drought applied at all growth stages (Table 3). This percentage increased to 9.88, 19.71, and 22.66% in 2021-22 and 9.79, 19.49, and 22.74% in 2022-23 when BGs and PGPR applied in combined form under drought at the same stages (Kuan *et al.* 2016). PGPR inoculations are a way to control plant aging and give plants a steady supply of N from the outside. PGPR keeps the plant's grain yield and protein content the same (Xie *et al.* 2004). CK increases the amount of protein in wheat by increasing the amount of growth hormones and nitrogen.

During all drought phases, the maximum photosynthetic rate was seen in the control treatments, followed by the combination of BGs and PGPR. The least amount of dryness was seen during the grain-filling stage of growth. Also, it was discovered that PGPR inoculation yields better results than BGs administration. The photosynthetic rate is lowest after being exposed to drought during all stages of plant development. The rate of photosynthesis went down as the leaves' water potential and relative water content went down (Keyvan, 2010). Inoculation with A. *brasilense* increases the amount of chlorophyll in the leaves, which in turn increases the rate of photosynthesis under water stress (Khalid *et al.* 2017). Figure 2 represents the data regarding chlorophyll content. It was observed that all treatments are significantly affected. Maximum chlorophyll content was observed in control treatment followed by PGPR + BGs application under drought stress conditions. In terms of drought at the growth stage, the minimum was noticed at the tillering stage. According to Gill and Tuteja, (2010) and Khakwani *et al.* (2013), the amount of chlorophyll in the plant's leaves went down due to drought. PGPR maintains plant water availability and improves soil fertility, which directly contributes to increased leaf area under drought stress, with higher leaf chlorophyll content (Müller *et al.* 2016; Delshadi *et al.* 2017). Gill and Tuteja, (2010) found that chlorophyll concentration dropped under water stress conditions due to reduced leaf area and the generation of reactive oxygen species, which killed chloroplasts.

The leaf area index was increased up to 10.30, 16.42, and 23.12% in 2021-22 and 10.21, 16.41, and 22.87% in 2022-23 when drought applied. This percentage was 9.88, 19.71, and 22.66% in 2021-22 and 9.79, 19.49, and 22.74% in 2022-23 when BGs and PGPR applied in combined form under drought at same. LAI is reliant on plant development, fertile soil, and enough water accessibility. In times of drought stress, PGPR stimulates plant growth to lower ethylene levels, preserve water availability, and enhance soil fertility (Müller *et al.* 2016; Delshadi *et al.* 2017). This directly contributes to an increase in leaf area index.

