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Research Article

Seed priming with bacterial and fungal biocontrol agents alter physio-chemical parameters to suppress leaf rust disease in bread wheat

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ABSTRACT

Wheat (*Triticum aestivum* L.), a staple crop in Pakistan and the third most cultivated crop globally suffers yield losses of up to 60-70% due to leaf rust (*Puccinia triticina*). This study explored sustainable management strategies using biocontrol agents (*Trichoderma sp.* and *Bacillus sp.*) by seed priming across four different wheat varieties i.e., Arooj-22, Subhani-21, Punjab-11, and Shafaq-06. The experiment was conducted using a Complete Randomized Block Design (RCBD). Morphological parameters, including fresh and dry shoot/root weights, spike length, number and weight of seeds per spike, yield per plant, 1000-grain weight, shoot/root length, plant height, leaf area, peduncle length and awn length were assessed alongside physiological parameters such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glycine betaine, chlorophyll content and free proline levels. Statistical analysis (ANOVA and LSD at 5% probability) revealed that Subhani-21 primed with *Trichoderma sp.* exhibited the best results by demonstrating reduced oxidative stress (lower MDA and H_2O_2 levels) and improved chlorophyll, glycine betaine, and free proline levels. These results highlighted the potential of eco-friendly treatments to mitigate leaf rust stress and enhancement of wheat growth and productivity.

Keywords: Wheat, Leaf rust, *Trichoderma* sp., *Bacillus* sp., Physio-chemical parameters

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INTRODUCTION

Being an agricultural country, Pakistan meets the food requirements through its crop resources. Among different crops, wheat has the pivotal position both in terms of production and total area which is being utilized for the cultivation of this major cereal in Pakistan (Khan *et al*., 2021). Wheat contains various vitamins along with zinc and fiber (Bird *et al*., 2018). Pakistan produced about 27 million tons of wheat by using 9.2 million hectares area in 2021-22 (FAO, 2022). Overall, many strategies have been made to enhance the production of wheat, but biotic and abiotic factors are still the main issues to achieve targeted production (Jabran *et al*., 2023). Different pathogens like fungi, nematodes, and bacteria cause severe crop losses in wheat (Martens *et al*., 2014), out of which fungal pathogens dominate significantly. Leaf rust caused by *Puccinia triticina* is one of the most severe fungal diseases in all wheat growing areas of Pakistan with special reference to plain areas (Din *et al*., 2017). It causes 5-20% yield losses which extend up to 50% during epidemics (Eversmeyer and Kramer, 2000; Ren *et al*., 2023). In 1978 leaf and stripe (*Puccinia striiformis*) rusts caused historical losses in wheat growing areas of Pakistan (Anonymous, 2000). Leaf rust is always a major threat to wheat yield (Afzal *et al*., 2020) and needs to manage in an effective way to retain wheat production level (Din *et al*., 2017). New pathogenic strains have the capability to surpass natural plant resistance (Draz *et al*., 2019).

On the other hand, application of fungicides has limited utilization as these synthetic chemicals have hazardous effects on the environment. Seed priming is an effective way to improve crop performance and disease management because it is efficient, easy to use and cost-effective. (Farooq *et al*., 2019). Major aspects of seed priming include, better seedling emergence, higher yields (Singh *et al*., 2015) and tolerance to both biotic and abiotic stresses (Arshad *et al*., 2022; Harris *et al*., 2007). Beneficial and environment-friendly microbial bio-control agents (BCAs) could contribute to sustainable agriculture (Warrior, 2000; Hyde *et al*., 2019). In this regard, *Trichoderma* sp. (Vinale *et al.,* 2008) and *Bacillus* sp. (Khan *et al*., 2022) play an important role in the management of crop diseases in a sustainable manner (Arshad *et al*., 2022). *Trichoderma* sp. improves soil texture and health and promotes plant growth by reducing the stress of biotic attacks (Eman *et al*., 2023; Reddy, 2012). On the other hand, a wide range of lipo-peptides and antibiotics are produced by *Bacillus* sp. BCAs can also induce systemic resistance in host plants (Albayrak, 2019) and help to tolerate the abiotic stresses (Mahmood *et al*., 2016). They also compete for nutrients and space in soil (Miljaković *et al.,* 2020) and seeds also with pathogens (Mahmood *et al*., 2016). Therefore, seed priming is a reliable technique to overcome pathogenic activity of plant pathogens in the field (Dubey *et al*., 2008). In this regard due to environmental and human health concerns, we used BCAs (*Trichoderma* sp. and *Bacillus* sp.) and their cultural filtrates to manage leaf rust disease in bread wheat.

MATERIALS AND METHODS

Collection of Seeds

Four different wheat varieties viz., Shafaq-06, Punjab-11, Arooj-22 and Subhani-21 were collected from Wheat Research Institute, Ayub Agriculture Research Institute (AARI), Faisalabad.

Preparation of BCAs' culture and extraction of their filtrates

Potato Dextrose Broth (PDB) and Nutrient Broth (NB) were used to culture the *Trichoderma* sp. and *Bacillus* sp. respectively. For *Trichoderma* sp. PDB was prepared and autoclaved in flask. The media was inoculated in mild warm conditions with culture and then incubated for 5-7 days at 25ºC approximately. After that, the culture was centrifuged at 6000 rpm for 10 mins and for treatment purpose supernatant was taken as *Trichoderma* Filtrate (TF). In the case of *Bacillus* sp., LB media was prepared in a flask and then the culture was inoculated in sterile conditions. The inoculated media was incubated at 25-30ºC for 2-3 days and then centrifuged the culture at 4000 rpm for 5 min. After that, the supernatant was collected for treatment as *Bacillus* filtrate (BF).

Priming of seeds with BCAs

Seeds of all wheat varieties were incubated in BCA filtrates and cultures overnight and then dried at room temperature for 4 hours before sowing into the field.

Sowing of collected varieties

After priming, the seeds were sown at the Experimental area of the Plant Pathology Department, University of Agriculture, Faisalabad (UAF). Sowing was laid out in Completely Randomized Block Design (RCBD), having ten plants in a single line with three replications.

Inoculum preparation for foliar application and scoring

The leaves infected with brown wheat rust were collected from Wheat Research Institute, AARI, Faisalabad. The infected leaves were submerged in distilled water for 12 hours overnight. After removing leaves from the water, spores were used for foliar application to spread disease in the field. After spraying inoculum in the field, the screening data was collected after two weeks of inoculum application.

Data recording of morphological parameters

The data were recorded for the following different morpho-physiological characters in all varieties.

Number of tillers per plant

Before harvesting, the number of tillers in three guarded plants was counted individually.

Fresh and dry root weight (g)

Randomly 4-5 plants of all varieties were brought to the lab. After removing all the dust from roots, the weight of fresh roots was taken with electronic weighing balance. Then these roots were kept in dry oven for 2-3 days for drying purpose and data of dry roots was measured on electronic weighing balance.

Spike and 1000 grains weight (g)

At maturity, the spikes were cut and weighed on electronic weighing balance randomly. Then, from spikes grains were threshed and counted up to 1000 manually and weighed.

Number of grains per spike

The spikes were threshed to get the grain which were counted manually for each spike.

