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Research Article

Synergetic Effect of Seed-Primed Biocontrol Agents and Biochar Soil Amendment on Wheat Productivity and Leaf Rust Suppression

Rabia Irshad[1], Ameer Hamza Aslam[1], Malaika Tariq[1], Ikhlas Shafique[2]*, Muhammad Usman[1], Muhammad Naveed[3], Amjad Abbas[1]*

[1]*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.*
[2]*Department of Sustainable Agriculture and Food Security, Institute of Science, Çukurova University, Adana, Türkiye.*
[3]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.*

ABSTRACT

Wheat (*Triticum aestivum*), a staple crop for over 2.5 billion people, suffers yield losses from leaf rust (*Puccinia triticina*). Effective management of this disease is essential. Therefore, the present study aimed to investigate the effects of biocontrol agents (*Trichoderma* sp. and *Bacillus* sp.), with or without biochar (1.5 g plant^{-1}), on four wheat genotypes under field conditions using a triplicate randomized complete block design (RCBD). Results showed that biocontrol treatments, particularly Trichoderma sp., significantly improved growth and yield traits, with increases of up to approximately 79% in yield per plant and 56% in grain weight per spike, while reducing brown rust severity by approximately 60% compared to the control. The magnitude of response varied among genotypes, with Anaj-2017 showing comparatively greater improvement across several traits. However, the effects of biochar were not consistently superior across all treatments, indicating a context-dependent response. These observations highlight the potential of eco-friendly strategies, particularly *Trichoderma* sp., along with biochar, for enhancing wheat productivity and resilience.

Keywords: *Bacillus*, Biochar, Brown rust, *Puccinia triticina, Trichoderma*, Wheat

Correspondence
Ikhlas Shafiq
iikhlas817@gmail.com
Amjad Abbas
amjad.abbas@uaf.edu.pk

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal crop in Pakistan, contributing nearly 37% of dietary energy intake and occupying about 40% of cultivated land (Baloch *et al*., 2018). However, many biotic and abiotic factors are continuously affecting its production (Jabran *et al*., 2023). Among biotic diseases, leaf rust, caused by *Puccinia triticina* (an obligate pathogen), is one of the most devastating foliar diseases of wheat, causing 25–50% yield losses depending upon the genotype (Prasad *et al*., 2020). This disease significantly reduces both wheat yield and grain quality. Symptoms of wheat leaf rust include small, yellow circular or oval spots that appear on the upper surface of the wheat leaf, which develop into small, orange pustules encircled by a yellow halo (Rahmatov *et al*., 2019). Upon maturity, the pustules release spores that are easily dislodged from the leaf surface and may be observed as orange dust. Over time, black spores can develop as the disease progresses, resulting in both orange and black lesions on the same leaf (Din *et al*., 2017). Conventional management strategies for leaf rust rely on fungicides and host resistance genes (*Lr* genes). Over 80 *Lr* genes have been identified, providing varying degrees of race-specific and adult-plant resistance (McCallum *et al*., 2024).

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However, the rapid evolution of new *P. triticina* races makes genetic resistance ineffective, while intensive fungicide use poses economic and environmental concerns. Consequently, sustainable and eco-friendly alternatives are increasingly required for integrated disease management in wheat (Dobhal *et al*., 2024; Jørgensen *et al*., 2024; Jabran *et al*., 2025). Biochar, a carbon-rich product from pyrolysis of organic matter, is a new potential tool for plant disease management (Lehmann *et al*., 2021). It can modify root exudates and activate plant defense pathways such as the jasmonic acid and phenylpropanoid systems, leading to increased resistance against foliar and root pathogens (Poveda *et al*., 2021; Dorjee *et al*., 2024).

Among biological control agents (BCAs), *Bacillus* species are known for producing cyclic lipopeptides (surfactin, iturin, fengycin), which have direct antifungal activity against *P. triticina* and *P. striiformis,* the causal agents of brown and yellow rust, respectively (Dehbi *et al*., 2023; Aslam *et al*., 2026). Simultaneously, *T. harzianum* produces hydrolytic enzymes such as chitinases and β-1,3-glucanases, which degrade fungal cell walls and contribute to pathogen suppression (Harman *et al*., 2004; Shoresh *et al*., 2010). The integrated use of BCAs with biochar can further enhance soil microbial activity and plant health by improving nutrient cycling, microbial colonization, and disease suppression. Combined application of BCAs and biochar has shown good promise to combat plant diseases (Arshad, 2024). For instance, Arshad *et al*. (2021) used rice husk biochar in combination with *T. harzianum* and *Bacillus* sp. to manage root-knot nematodes in tomato with the activation of defense-related genes.

