



INTERACTION OF PSEUDOMONAS AND AZOSPIRILLUM SPECIE WITH WHEAT PLANT ROOTS TO ENHANCE PLANT PRODUCTIVITY AGAINST DROUGHT STRESS

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ABSTRACT

Wheat (*Triticum aestivum* L) is regarded as the main staple crop of Pakistan. Drought is one major environmental stress that badly affects crop production. Plant-growth-promoting rhizobacteria are proven to increase crop productivity and plant resistance. The zone of continuing interaction between soil bacteria and plant roots is known as the rhizosphere. Plants draw rhizosphere microorganisms to accumulate in the internal and surface tissues of their roots through root exudates. Numerous of these microbes, often referred to as plant growth promoting rhizobacteria, support plant growth by a variety of direct and indirect procedures such as biological nitrogen fixation, nutrient solubilization, and disease management. A field experiment was conducted at the Agronomic research area, Faculty of Agriculture and environment, The Islamia University of Bahawalpur. Crop was sown on 21st November 2022 under normal management practices. However, drought was created at tillering and anthesis stage and *Pseudomonas* and *Azospirillum* both are applied alone and in combination. The treatments included T₀= Control, T₁= Drought + *Azospirillum*, T₂= Drought + *Pseudomonas*, T₃= Drought + *Azospirillum* + *Pseudomonas*. The experiment was held out in randomized complete block design (RCBD) with three replications having a net plot size of 5m*3m. Fisher's analysis of variance was used to statistically analyze all the acquired data at a 5% probability level. Findings of the present study indicated that plant height (101.87cm), number of fertile tillers (360.00m⁻²), spike length (11.8cm), number of grains spikes (46.00⁻²), number of spikelet spikes (20.993⁻²), grain weight (40.00g), biological yield (13.407 t ha⁻¹), harvest index (34.987%), 1000-grain protein contents (40.00g), grain yield (5.63 t ha⁻¹), grain nitrogen contents (17.36%), grain phosphorus contents (3.7567%), grain potassium (4.25%), total chlorophyll contents (46.500mg g⁻¹), osmotic (0.77 MPa) and water potential (0.5 MPa) were significantly increased in Drought + *Azospirillum* + *Pseudomonas* group as compared to control group. Finally, it was concluded that wheat growth is significantly impacted by drought stress. However, when *Azospirillum* and *Pseudomonas* are used correctly, wheat becomes more drought-tolerant and Plant Growth-Promoting Rhizobacteria (PGPR) can decrease the impact of drought stress and increase crop yield.

Keywords: drought stress, growth, plant roots, wheat

INTRODUCTION

Wheat, being among the top three most widely grown crops globally after maize and rice, is considered a staple food for humans. It is the third-largest crop in the world and a crucial source of carbohydrates for millions of people. However, wheat production faces challenges, especially in saline soil, leading to decreased yields due to improper plant nutrition, drought,

and osmotic stress (Ansari *et al.*, 2021). In the context of the global population explosion, food production has emerged as a critical concern for the 21st century. Pakistan, being among the top 10 wheat producers globally, faces challenges in meeting the demands of its populace, given that wheat constitutes a significant portion of the daily diet. Punjab, Sindh, Baluchistan, and Kheybar Pakhtunkhwa are Pakistan's main wheat-producing areas (Fioreze *et al.*, 2020). Even if intensive agriculture techniques increase output, the use of chemical fertilizers puts