Plant height, spike, root, peduncle, awn and internode length (cm)

Plant height and lengths (spike, root, peduncle, awn and internode length) were recorded by using measuring rod. The data were recorded from randomly selected plants and average was taken from the replicates.

Number of grains per plant

By threshing all spikes of a single plant, the collected seeds were counted. In this way per plant yield was observed.

Data recordings of physio-chemical parameters

Determination of Malondialdehyde (MDA) contents

By following the method of Cakmak and Horst, (1991) MDA content was determined. In this method, 0.5g leaves sample was grinded in trichloro-acetic acid (TCA) solution and then was centrifuged for 10 minutes at 12000 rpm. By removing supernatant, 0.5ml of sample was mixed with 3ml of 20% TCA and 0.5% thiobarbituric acid (TCA). After that, this mixture was placed in water bath for 30 minutes at 95^0C and readings were taken in spectrophotometer at 532nm and 600nm wavelengths.

Determination of Hydrogen Peroxide (H_2O_2)

The method described by Velikova *et al*. (2000) was used to compute H_2O_2. To prepare H_2O_2 solution, 0.5g fresh leaves were grinded in TCA (5ml of 0.1% w/v). This mixture was centrifuged at 12000 rpm for 15 minutes. After that, 0.5 ml supernatant was mixed with 0.5ml phosphate buffer (7.0 pH) and 1ml of iodide potassium (1M). UV-visible spectrophotometer was used to record the data at 390nm vortex wavelength.

Determination of glycine betaine contents

To determine the glycine betaine contents, procedure of Grieve and Gratan, (1983) was used. Fresh leaves (0.5g) were mixed with 0.5% solution of 10ml toluene and 1ml of 2N sulphuric acid (H_2SO_4). 0.5ml of this mixture was transferred in a glass tube and mixed with 0.2ml potassium triiodide solution. After stirring the mixture was cooled for 90 minutes. After that, 2.8ml chilled distilled water and 6ml 1,2-dichloroethane were added. This mixture was centrifuged at 1000rpm for 10 minutes and supernatant was removed. The readings were taken for lower organic matter in spectrophotometer at 365nm.

Determination of Chlorophyll contents

In 1949, Arnon used the method to identify the contents of chlorophyll 'a' and chlorophyll 'b'. Fresh leaves of weight 0.5g were masturbated and 10ml acetone (80%) was added and left them at 0-4^0C for overnight. Later on, the samples were centrifuged at 10000rpm for 5 minutes and supernatant was used to take readings in spectrophotometer at 645 and 663nm wavelengths.

Chlorophyll 'a' and 'b' contents were measured by given formulas

Chl. a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000 × W

Chl. b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000 × W

W = weight of the fresh leaf tissue (g)

V = volume of the extract (mL)

Determination of free proline contents

The leaf sample (0.5g) was ground in 5 ml sulfosalicylic acid (3%) and was filtered by using Whatman No. 2 filter paper. Later 2ml of this filtrate was mixed with 2ml glacial acetic acid and 2ml acid ninhydrin. This mixture was heated for 30 minutes at 95C in the water bath. After water bathing, 4ml of toluene was added and the supernatant was used for data readings after vertexing for 5 seconds. At 520nm wavelength in spectrophotometer calculations were calculated (Bates *et al*., 1973). Determination of proline concentration in leaf tissues was done by using formula:

$$Proline\ content = \left(\mu \frac{\text{g}}{\text{g FW}}\right) \times = \text{Concentration from standard curve } \times \frac{\text{Volume of extraction solution}}{\text{Weight of sample (g)}} \times 100$$

Statistical analysis

All the collected data were statistically analyzed by using Statistix version 8.1 and Analysis of Variance (ANOVA) was performed under a Randomly Complete Block Design (RCBD). Finally, the least significant difference (LSD) test at 5% level of probability was used to compare the mean values of each treatment (Steel *et al*., 1997). The graphs were carried out by using Origin-Pro 2024 software. The correlation plot on wheat parameters was carried out by using RStudio software.

RESULTS

Effect on peduncle, intermodal, spike, awn and root lengths and plant height

Peduncle length showed maximum results in response to *Trichoderma* sp. in Subhani-21(Figure 1A); same treatment performed maximum in same varieties of plant height (Figure C) and root length (Figure E). In case of internode length (Figure B), BF (*Bacillus* filtrate) was with maximum results in Punjab-11 while in spike length (Figure D), Arooj-22 revealed outstanding results in response to TF (*Trichoderma* filtrate). Awn length (Figure F) was observed maximum in Subhani-21, treated with *Bacillus* sp. On the other hand, Punjab-11 treated with TF showed minimum results in case of peduncle length while *Bacillus* sp. was with minimum effects in Subhani-21 of internode length. Interestingly in case of plant height, all treatments in Punjab-11, *Bacillus* sp. in Shafaq-06 and *Trichoderma* sp. in Arooj-22 exhibited almost same results with just very slight differences and BF in Arooj-22 performed the least. Same as in spike length, all treatments in Subhani-21, TF in Punjab-11 and all treatments in Shafaq-06 (excluding *Trichoderma* sp.) performed almost same with slight differences and *Trichoderma* sp. and BF were in Punjab-11 were minimum, compared with other treatments. In root length, along Subhani-21 treated with *Trichoderma* sp. was with same results of TF in Shafaq-06 as best treatments while BF was with least output in Arooj-22. Finally in awn length, Subhani-21 treated with BF, Punjab-11 with *Trichoderma* sp. and Arooj-22 with TF and BF showed almost same outputs while BF in Punjab-11 and TF in Shafaq-06 were with least results.

Fresh and dry root weight response to various seed priming

Fresh root weight (Figure A) was observed maximum in Punjab-11 in response to *Trichoderma* sp. treatment while Arooj-22 was with least fresh root weight treated with BF. On the other hand, in dry root weight (Figure B), again Punjab-11 was with great results in *Trichoderma* sp. treatment. BF showed minimum results in Arooj-22.

Response to seed priming against leaf rust of various yield factors

In Figure 3, all the given factors directly relate to crop yield, which includes number of tillers per plant (A), number of grains per spike (B), 1000 grains weight (C) and number of grains per plant (D).In number of tillers per plant, we observed maximum response in TF treatment in Shafaq-06 cultivar while in Punjab-11 with treatment BF was with least output. In case of number of grains per spike, Punjab-11 output was observed outstanding in about all treatments as compare to other varieties. In Punjab-11 *Bacillus* sp. and TF showed almost similar results with a very minor difference and other both treatments (BF and *Trichoderma* sp.) also exhibited significant response in same variety respectively. In case of 1000 grains weight, controls of all varieties responded very well in compare to all treatments but among treatments Arooj-22 gave maximum results in response to *Trichoderma* sp. treatment. *Trichoderma* sp. also responded very well in Shafaq-06 just as aligning with TF in Arooj-22. Finally, in number of grains per spike Punjab-11 was with excellent results in case of TF treatment. In same variety with a small gap *Bacillus* sp. responded well also. In same character least results were shown by *Trichoderma* sp. and *Bacillus* sp. in Subhani-21 and *Bacillus* sp. and TF in Arooj-22.