The application of PGPR and BGs significantly affects the transpiration rate. The transpiration rate decreased to 63.14, 65.32, and 48.59% in 2022-21 and 63.12, 65.89, and 51.13% in 2022-23 when drought applied at all growth stages. The transpiration rate was increased to 17.51, 14.51, and 0.18% in 2020-21 and 15.92, 13.91, and 0.19% in 2022-23 when BGs and PGPR were applied in combined form under drought at the same stages. Stomatal conductance decreased together with the rate of transpiration (Sheng *et al.* 2005). Under drought stress, PGPR increases root density and water intake, which improves the root's hydraulic characteristics and maintains the plant's water relationship (Raza *et al.* 2012). Leaf relative water contents were decreased up to 11.57, 14.09, and 12.06% in 2021-22 and 11.57, 14.09, and 10.09% in 2021-23 when water stress was applied at all three growth stages. And increase up to 3.07, 1.18, and 0% in 2021-22 and 3.01, 1.18, and 0% in 2022-23 when BGs and PGPR are applied in combined form under drought at the same stages. A key indication of plant water condition is RWC. According to Khakwani *et al.*, [40], a plant's root has a significant role in the water content of its leaves. Under water-limited conditions, deeper roots and a higher root density will draw more water from the soil. Root length and density are increased as a result of PGPR inoculation (Llorente *et al.* 2016). The synthesis of plant hormones like IAA by PGPR inoculation increases leaf water content. Stomatal Conductance (Figure 5) was decreased to 11.57, 14.09, and 12.06% in 2021-22 and 11.57, 14.09, and 10.09% in 2021-23 when water stress was applied. This percentage remained at 3.07, 1.18, and 0% in 2021-22 and 3.01, 1.18, and 0% in 2022-23 when BGs and PGPR applied in the combined form under drought at the same stages. The stomatal function serves as a bridge between the plant and the atmosphere, contributing significantly to how plants respond to environmental circumstances (Nilson and Assmann, 2007). Therefore, measuring stomatal function is crucial to understanding how plants respond physiologically to drought stress (Ryan *et al.* 2001). Plant hormone synthesis, such as the generation of IAA, increases stomatal conductance. PGPR inoculation enhances stomatal conductance, lateral root development, root growth, and water and nutrient absorption under drought (Arzanesh *et al.* 2011). Wheat (Triticum aestivum) is prone to the build-up of reactive oxygen species (ROS) that are produced when the plant experiences drought stress (Abid *et al.* 2018; Hasanuzzaman *et al.* 2020). Elevated levels of reactive oxygen species (ROS) lead to oxidative stress, which in turn stimulates the production of antioxidants such as superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione reductase (GR), and proline to mitigate water stress (Ahmad *et al.* 2023; Ahmed *et al.* 2023).

PGPR (Plant Growth Promoting Rhizobacteria) and biogas slurry are two potential sources that can enhance plant growth and yield, especially under stress conditions. The impact of PGPR and biogas slurry on the antioxidant enzyme activity of wheat under drought has been investigated by several studies. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) play a crucial role in scavenging reactive oxygen species (ROS) generated during stress conditions like drought. PGPR can induce the production of these enzymes, leading to better ROS scavenging ability, and consequently, better plant growth and yield. Similarly, biogas slurry contains essential plant nutrients, organic matter, and beneficial microorganisms that can improve soil fertility, water-holding capacity, and plant growth. Kasim *et al.* (2012) reported that drought stress raises the activity of many enzymes (APX, DHAR, MDHAR, and GR) that are involved in the ascorbate-glutathione redox cycle. Our data revealed that drought generally increased the levels of antioxidant enzymes as compared to control in wheat. Such enzymatic changes might be due to ROS overproduction and a heightened antioxidant defense system (Almeselmani *et al.* 2006; Raza *et al.* 2020). Accordingly, PGPR and biogas slurry application further increased wheat antioxidant enzymes under drought (Figure 6): POD at 27 and 26%, CAT at 19 and 35%, APX at 28 and 14%, and SOD at 33 and 43%, at GFS with combine application of PGPR and biogas slurry respectively, over respective control treatment. Enhancing the activity of catalase (CAT) and peroxidase (POX) in plants is a crucial strategy to mitigate the detrimental effects of water stress on wheat (Mosalem *et al.* 2021). A study conducted by Hassan *et al.* (2018) concluded that PGPR and biogas slurry could enhance the antioxidant system of wheat plants, leading to better tolerance to drought stress. Application of PGPR and biogas slurry significantly increased the activity of SOD, CAT, and POD enzymes, leading to better growth and yield of wheat plants (Yadav *et al.* 2020). The study suggested that PGPR and biogas slurry could be used as a potential strategy to mitigate the adverse effects of drought stress on wheat plants.

6. Conclusions

Our study found that drought stress has a significant impact on wheat physiology and yield-related parameters at any growth stage. However, we also found that soil application of biogas slurry and seed inoculation with PGPR can significantly enhance the potential of wheat to withstand drought by improving water relations, photosynthesis, stomatal conductance, and leaf area index. These improvements also increase nutrient availability, leading to an increase in grain yield. Therefore, it can be concluded that the application of biogas slurry and PGPR seed inoculation can effectively improve wheat's ability to withstand drought and increase its yield potential.

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