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human health and the environment at danger. These difficulties are made worse by environmental factors, especially drought, which lowers agricultural productivity (Ahmad *et al.*, 2021). Osmotic stress brought on by drought, a result of climate change, prevents cell development and division. Furthermore, plant development is further hindered by oxidative stress caused by reactive oxygen species during drought (Akhtar *et al.*, 2021). In response to these worries, the effectiveness of *Pseudomonas* inoculants has been evaluated in a number of commercially relevant crops, including wheat, beans, peanuts, soybeans, and soybean oil, which has improved yield and growth (Diaz *et al.*, 2022). It is well known that certain strains of *Pseudomonas* exhibit fast growth, genetic diversity, and metabolic flexibility. In the rhizosphere of plants, several species of pseudomonads flourish, often attaining high densities of 10⁸ CFU/g of roots for crop species like maize or soybeans in field settings (Alam *et al.*, 2021). These rhizospheric pseudomonads defend roots from fungal infections, among other probiotic processes that promote plant development and health. *Pseudomonads*' physiological, metabolic, and genetic characteristics have been extensively studied due to their comparatively simple isolation and development in lab settings (Hakim *et al.*, 2021). According to statistical calculations, rising temperatures and dwindling water supply have resulted in a 5.5% drop in wheat yield worldwide, which has a substantial effect on cereal exports. Consequently, in the face of inadequate moisture and antiquated methods, creative approaches are essential to boosting agricultural output (Kabiraj *et al.*, 2020). Drought intensity and length are predicted to increase globally, placing more pressure on agricultural production. Drought effects differ depending on the stage of crop growth; pre-flowering drought stress in wheat causes a delay in flowering. One major environmental stressor that affects plant development and metabolic processes is drought (Karimzadeh *et al.*, 2021). Osmotic and oxidative stress are examples of secondary stressors that intensify the overall impact on plants (Sood *et al.*, 2020). The nitrogen-fixing bacteria *Azospirillum* is essential for enhancing soil fertility and assisting plants in withstanding abiotic stress. It compensates for ionic, osmotic, and oxidative stressors brought on by salt and drought by transforming atmospheric nitrogen into a form that plants can utilize. Plant failure

may eventually arise from these connected stressors, which are often brought on by salt and drought (Khan *et al.*, 2021). Additionally, drought can curtail grain filling time and ultimately reduce yield. The hormone auxin plays a pivotal role in plant growth, and its application through the soil aids in nutrient uptake. Plant Growth-Promoting Rhizobacteria (PGPR) contributes to a healthy rhizosphere, thereby supporting plant growth. Abiotic stresses, such as heavy metal presence, nitrogen deficiency, drought, and salinity, all contribute to challenges in agricultural production (Zaib *et al.*, 2023).

The objective of the research is:

- Assess and evaluate the impact of PGPR (*Pseudomonas* + *Azospirillum*) on the Morphological and physiological character of wheat and the production potential of wheat under Drought stress.
- To assess the effect of growth regulators on the quality and quantity parameters of wheat.

MATERIALS AND METHODS

The proposed study was conducted at the research area, Department of Agronomy, The Islamia University of Bahawalpur Nov 2021-April. The experiment is performed in Randomize Complete Block Design (RCBD) in three replications. Seed of genotype will be collected from the Punjab Seed Corporation Department Bahawalpur.

Climatic data

Data of environmental variables such as rainfall, temperature during the research trial is as following.

Temperature

Minimum and maximum temperature recorded during the whole research Nov 2021- April 2022. Below, you will find the information pertaining to temperature.

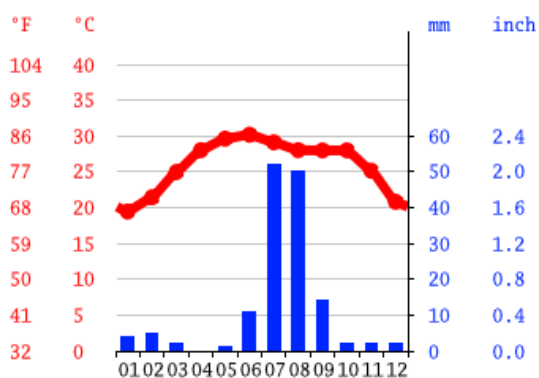
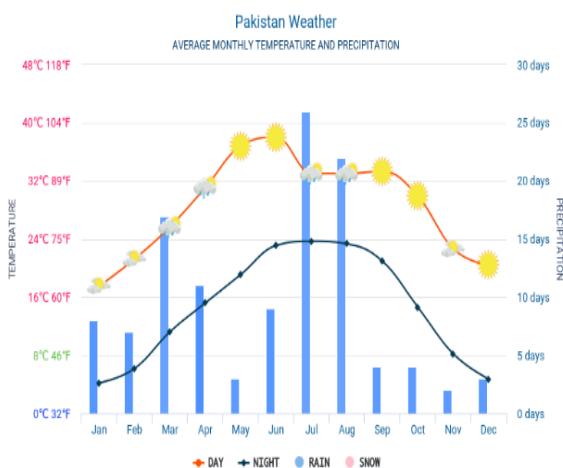
Variety and sowing date

Aas-2011 were used planted on 21st November 2021 drought was given artificially in control all irrigation as per needs were given but drought irrigation was stopped after 1st three irrigation.