Effect on Chlorophyll contents

The contents of Chl-a (A) and Chl-b (B) interaction among treatments and varieties as shown in Figure 4. Chl-a showed the most effective response in Shafaq-06 variety treated with BF. Mostly results showed significance with each other like all treatments (excluding BF) in Subhnai-21 and TF and BF treatments in Arooj-22. On the other hand, all treatments (excluding *Bacillus* sp.) in Punjab-11, TF in Shafaq-06 and *Trichoderma* sp., *Bacillus* sp. and TF in Arooj-22 were with almost same results with very slight differences. Punjab-11 treated with *Bacillus* sp. and Shafaq-06 treated with *Trichoderma* sp. and *Bacillus* sp. were with least results observed in Chl-a. In case of Chl-b, *Trichoderma* sp. gave maximum results in Shafaq-06 while Arooj-22 treated with BF exhibited most well at second number. In case of Chl-b many results were significant among themselves with least differences. Like *Trichoderma* sp. and *Bacillus* sp. in Subhani-21, *Bacillus* sp., TF and BF in Punjab-11 and Shafaiq-06 and at the end *Trichoderma* sp., *Bacillus* sp. and BF in Arooj-22. Least results were observed in Subhnai-21 treated with TF.

Effect on Malondialdehyde (MDA) and Hydrogen Peroxide (H_2O_2)

Malondialdehyde (A) and Hydrogen Peroxide (B) are shown in Figure 5. MDA exhibited most efficient results in Punjab-11 treated with BF and least results were observed in same cultivar treated with *Trichoderma* sp. Interestingly, Subhani-21 treated with *Bacillus* sp., Punjab-11 with BF, Shafaq-06 with all treatments (except BF) and Arooj-22 with BF showed alphabetically same significance in results with slight changes in their results among themselves. On the other hand, in results of H_2O_2 BF showed most good results in Subhani-21. However, least results were observed in Shafaq-06 treated with *Bacillus* sp. As least results, *Trichoderma* sp. in all four varieties and *Bacillus* sp. in Shafaq-06 and Arooj-22, shared same significance alphabetically.

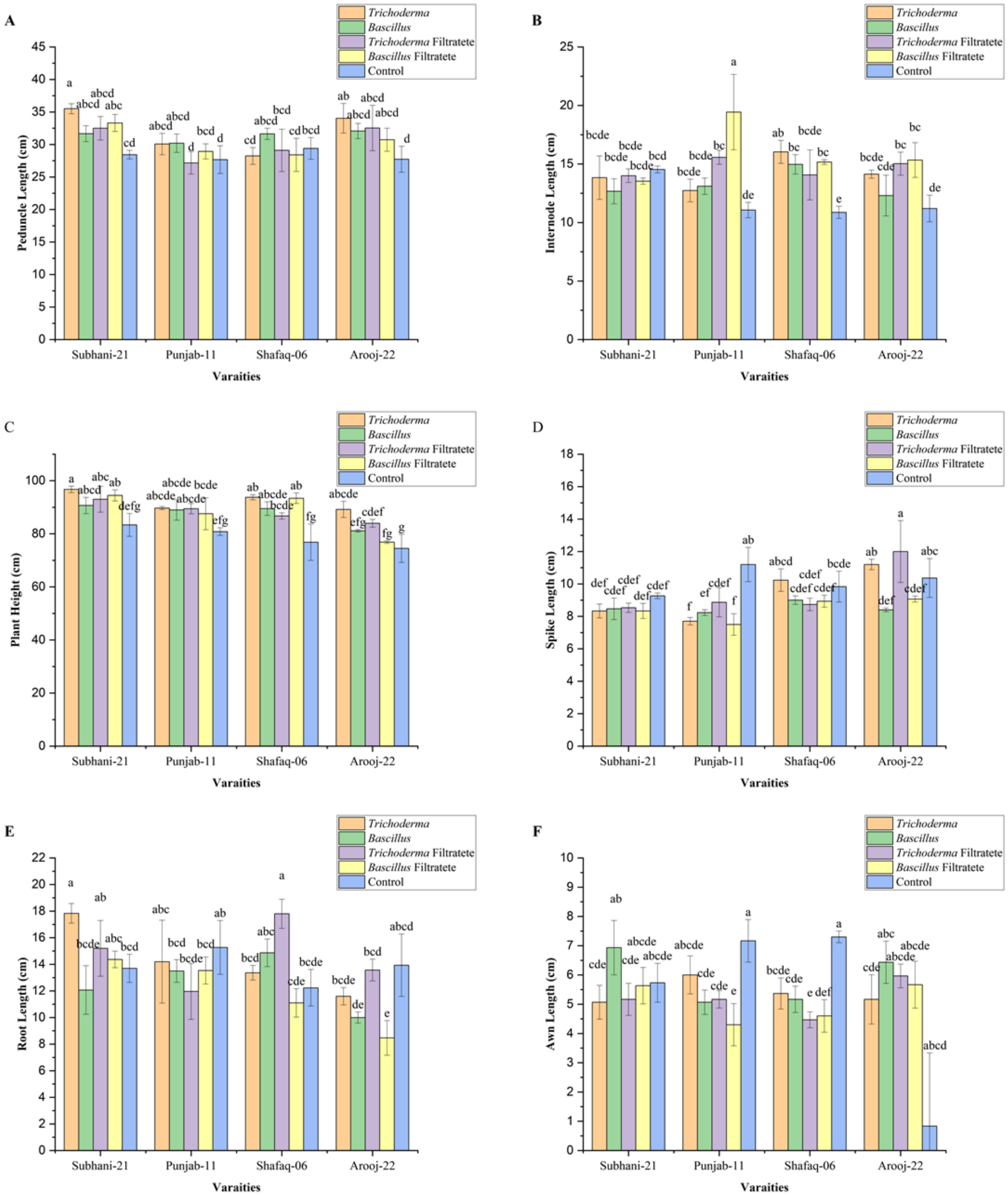

Figure 1. Peduncle length (A), internode length (B), plant height (C), spike length (D), root length (E) and awn length (F) data are shown against leaf rust in response to *Trichoderma* sp., *Bacillus* sp., TF and BF. Error bars represent the standard error of mean and alphabets are showing significance of treatments against leaf rust.