Although biochar and BCAs have independently shown potential in improving plant resilience and suppressing pathogens, their combined effects on the management of wheat leaf rust remain insufficiently explored. Therefore, this study evaluated the individual and combined effects of biochar and BCAs on wheat leaf rust and assessed their impact on plant growth and yield-related traits.

MATERIALS AND METHODS

Collection of Wheat Seeds

Seeds of four wheat genotypes with contrasting resistance to leaf rust were used in this study, including two rust-resistant varieties (Dilkash-2020 and Anaj-2017) and two rust-susceptible varieties (Galaxy-2013 and Seher-2006). All seeds were obtained from the Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, where these varieties were developed and released. Detailed pedigree (parentage) information for these cultivars was not available in the accessible literature; however, all selected genotypes are officially released and widely cultivated, with documented resistance profiles and agronomic performance.

Preparation and Culturing of BCAs

Pure cultures of *Bacillus* sp. and *Trichoderma* sp. were obtained from the Environmental Microbiology Laboratory, University of Agriculture, Faisalabad (UAF). *Bacillus* sp. was cultured on Liquid broth (LB) medium (10 g tryptone, 10 g NaCl, 5 g yeast extract per liter), while *Trichoderma* sp. was grown on potato dextrose broth (PDB) (200 g potato extract and 20 g dextrose per liter). Media were autoclaved at 121 °C and 15 psi for 15 min. After inoculation, *Bacillus* plates were incubated at 30 °C for 24 h, and *Trichoderma* cultures at 28 °C for 5-7 days under sterile conditions.

Seed Priming with BCAs

Wheat seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and rinsed three times with sterile distilled water. For priming, seeds were soaked for 8 h in BCA suspensions prepared at approximately 10^8 CFU mL^{-1} for *Bacillus* sp. and 10^6 spores mL^{-1} for *Trichoderma* sp. Three treatments were prepared: *Bacillus* alone, *Trichoderma* alone, and a combination of both (1:1 v/v). Control seeds were soaked in sterile broth. After priming, seeds were air-dried aseptically at room temperature.

Biochar Preparation Protocol

Biochar was produced from dried lawn grass through slow pyrolysis following Xi *et al*. (2020). The biomass was sun-dried to below 10% moisture content and pyrolyzed at 500 °C for 3 h in a muffle furnace under limited oxygen. The resulting biochar was cooled, ground, and sieved (2 mm), then sterilized at 105 °C for 2 h before use. The application rate of biochar (1.5 g $plant^{-1}$) was selected based on preliminary trials and previous studies reporting improvements in plant growth and soil microbial activity at similar dosages (Lehmann *et al*., 2021; Poveda *et al*., 2021). Biochar characterization (e.g., SEM, FTIR, and elemental analysis) was not conducted in the present study; however, the production conditions (feedstock type, 500 °C, and residence time) were standardized following established protocols (Xi *et al*., 2020), which are known to produce biochar with consistent physicochemical properties. This is acknowledged as a limitation and should be considered in future studies.

Application of Leaf Rust Inoculum

To ensure uniform disease pressure, spreader rows of the susceptible variety Morocco were artificially inoculated with urediniospores of *P. triticina* at the tillering stage using a spore suspension. Disease spread was monitored, and uniform rust severity across plots was confirmed before recording observations.

Sowing of Primed Seeds and Experimental Layout

The experiment was conducted in the month of November at the Department of Plant Pathology, UAF. A randomized complete block design (RCBD) with three replications was used. Treatments consisted of two biochar conditions (with and without biochar), four wheat genotypes, and three BCA treatments (*Bacillus*, *Trichoderma*, and *Bacillus* + *Trichoderma*), along with an untreated control. Each plot contained four rows (30 cm apart), each with 10 plants. Biochar was incorporated into the soil at a rate of 1.5 g plant^{-1} at sowing. The highly susceptible variety Morocco was sown around each block to serve as a natural source of *Puccinia triticina* inoculum.