Rainfall

The number of rainfalls during the entire research mid of November 2021-April 2022. Highest rainfall recorded in the month of February and lowest rainfall also we can say no

rainfall recorded in the month of December and January.



Irrigation

Total 3 irrigations were applied
 First irrigation was applied at 21st November 2021
 Second irrigation was applied at December 21, 2021
 Third irrigation was applied at January 15, 2022
 First irrigation (pre-drought stress): 600m³ ha⁻¹
 Second irrigation (initiation of drought stress): 300m³ ha⁻¹
 Third irrigation (maintaining drought stress): 300m³ ha⁻¹

Method of irrigation

Drip irrigation method was used to irrigate plants.

Soil analysis

A soil analysis of the experimental soil was done before the wheat crop was sown. With the use of an auger, soil samples were obtained at depths of 0-15cm and 16-30cm. Sample was collected, placed in a polythene bag, and then moved to the RARI (Regional Agricultural Research Institute) Bahawalpur.

T.S.S	0.81	0.11
Available-P	7.1	5.2
Available-K	113	111
Saturation Percentage	35	35
Soil separates and Percent	39	37
Silt Percent	40	37
Clay Percent	22	27
Textural	Loam class	Loam class

Net plot size

The area of a rectangular plot of land is determined by multiplying its length by its width. For example, if a plot measures 5 feet long and 3 feet wide.

Plant height

Plant height, leaf size and spikes length were measure by measuring tape. ut of each plot, ten plants were randomly chosen. At maturity, plant heights were measured using a measuring Tape; their averages would be calculated.

Number of fertile tiller m⁻²

Spike length

Ten randomly chosen plants from each plot were measured for their spike length from the base to the top that used a foot rod, and an average was calculated.

Number of spikelets spike⁻¹

From each plot ten plants were randomly chosen and counted the number of spikelets from the spike. It was calculated the average number of spikelets per spike. the number of spikelets from the spike was measured at maturity stage.

Number of grains spike⁻¹

From each plot, ten plants were picked at random, and each spike was manually separated and threshed. Calculations and averages were made for the number of grains from each spike.

1000-grain weight (g)

Counted and separated thousands of grains while threshing the grain at maturity stage. Use an electric balance to weigh them. We computed the weight in grains.

Grain yield (t ha⁻¹)

Each plot's one m portion was chosen, and it was harvested. Manually threshed, grain weight taken using an electronic scale, and grain yield converted to t ha⁻¹.

Biological yield (t ha⁻¹)

It was measured at maturity stage. Plant samples from 1 m² were harvested from every plot. Use an electronic scale to weigh it and convert the biological yield to t ha⁻¹.

Harvest index (%)

Harvest index was measured at maturity stage under drought stress and calculated as the ratio of grain yield to total (above ground) biological yield using the formula:

$$\text{Harvest Index} = \frac{\text{Grain Yield}}{\text{Total Biological Yield}} \times 100$$

Determination of total chlorophyll content

The method developed by Nagata and Yamashta was used to determine the amount of chlorophyll in plant leaves (1992).

Reagents required

Hexane
Acetone

Procedure

Extraction of chlorophyll

Chlorophyll content was measured at flowering stage. One gram of plant material was thoroughly homogenized in a mortar and pestle with an acetone-hexane solution (4:6 ratio) to remove the chlorophyll content. The resulting combination will next undergo a 10-minute centrifugation process in a Clandon T53 centrifuge at 3000 rpm.

Measurement of chlorophyll

The supernatant will be taken in cuvette and the absorbance will be measured at 663 nm and 645nm by spectrophotometer using 80% acetone as blank.

Calculations

Following equations was used for the calculations of chlorophyll a and b (mg/100ml).
Total Chlorophyll (mg g⁻¹) = $8A_{663} + 20A_{645}$
Chlorophyll a (mg g⁻¹) = $0.999 A_{663} - 0.0989A_{645}$.