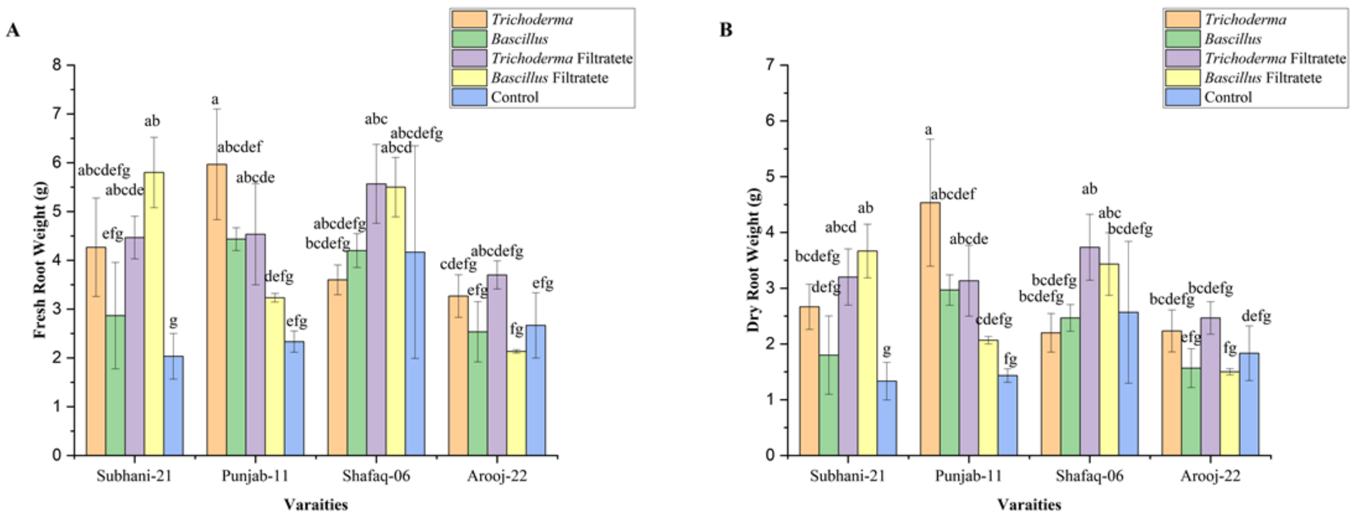

Figure 2. Fresh root weight (A) and dry root weight (B) of different wheat varieties in response to different seed priming treatments. Error bars are representing the standard error of mean and alphabets are showing significance of treatments against leaf rust.

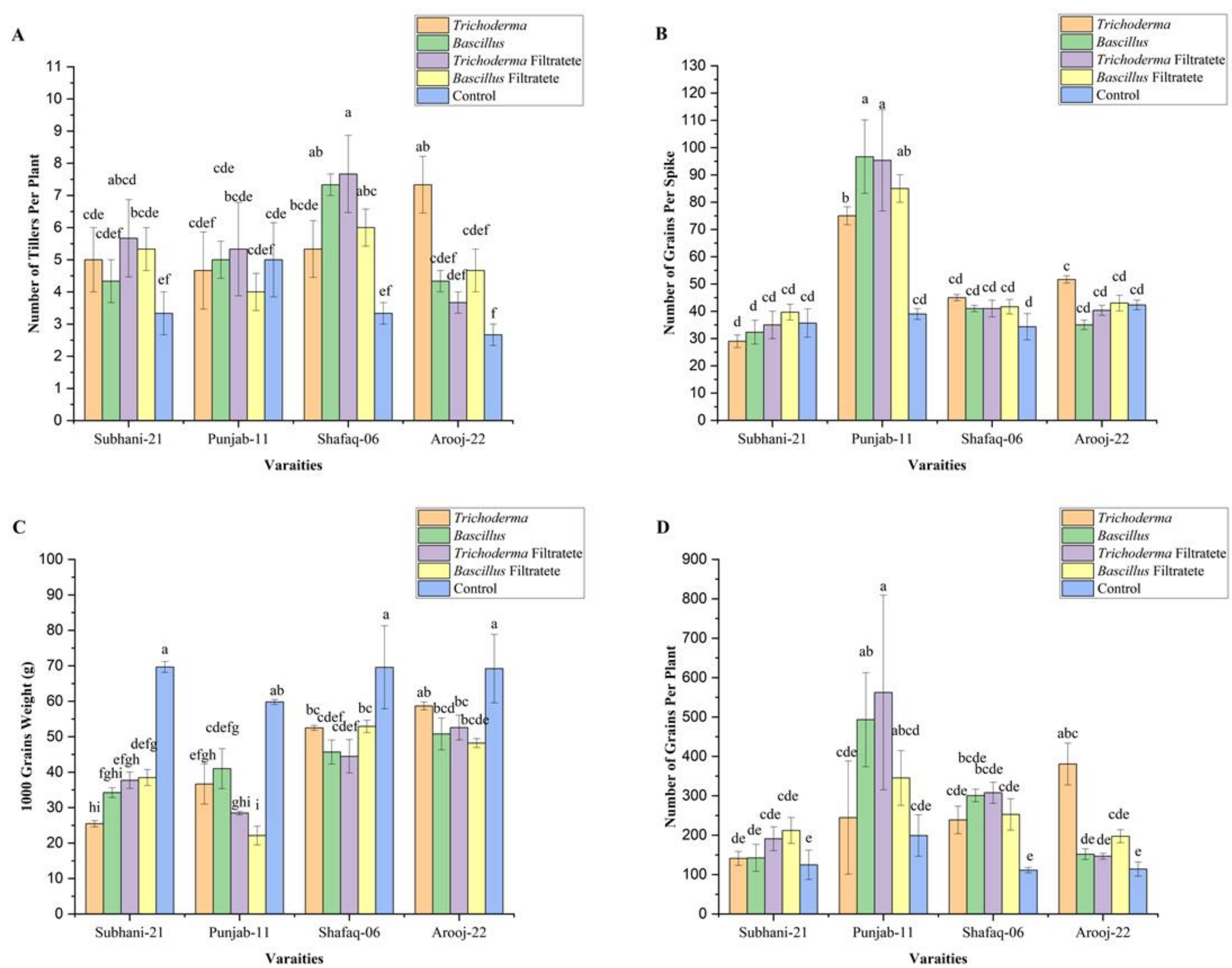

Figure 3. Different characters such as number of tillers per plant (A), number of grains per spike (B), 1000 grain weight (C) and number of grains per plant (D) showing their response to different wheat varieties treated with different biocontrol agents.. Error bars are representing the standard error of mean and alphabets are showing significance of treatments against leaf rust.

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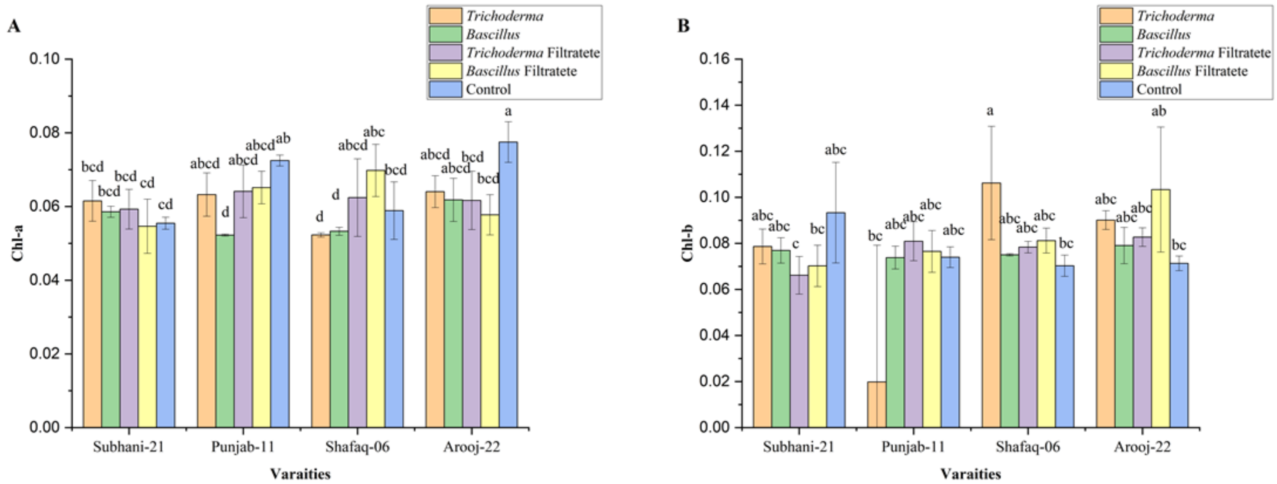

Figure4. Chlorophyll contents (Chl-a and Chl-b) showing their response to different wheat varieties in response to treated with different biocontrol agents. Error bars are representing the standard error of mean and alphabets are showing significance of treatments against leaf rust.