Grain protein content

Grain protein content was measured at the maturity stage. By applying Kajeldahl's approach, the protein content of grains was determined. Protein contents were calculated using the method for determining the nitrogen content in grains by which involved multiplying the nitrogen content by a factor of 5.70. (1966; Tkachuk)

Grain nitrogen content (mg g⁻¹ Dw)

Grain Nitrogen content was measured at the maturity stage. Took 0.1g of dried, powdered wheat in a digestion tube. Each tube with 5ml of pure H₂SO₄. They were left at room temperature for the night. The sides of the digesting tube were injected with 1ml of H₂O₂ (35% concentration). The tubes were placed in a digesting block and heated to 350°C until fumes began to emerge began to come out. Once more, keep heating it for 30 minutes. The digestion tube was then removed from the block, allowed to cool, and 1ml of H₂SO₄ the tubes were reinserted into the digesting block after the addition. The selected digestion material is constantly put through these procedures until it loses its colour. 50ml of the extract were prepared in volumetric flasks. The extract was purified using Kajeldahl's method and then used for calculating nitrogen content.

Grain phosphorus content

Grain phosphorous content was measured at the maturity stage. 50mL of a volumetric flask containing 5ml of sample was used. distilled water was used to get the volume up to the required level after adding 10mL of Barton reagent. KH₂PO₄ was used to create volume, and to make the standards, 10mL of Barton reagents were combined with distilled water. This sample was left for a short while so the colors could develop. By using a standard curve and a spectrophotometer at 420nm, phosphorus was determined.

Grain potassium content

Grain potassium content was measured at the maturity stage. Took 0.1g of dried, powdered wheat in a digestion tube. Each tube with 5ml of pure H₂SO₄. They were left at room temperature for the night. The sides of the digesting tube were injected with 1ml of H₂O₂ (35% concentration). The tubes were placed in a digesting block and heated to 350°C until fumes began to emerge began to come out. Once more, keep heating it for 30 minutes. The digestion tube was then removed from the block, allowed to cool, and 1ml of H₂SO₄ the tubes were reinserted into the digesting block after the addition. The selected digestion material is constantly put through these procedures until it loses its colour. 50ml of the extract were prepared in volumetric flasks. The extract was purified using Kajeldahl's method and then used for calculating nitrogen content.

Determination of water potential

Water potentials have been measured on the same leaf or plant using a pressure chamber, a sample dew-point hygrometer and an in-situ hygrometer. Relatively good agreement was found in plants with zero or low transpiration, but results diverged widely when transpiration was high. Leaf water potential measurements are easily and accurately obtained using the chilled-mirror dew point technique of the WP4C. The recommended procedure involves the abrasion of the leaf cuticle to speed equilibration. Essentially, there are only two primary measurement methods for water potential tensiometers and vapor pressure methods. Tensiometers works in the wet range special tensiometers that retard the boiling point of water (UMS) have a range from 0 to about -0.2 MPa. Vapor pressure methods work in the dry range from about -0.1 MPa to -300 MPa. Both methods were used as per requirement. It was measured at the Flowering stage.

Determination of osmotic potential

The value of the osmotic potential can be determined using the Van't Hoff equation:

$$\Psi_s = -CiRT$$

Where: C is the molar concentration of the solutes (molarity = moles L⁻¹), i is the osmotic coefficient (the value of i is 1 for molecules that do not dissociate in solution (sucrose) and can be 2 or more for molecules. It was measured at Flowering stage.

Water analysis

Before the sowing of wheat water testing was also held. The crop was irrigated using water from a tube well. The crop was irrigated with tube well water. Water was put into plastic bottles, which were then marked. transferred these bottles to the Regional Agricultural Research Institution (RARI) Bahawalpur's soil and water testing lab. Information regarding the analysis of water is available on this platform.

PGPR inoculation method

PGPR (*Pseudomonas* and *Azospirillum*) was applied through the process of seed priming. The concentration used was 10⁸ CFU/ml. The seeds were soaked for 24 hours in the PGPR solution before sowing.

Statistical analysis

The data was analyzed using the least significant difference (LSD) test at a 5%

probability level to compare the treatment means with the results of the analysis of variance method.

Characteristics	Value
EC	1103
pH	7.4
Ca ²⁺ + Mg ²⁺ (milli equivalent per liter)	7.9
Na ⁺ (milli equivalent per liter)	3.12
CO ₃ ²⁻ (milli equivalent per liter)	Nil
HCO ₃ ⁻ (milli equivalent per liter)	5.3
Cl ⁻ (milli equivalent per liter)	3.02
SO ₄ ²⁻ (milli equivalent per liter)	1.03
SAR (milli mol liter)	1.56
RSC (milli equivalent per liter)	Nil

RESULTS

Plant height of wheat which showed a non-significant effect of interaction of PGPR with wheat crop under drought and normal condition. Three different treatments were performed compared to T₀ (Control) group which showed normal growth and plant gained normal height (Table 1). T₁ group resulted in the increased growth as compared to control. As in T₁ (100.32 cm), there was a small change in mean values that means there was an increased growth. While the results of treatment T₂ showed the maximum growth (102.33cm). Number of Fertile Tillers m⁻² of what showed a significant effect of PGPR on wheat plant's fertile tiller. T₁ showed the greater (340.50m⁻²) mean values as compared to control (320.00m⁻²) and T₃ appeared with greater mean values (360.00m⁻²) as compared to T₂ means that it gained a maximum height and growth. Spike Length of what showed a significant impact of PGPR on the length of the spikes on wheat plants under drought stress. Minimum mean values were recorded in control group (9.00cm) whereas maximum value was recorded in T₃ (11.8cm). Number of Grains per spike of wheat crop under drought stress was also recorded and control group (35.16 GNS) showed normal growth and number of grains per spike. T₁ resulted in the increased as compared to T₂. While the results of T₃ (46.00 GNS) showed the maximum significant results in grain per spike. Number of Spikelet spike⁻¹ of wheat results under drought and control conditions. Maximum values were shown at (Control and compared to that T₁ showed larger values. T₂ group shown a somewhat decreased number of spikelets. Compared to all, T₃ group showed the maximum values. In case of grain weight spike length of wheat crop under drought stress, all applied treatments showed significant effect on grain

spike length than control treatment under drought condition. The treatments included were Control, in which the crop showed its proper growth, and 1000-grain weight was also maximum. Upon second treatment T₁ the mean values appeared were larger than control that means the crop showed its increased growth i.e, greater 1000 brain weight as well. In T₂ group greater mean value was observed as compared to T₀ and T₁ T₃ groups revealed the maximum (40.83g) grain weight.

Wheat crop biological yield under drought stress was observed and the mean values of yield were increased as treatments were performed from control group (4.61 t ha⁻¹) followed by T₁ (11.247 t ha⁻¹), T₂ (12.5 t ha⁻¹), T₃ (13.407 t ha⁻¹). The positive impact of PGPR Pseudomonas and Azospirillum on harvest index (%) of wheat under drought conditions was observed (Table 2). Grain protein content of wheat crop under control and drought stress conditions was checked and results revealed that all the findings of T₃ (14.23%) represents the maximum growth of the crop. Different treatments were performed compared to the control (4.61 t ha⁻¹) group which showed normal growth and grain yield of the wheat crop under normal conditions. While the results of treatment T₃ (showed the maximum growth (5.63 t ha⁻¹).

As concerned the grain nitrogen content of wheat crop under control and drought stress conditions, three different treatments were performed. Control group (13.66%) showed normal growth and grain nitrogen content. T₁ group resulted in the increased growth of plant followed by T₂ there was a small change in mean values that means there was an increased growth. While the results of treatment T₃ (17.36%) showed the maximum significant results. Maximum phosphorous content was showed in T₃ (3.756%). In case of grain potassium of wheat crops during drought stress, control (1.54%) showed the normal growth of crop. While the results of T₃ group (4.25%) showed the maximum growth (Table 3).