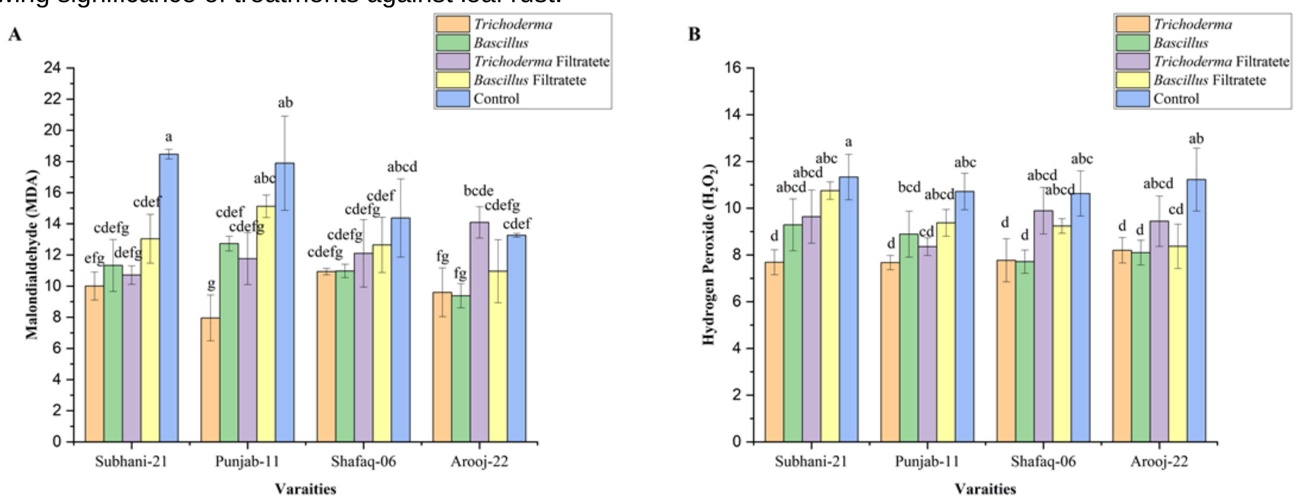

Figure 5. Malondialdehyde (A) and hydrogen peroxide (H_2O_2) (B) of different wheat varieties in response to various seed priming treatments. Error bars are representing the standard error of mean and alphabets are showing significance of treatments against leaf rust.

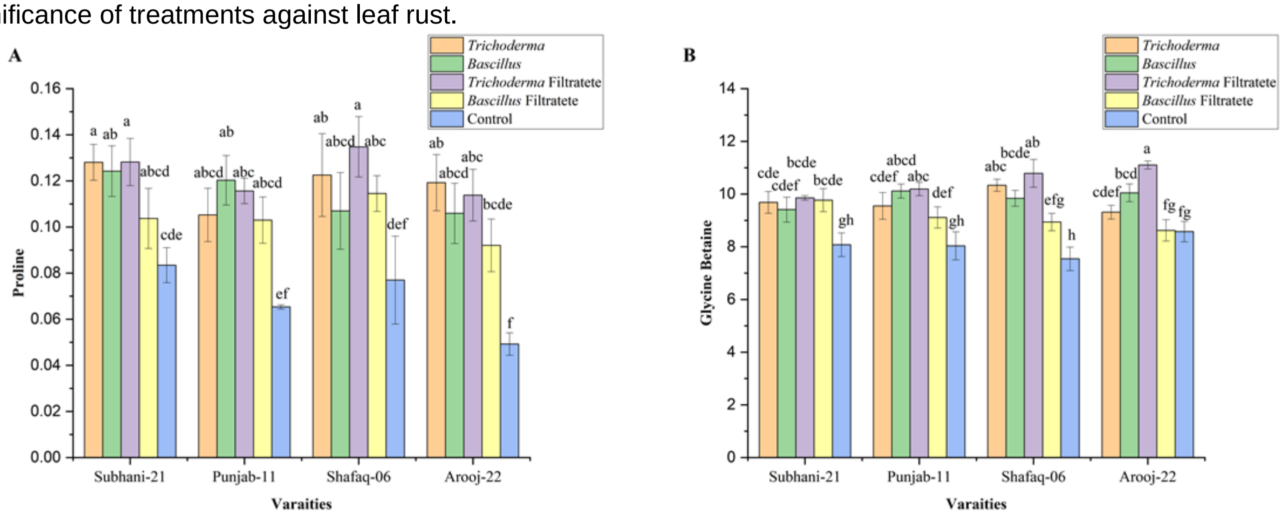

Figure 6. Proline (A) and glycine betaine (GB) of various wheat varieties in response to different seed priming treatments. Error bars are representing the standard error of mean and alphabets are showing significance of treatments against leaf rust.

Effect on proline and glycine betaine (GB) contents

Most outstanding results of proline were observed in Shafaq-06 and Subhnai-21 respectively, treated with TF as shown in Figure 6A. Arooj-22 treated with BF revealed minimum results. However, Subhani-21 treated with BF, Punjab-11 treated with *Trichoderma* sp. and BF and at last Shafaq-06 and Arooj-22 treated with *Bacillus* sp. gave alphabetically same results with minute variations among them. Additionally, in GB contents as shown in Figure 6B, TF dominated with results in Arooj-22 and Shafaq-06 respectively. In contrast, Arooj-22 treated with BF was with least results. Almost all other results were alphabetically different from each other.

Correlation among various physio and morphological characters

Different physiological and morphological character associations are given in Figure 7 by using Pearson's correlation graph. Internode length and plant length, plant length and number of tillers and fresh root weight and 1000 grains weight have positive correlations among themselves. Additionally, proline and MDA have also positive correlation among them and indicate the increase of their levels, if anyone of them increases. Comparatively, awn length and root length and fresh root weight and chl-a showed very negative correlation among them. All other remaining characters have no or very week correlations.