The total Leaf Chlorophyll content in wheat crops under drought stress in the maximum mean values were displayed in T₂ group (46.867) whereas in T₃ shows a slight difference (46.500) as compared to T₂. The effect of PGPR Azospirillum and Pseudomonas on Osmotic potential of wheat crops under drought stress. T₁ group showed the greater value (0.9167 MPA) as compared to T₂ (0.77 MPA). Wheat plant's water potential was increased in T₂ (0.5 MPA) as compared to T₀ (Control) (0.25 MPA) (Table 4).

Table 1. Impact of Azospirillum and Pseudomonas on plant height (cm), Number of fertile tillers m⁻², Spike length (cm), Number of grains spike⁻², Number of spikelet spike⁻², 1000-Grain weight (g) index of wheat crop under drought stress

Treatment	Plant height (cm)	Number of fertile tillers m ⁻²	Spike length (cm)	Number of grains spike ⁻²	Number of spikelet spike ⁻²	1000-Grain weight (g)
T ₀ = Control	100.84 c	320.00 d	9.007 d	35.16 d	15.91 d	35 d
T ₁ = Drought + Azospirillum	100.32 d	340.50 c	9.773 c	39.00 c	18.78 b	37 c
T ₂ = Drought + Pseudomonas	102.3 a	350.33 b	10.94 b	41.33 b	18.3 c	39 b
T ₃ = Drought + Azospirillum + Pseudomonas	101.8 b	360.00 a	11.8 a	46.00 a	20.993 a	40.83 a

Means not sharing a common letter differ significantly at 5% level of probability

Table 2. Impact of Azospirillum and Pseudomonas on biological yield (t ha⁻¹), Harvest index (%), Grain protein content, Grain yield (t ha⁻¹) index of wheat crop under drought stress

Treatment	Biological yield (t ha ⁻¹)	Harvest index (%)	Grain protein content (%)	Grain yield (t ha ⁻¹)
T ₀ = Control	4.61 d	30.363 d	9.9 d	4.61 d
T ₁ = Drought + Azospirillum	11.247 c	33.177 b	10.31 c	5.06 c
T ₂ = Drought + Pseudomonas	12.517 b	33.137 c	13.67 b	5.07 b
T ₃ = Drought + Azospirillum + Pseudomonas	13.407 a	34.987 a	14.237 a	5.63 a

Means not sharing a common letter differ significantly at 5% level of probability

Table 3. Impact of Azospirillum and Pseudomonas on grain nitrogen content, Grain phosphorus content, Grain potassium index of wheat crop under drought stress

Treatment	Grain nitrogen content (%)	Grain phosphorus content (%)	Grain potassium (%)
T ₀ = Control	13.66 d	2.9233 d	1.54 d
T ₁ = Drought + Azospirillum	14.48 c	3.1733 c	3.5033 c
T ₂ = Drought + Pseudomonas	14.99 b	3.4333 b	3.8967 b
T ₃ = Drought + Azospirillum + Pseudomonas	17.36 a	3.7567 a	4.25 a

Means not sharing a common letter differ significantly at 5% level of probability

Table 4. Impact of Azospirillum and Pseudomonas on Total Leaf Chlorophyll content, Osmotic potential, Water potential index of wheat crop under drought stress

Treatment	Total leaf chlorophyll content (mg g ⁻¹)	Osmotic potential (MPa)	Water potential (MPa)
T ₀ = Control	35.543 d	0.6867 c	0.25 c
T ₁ = Drought + Azospirillum	45.743 c	0.9167 a	0.2767 b
T ₂ = Drought + Pseudomonas	46.867 a	0.77 b	0.5 a
T ₃ = Drought + Azospirillum + Pseudomonas	46.500 b	0.3533 d	0.04 d

Means not sharing a common letter differ significantly at 5% level of probability

DISCUSSION

According to our findings Azospirillum and Pseudomonas had positive effect under the draught condition. Findings of the present study indicated that plant height, number of fertile tillers, spike length, number of grains spikes, number of spikelet spikes, grain weight, biological yield, harvest index, grain protein contents, grain yield, grain nitrogen contents, grain phosphorus contents, grain potassium, total chlorophyll contents, osmotic and water potential were significantly increased in T₃ group (Drought + Azospirillum + Pseudomonas) as compared to control group.