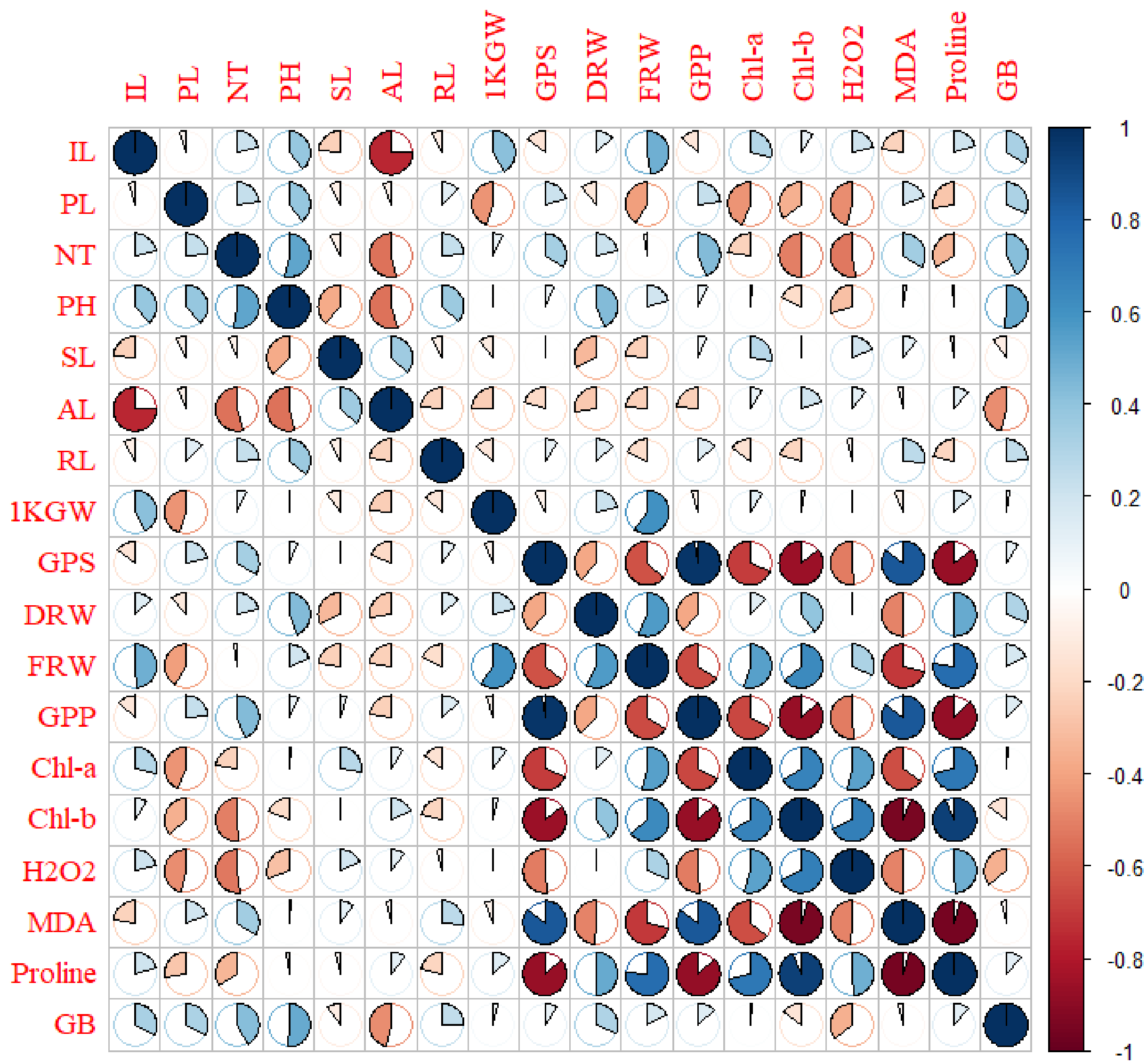

Figure 7. Pearson's correlation for various physio and morphological parameters of wheat under leaf rust stress. Circles in figure showing the distribution of all parameters. Abbreviations in this figure are used as follows: IL (internode length), PL (plant length), NT (number of tillers), PH (plant height, SL (shoot length), AL (awn length), RL (root length), 1KGW (1000 grains weight), GPS (grains per spike), DRW (dry root weight), FRW (fresh root weight), GPP (number of grains per plant), Chl-a (chlorophyll a), Chl-b (chlorophyll b), H_2O_2 (hydrogen peroxide), MDA (malondialdehyde) and GB (glycine betaine).

DISCUSSION

Different pathogens attack on wheat crop and cause significant yield losses from which leaf rust is one of the most important foliar disease and causes significant yield losses (Jabran *et al*., 2023).The present study was aimed to assess different biocontrol agents to manage the impact of leaf rust disease on various wheat varieties by seed priming. Seed priming techniques have been used to increase seed germination efficacy, plant biomass and resistance to many seed-borne diseases (Arshad *et al*., 2022). For that purpose, we used two different biocontrol agents and their filtrates i.e., *Trichoderma* sp. and *Bacillus* sp. and four different wheat varieties (Shafaq-06, Punjab-11, Subhani-21 and Arooj-22).

As biocontrol agents, *Trichoderma* sp. helps to enhance yield and plant growth as well and *Bacillus* sp. has been used to manage plant pests and diseases (Khan *et al*., 2020; Mir *et al*., 2022). This current research was conducted to analyze the impact of different bio-control agents via seed-priming on different morpho and physiological factors in response to *P. triticina* in local wheat varieties. For instance, *Trichoderma* sp. enhanced the plant height, peduncle length, root length and dry root biomass in wheat which are promising with previous studies Merwad and Abdel-Fattah (2017); Anjum *et al*. (2020); Tiwari *et al*. (2022). Enhancement of radicular and aerial growth in various field crops has been reported by *Trichoderma*-induced enhancement (Moya *et al*., 2020; Singh *et al*., 2016). These factors enhancement in wheat may possessed due to *Trichoderma* sp. priming by elevating the capability of nutrients availability and growth hormone production like Indole acetic acid (IAA) (Singh et al., 2016).

Grain yield is the main output of any grain crop (Ali et al., 2009; Rajaram and Braun, 2007). In current research, wheat seeds primed with both *Trichoderma* sp. and *Bacillus* sp. improved the yield factors significantly. Same promising results were reported in previous studies by Nzanza*et al*. (2011); Azarami *et al*. (2011); Lastochkina *et al*. (2023). The increase in yield by seed priming of *Trichoderma* sp. and *Bacillus* sp. was probably related to the increased establishment of plant and its growth (Cuvas, 2006; Rahman *et al*., 2015; Mir *et al*., 2022; Lastochkina *et al*., 2023). Seeds without priming showed some good results of chlorophyll contents but in some cases, as compared with treated seeds. Interestingly, primed seeds also exhibited the enhancement of chlorophyll contents as already reported results by Tiwari *et al*. (2022). Increase in chlorophyll contents could enhance the photosynthetic efficacy in plants (Jisha and Puthur, 2016).

Moreover, wheat diseased with leaf rust was evaluated to quantify the MDA content. *P. triticina* increased the MDA level in non-primed seeds but only a single variety treated with *Bacillus sp.* exhibited more effect than control. MDA is produced as a consequence of cell membrane lipid peroxidation leading to electrolyte leakage and eventual cell death (Basit *et al*., 2022). However, in a recent study by Tiwari *et al*. (2022) reported the decrease of MDA content in primed seeds. For cell structures hydrogen peroxide (H_2O_2) generally considered to be a toxic agent but also meant to be a vital signal molecule which triggers acclimation under stress conditions (Ellouzi *et al*., 2017).