This outcome is comparable to that of an experiment conducted by Reyes *et al.* (2020), in which the maximum plant height was recorded in the control treatment during the tillering stage, a water deficiency was observed because of the drought. It is important to note that the drought did not occur during any other growth stage. Analysis of data regarding the quantity of productive tillers per square meter revealed that the significant variations previously observed in the wide range of tillers per square meter were no longer present when drought conditions coincided with exceptional growth levels. However, significant disparities were still evident when different nitrogen doses were combined with PGPR (Lin *et al.*, 2020). Longer spikes with more spikelets ultimately lead to higher grain productivity. According to observations, PGPR (Pseudomonas and Azospirillum) can successfully lengthen spikes when drought stress circumstances are met (Wittern *et al.*, 2022). The number of spikelets per spike significantly decreases by around 28% during the inadequate rainfall-characterized grain filling and anthesis phases. According to research by Chowdhury *et al.* (2021), there is a significant interaction between drought and PGPR (Plant Growth Promoting Rhizobacteria) throughout the drought phase. A crucial component of wheat development is the quantity of grain per spike, which is significantly impacted by drought. However, as Thakur *et al.* (2023) show,

researchers have found that appropriate use of PGPR may increase spike counts even in dry situations. Assessing the weight of a thousand grains offers significant understanding of the degree of grain formation, which is critical to the growth and quality of the wheat crop and directly related to the profitability of wheat production. According to Mitura *et al.* (2023), inoculation with Azospirillum significantly enhanced total productivity during drought conditions. Drought during the grain filling stage was shown to reduce grain output by 36%. Grain production is greatly increased by inoculating wheat seeds with Plant Growth-Promoting Rhizobacteria, as shown by Zahra *et al.* (2021). According to Usman *et al.* (2023), biological yield is the total biomass generated by a plant over its lifespan that is attained by effectively using the resources that are available. According to Wieser *et al.* (2023), protein concentration is crucial for wheat grain quality and is regulated by nitrogen and enzymatic availability. According to Vafa *et al.* (2021), the application of PGPR has a considerable impact on grain nitrogen content, with strong impacts reported when paired with other parameters. Nowadays, inoculation with rhizospheric soil microorganisms, namely plant growth promoting rhizobacteria (PGPR), became a fastest and recommended practice to sustain crops growth and productivity, especially in stress condition soils (Bargaz *et al.*, 2021, Notununu *et al.*, 2022). Moreover, the application of PGPR has a significant effect on grain phosphorus content; it increases noticeably in comparison to the control group, reaching 3%, as reported by Sedri *et al.* (2022). Increased formation of reactive oxygen species (ROS) during dry spells might negatively affect photosynthetic pigment activity and composition (Bukhari *et al.*, 2021). This could lead to a decrease in the amount of chlorophyll in leaves. Temperature variations in conjunction with shifts in water potential have a big effect on seed germination (Saeed *et al.*, 2022). When PGPR, especially Azospirillum and Pseudomonas, are used appropriately, wheat plants become more drought-tolerant and produce more grain than

when they are left untreated. Recently, application of more than two microbial inoculants, is gaining huge interest as it was reported to be more significant in promoting crops growth compared to single inoculation (Elhaissofi *et al.*, 2022). Rhizobacteria increase plant growth and productivity by one or more mechanisms. In this context, P-solubilizing bacteria (PSB) promote specific mechanisms to improve P uptake by the plant. Secretion of protons, organic acids, hydrogen cyanide (HCN), and phosphatases are among the main mechanisms that increase solubilization and mineralization of insoluble forms of P (Elhaissofi *et al.*, 2020, Ibyasser *et al.*, 2024, Benmrid *et al.*, 2024).

CONCLUSION

Wheat growth is significantly impacted by drought stress. However, when *Azospirillum* and *Pseudomonas* are used correctly, wheat becomes more drought-tolerant and produces more crops than it would if it weren't treated. When both PGPR kinds are used together, the outcomes are better than when they are applied separately.

AUTHOR'S CONTRIBUTION

A. Shakoor: Conceived and designed the experiments and wrote the manuscript.

A. S. Raza: Performed the experiments.

M. Hameed: Contributed materials.

R. Jameel: Research drafting.

M. M. Ahmad: Analysed the data.

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(Received: June 25, 2024; Accepted: August 09, 2024)