Our treated seeds with bio-control agents exhibited significant decline of H_2O_2 content. Increased in proline content enhances the plant ability to mitigate the pathogen activity (Vujanovic *et al*., 2022). In our study, all treated seeds showed the increase in proline content in contrast to non-treated seeds. Same results have been reported in a study by Ambreen *et al*. (2021). Multiple studies have reported the glycine betaine (GB) content enhancement by seed-priming in wheat and other crop plants but in abiotic stresses (Ahmed *et al*., 2021; Chakraborti *et al*., 2022; Moradi and Siosemardeh, 2023). Our results also demonstrated the significant growth of GB content in primed wheat seeds and then having biotic stress of *P. triticina.*

CONCLUSION

This work demonstrates the importance of *Trichoderma* sp. seed priming as a strategy and *Bacillus* sp. in enhancing wheat growth and treatment to leaf rust disease (*P. triticina*). In addition, seed treatment with these biocontrol agents improved many vital traits such as plant height, root length, biomass, and yield factors, indicating that these plant growth-promoting agents could be useful in promoting plant growth and productivity.

The higher chlorophyll content in treated seeds indicates increased photosynthetic activity, and decreases in MDA and H_2O_2 content of primed seeds suggest reduced oxidative stress and better health of plants under disease conditions. Moreover, the elevated levels of proline and glycine betaine observed in treated seeds further emphasize their contribution to increased stress tolerance and pathogen resistance in these plants. Overall, the findings confirm that seed priming with *Trichoderma sp.* and *Bacillus sp.* is an effective strategy for managing leaf rust disease while improving wheat yield and stress resilience. These results pave the way for using biocontrol agents as sustainable and eco-friendly solutions in wheat cultivation.

AUTHOR CONTRIBUTIONS

MUR and IS mainly conducted the research and contributed equally. EN and MA helped in data analyses, AJ and UM assisted in data recording and SJ reviewed the manuscript. AA supervised the study.

CONFLICT OF INTEREST

The authors declare no competing interests.

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REFERENCES

Afzal, A., Ijaz, M., & Shah, S. R. A. (2020). Determination of suitable growth stage for application of fungicide against stripe rust in wheat. Pakistan Journal of Agricultural Research, 33(4), 714–719.

Ahmed, N., Zhu, M., Li, Q., Wang, X., Wan, J., & Zhang, Y. (2021). Glycine betaine-mediated root priming improves water stress tolerance in wheat (*Triticum aestivum* L.). Agriculture, 11(11), 11–27.

Albayrak, Ç. B. (2019). Bacillus species as biocontrol agents for fungal plant pathogens. In Bacilli and Agrobiotechnology. Phytostimulation and Biocontrol (pp. 239–265).

Ali, H., Khan, M. S., & Ahmed, Z. (2020). Role of biofertilizers in enhancing chlorophyll content in wheat under stress. Journal of Crop Improvement, 34(2), 89–96.

Ali, M. A., Nawab, N. N., Abbas, A., Zulkiffal, M., & Sajjad, M. (2009). Evaluation of selection criteria in *Cicer arietinum* L. using correlation coefficients and path analysis. Australian Journal of Crop Science, 3(2), 65–70.

Ambreen, S., Athar, H. U. R., Khan, A., Zafar, Z. U., Ayyaz, A., & Kalaji, H. M. (2021). Seed priming with proline improved photosystem II efficiency and growth of wheat (*Triticum aestivum* L.). BMC Plant Biology, 21, 1–12.

Anjum, Z. A., Hayat, S., Ghazanfar, M. U., Ahmad, S., Adnan, M., & Hussain, I. (2020). Does seed priming with *Trichoderma* isolates have any impact on germination and seedling vigor of wheat? International Journal of Botany Studies, 5(2), 65–68.

Anonymous. (2000). Wheat rust (in Urdu). CDRI, TARC, Karachi, pp. 25270.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology, 24(1), 1.

Arshad, U., Raheel, M., Shakeel, Q., Jabran, M., Ahmed, S., Abbas, A., Jabbar, A., Zahid, M. S., & Ali, M. A. (2022). Seed-priming: A novel approach for improving growth performance and inducing resistance against root-knot nematode *(Meloidogyne incognita*) in bread wheat (*Triticum aestivum* L.). Gesunde Pflanzen (Healthy Plants).

Azarmi, R., Hajieghrari, B., & Giglou, A. (2011). Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. African Journal of Biotechnology, 10(31), 5850–5855.

Basit, F., Bhat, J. A., Ulhassan, Z., Noman, M., Zhao, B., & Zhou, W. (2022). Seed priming with spermine mitigates chromium stress in rice by modifying the ion homeostasis, cellular ultrastructure, and phytohormone balance. Antioxidants, 11(9), 1704.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1), 205–207.

Bird, A. R., & Regina, A. (2018). High amylose wheat: A platform for delivering human health benefits. Journal of Cereal Science, 82, 99–105.

Bolton, M. D., Kolmer, J. A., & Garvin, D. F. (2008). Wheat leaf rust caused by *Puccinia triticina*. Molecular Plant Pathology, 9(5), 563–575.

Cakmak, I., & Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum, 83(3), 463–468.

Chakraborti, S., Bera, K., Sadhukhan, S., & Dutta, P. (2022). Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical, and molecular mechanisms. Plant Stress, 3, 100052.

Din, G. M., Ali, A., Abbas, A., Naveed, M., Anwar, J., & Tanveer, M. H. (2017). Effect of leaf rust disease on various morpho-physiological and yield attributes in bread wheat. Pakistan Journal of Phytopathology, 29(1), 117–128.

Draz, I. S., Elkhwaga, A. A., Elzaawely, A. A., El-Zahaby, H. M., & Ismail, A. W. A. (2019). Application of plant extracts as inducers to challenge leaf rust of wheat. Egyptian Journal of Biological Pest Control, 29, 1–8.

Dubey, N. K., Srivastava, B., & Kumar, A. (2008). Current status of plant products as botanical pesticides in storage pest management. Journal of Biopesticides, 1(2), 182–186.

Ellouzi, H., Sghayar, S., & Abdelly, C. (2017). H2O2 seed priming improves tolerance to salinity, drought, and their combined effect more than mannitol in *Cakile maritima* when compared to *Eutrema salsugineum*. Journal of Plant Physiology, 210, 38–50.

Eman, A. A. F., Mohamed, I. A., Allah, S. F., Shams, A. H., & Elsokkary, I. H. (2023). *Trichoderma* species: An overview of current status and potential applications for sustainable agriculture. Indian Journal of Agriculture Sciences, 57, 273–282.

Eversmeyer, M. G., & Kramer, C. L. (2000). Epidemiology of wheat leaf and stem rust in the central Great Plains of the USA. Annual Review of Phytopathology, 38(1), 491–513.

FAO. 2022. FAOSTAT. Food and Agriculture Organization. https://www.fao.org/faostat/en/.

Farooq, M., Usman, M., Nadeem, F., Rehman, H., Wahid, A., Basra, S. M., & Siddique, K. H. (2019). Seed priming in field crops: Potential benefits, adoption, and challenges. Crop and Pasture Science, 70(9), 731–771.

Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for determination of water-soluble quaternary ammonium compounds. Plant and Soil, 70(2), 303–307.

Harris, D., Rashid, A., Miraj, G., Arif, M., & Shah, H. (2007). On-farm seed priming with zinc sulfate solution: A cost-effective way to increase the maize yields of resource-poor farmers. Field Crops Research, 102(2), 119–127.

Hussain, T., Ishtiaq, M., Khan, F. A., Ullah, T. S., & Shabir, I. (2023). Identification of aggressive microfungal pathogens of wheat crop from Bhimber Azad Kashmir and effective bio-management of a dominant pathogen *Alternaria solani*. Nature Resources Conservation Research, 1, 6–2130.

Hyde, K. D., Xu, J., Rapior, S., Jeewon, R., Lumyong, S., Niego, A. G. T., & Stadler, M. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity, 97, 136–146.

Jabran, M., Ali, M. A., Zahoor, A., Muhae-Ud-Din, G., Liu, T., Chen, W., & Gao, L. (2023). Intelligent reprogramming of wheat for enhancement of fungal and nematode disease resistance using advanced molecular techniques. Frontiers in Plant Science, 14, 1132699.

Javed, M. T., Khan, M. A., Ehetisham-ul-Haq, M., & Atiq, M. (2013). Biological management of bacterial blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum* through plant extracts and homeopathic products. Journal of Plant Disease Pathology, 1, 1–10.

Jisha, K. C., & Puthur, J. T. (2016). Seed priming with BABA (β-amino butyric acid): A cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) Wilczek. Protoplasma, 253(2), 277–289.

Khan, A. R., Mustafa, A., Hyder, S., Valipour, M., Rizvi, Z. F., Gondal, A. S., & Daraz, U. (2022). *Bacillus* spp. as bioagents: Uses and application for sustainable agriculture. Biology, 11, 1763.

Khan, M. R., Imtiaz, M., Munir, I., Hussain, I., & Ali, S. (2021). Differential distribution of leaf rust across major wheat-growing regions of Pakistan revealed through a three-year surveillance effort. Pakistan Journal of Botany, 53(1), 261–266.

Khan, S., Basra, S. M. A., Nawaz, M., Hussain, I., & Foidl, N. (2020). Combined application of moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum* L.). South African Journal of Botany, 129, 74–81.

Lastochkina, O., Yakupova, A., Avtushenko, I., Lastochkin, A., & Yuldashev, R. (2023). Effect of seed priming with endophytic *Bacillus subtilis* on some physio-biochemical parameters of two wheat varieties exposed to drought after selective herbicide application. Plants, 12(8), 1724.

Mahmood, A., Turgay, O. C., Farooq, M., & Hayat, R. (2016). Seed biopriming with plant growth-promoting rhizobacteria: A review. FEMS Microbiology Ecology, 92(8), 112.

Martens, G., Lamari, L., Grieger, A., Gulden, R. H., & McCallum, B. (2014). Comparative yield, disease resistance, and response to fungicide for forty-five historic Canadian wheat cultivars. Canadian Journal of Plant Science, 94(2), 371–381.

Merwad, M. A., & Abdel-Fattah, M. K. (2017). Improving productivity and nutrient uptake of wheat plants using *Moringa oleifera* leaf extract in sandy soil. Journal of Plant Nutrition, 40, 1397–1403.

Miljaković, D., Marinković, J., & Tubić, S. B. (2020). The significance of *Bacillu*s spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8, 1037.

Mir, R. A., Argal, S., Ahanger, M. A., Jatav, K. S., & Agarwal, R. M. (2022). Differential activity of wheat antioxidant defense system and alterations in the accumulation of osmolytes at different developmental stages as influenced by marigold (*Tagetes erecta* L.) leachates. Frontiers in Plant Science, 13, 1001394.

Moradi, L., & Siosemardeh, A. (2023). Combination of seed priming and nutrient foliar application improved physiological attributes, grain yield, and biofortification of rainfed wheat. Frontiers in Plant Science, 14, 1287677.

Moya, P., Barrera, V., Cipollone, J., Bedoya, C., Kohan, L., & Toledo, A. (2020). New isolates of *Trichoderma* spp. as biocontrol and plant growth-promoting agents in the Pyrenophora teres-barley pathosystem in Argentina. Biological Control, 141, 104152.

Nzanza, B., Marais, D., & Soundy, P. (2011). Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi. African Journal of Microbiology Research, 5(4), 425–431.

Rahman, M., Ali, J., & Masood, M. (2015). Seed priming and Trichoderma application: A method for improving seedling establishment and yield of dry direct-seeded boro (winter) rice in Bangladesh. Universal Journal of Agricultural Research, 3(2), 59–67.

Rajaram, S., & Braun, J. H. (2008). Wheat yield potential. In International Symposium on Wheat Yield Potential: Challenges to International Wheat Breeding (pp. 103–107). CIMMYT (International Maize and Wheat Improvement Center), Mexico.

Reddy, P. P. (2012). Biological priming of seeds. In Recent advances in crop protection (pp. 45–56). Springer, New Delhi, India.

Ren, X., Wang, C., Ren, Z., Wang, J., Zhang, P., Zhao, S., & Wang, X. (2023). Genetics of resistance to leaf rust in wheat: An overview on a genome-wide level. Sustainability, 15(4), 3247.

Senthamizhlselvan, P., Sujeetha, J. A. R., & Jeyalakshmi, C. (2010). Growth, sporulation, and biomass production of native entomopathogenic fungal isolates on a suitable medium. Journal of Biopesticides, 3(2), 466.

Singh, V., Upadhyay, R. S., Sarma, B. K., & Singh, H. B. (2016). Seed bio-priming with *Trichoderma asperellum* effectively modulates plant growth promotion in pea. International Journal of Agriculture, Environment and Biotechnology, 9(3), 361–365.

Singh, H., Jassal, R. K., Kang, J. S., Sandhu, S. S., Kang, H., & Grewal, K. (2015). Seed priming techniques in field crops: A review. Agricultural Reviews, 36(4), 251–264.

Steel, R. G. D., Torrie, J. H., & Dicky, D. A. (1997). Principles and procedures of statistics: A biometrical approach (3rd ed.). McGraw Hill Book Co. Inc., New York.

Tiwari, M., Singh, R., Jha, R., & Singh, P. (2022). Heritable priming by *Trichoderma*: A sustainable approach for wheat protection against *Bipolaris sorokiniana*. Frontiers in Plant Science, 13, 1050765.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Science, 151(1), 59–66.

Vinale, F., Sivasithamparam, K., Ghisalberti, L. E., Marra, R., Woo, L. S., & Lorito, M. (2008). *Trichoderma*-plant-pathogen interactions. Soil Biology and Biochemistry, 40, 1–10.

Vujanovic, S., Vujanovic, J., & Vujanovic, V. (2022). Microbiome-driven proline biogenesis in plants under stress: Perspectives for a balanced diet to minimize depression disorders in humans. Microorganisms, 10(11), 2264.

Warrior, P. (2000). Living systems as natural crop-protection agents. Pest Management Science, 8, 681–687.