Recording of Morpho-physiological Traits

Data were recorded at appropriate growth stages from three randomly selected plants per replicate from central rows to avoid border effects. Productive tillers were counted manually at maturity. Plant height (cm) was measured from the soil surface to the spike tip (excluding awns), spike length (cm) from base to tip, peduncle length (cm) from the uppermost node to spike base, awn length (cm) from spikelet tip to awn end, and internodal length (cm) as the distance between successive nodes. Plants were carefully uprooted for root measurements; root length (cm) was measured with a ruler, fresh root weight (g) was recorded immediately, and dry root weight (g) was determined after oven drying at 70 °C for 72 h to constant weight. At harvest, spike weight (g) was measured using an electronic balance, grains per spike were counted after threshing, grain weight per spike (g) was recorded, and grain yield per plant (g) was calculated as total grain weight per plant. The use of three plants per replicate is adopted in field experiments and was considered sufficient to capture within-plot variability while maintaining manageable data collection.

Data Recording for Brown Rust Severity

Leaf rust severity was evaluated during the grain-filling stage using the modified Cobb scale (Saari and Wilcoxson, 1974). Disease severity was expressed as the percentage of leaf area affected by *P. triticina* infection.

Statistical Analysis

Statistical analysis was performed using Statistix 8.1. Analysis of variance (ANOVA) was conducted under a RCBD, and treatment means were compared using the least significant difference (LSD) test at a 5% significance level (Steel *et al.*, 1997). Data visualization and correlation analysis were performed in R (version 4.3.2) using the ggplot2 package, with Pearson's correlation coefficients calculated using the cor() function. Principal component analysis (PCA) was carried out using the FactoMineR package (version 2.9) to identify major sources of variation among traits. All graphical outputs were exported as high-resolution PNG files.

RESULTS

Integrated Effect of Seed Priming and Biochar on Growth Parameters

Significant variations were observed among wheat genotypes and treatment combinations for all recorded morphological traits (Table 1). The longest awn was recorded in Dilkash-2020 and Anaj-2017, particularly when seeds were treated with *Trichoderma* sp. and *Bacillus* sp. combined with biochar, reaching 8.33 cm and 8.17 cm, respectively. In contrast, Seher-2006 and Anaj-2017 under control exhibited the shortest awn lengths (5.73 cm and 6.23 cm). Anaj 2017 showed the highest fresh (3.5 g) and dry (3.0 g) root weights under *B* + biochar, whereas Galaxy 2013 recorded the lowest (1.0 g) with mixed culture plus biochar, as shown in Table 1.

The highest internodal length (15.0 cm) was observed in Seher-2006 with the *T* + *B* + Biochar treatment, whereas the shortest (8.4 cm) was observed in Galaxy-2013 under the same treatment. Peduncle length was highest in Anaj-2017 with mixed cultures of BCAs (*T* +*B*) and lowest in Galaxy-2013 treated with *Trichoderma* sp. alone. Similarly, plant height ranged from 75.06 cm (Dilkash-2020, *B* + biochar) to 90.27 cm (Dilkash-2020, *T* + *B*), highlighting the genotypic response to seed treatment. Root length was greatest in Seher-2006 (15.53 cm) and Anaj-2017 (14.83 cm) under *T* + *B* treatment, with and without biochar, respectively. Only statistically significant differences ($p \leq 0.05$) were interpreted, while non-significant variations were not considered biologically meaningful.

Seed Priming and Biochar Effects on Wheat Yield and Rust

The results of yield-related traits summarized in Table 2 showed significant varietal differences in number of tillers (NOT), spike weight (SW), grain weight per spike (GWPS), number of grains per spike (NOGPS), yield per plant (YPP), and brown rust severity (BR). However, no significant differences were observed for spike length (SL) among treatments ($p > 0.05$); therefore, this trait was not interpreted further.

Table 1. Impact of different biological control agents and biochar treatments on growth performance across four wheat genotypes

Varieties	Treatments	AL (cm)	DRW (g)	FRW (g)	IL (cm)	PL (cm)	PH (cm)	RL (cm)
Seher-2006	*B*	7.6^{a-f}	1.3^{d-g}	2.3^{d-i}	11.2^{f-j}	27.7^{cdef}	77.4^{d-g}	12.3^{a-d}
	T	7.5^{a-f}	1.4^{d-g}	2.4^{c-h}	12.8^{b-h}	30.2^{a-e}	80.8^{b-g}	14.1^{ab}
	T + *B*	7.1^{a-g}	1.5^{defg}	2.5^{c-g}	13.1^{a-g}	29.6^{a-e}	85.2^{a-d}	15.5^{a}
	B + BC	6.6^{d-g}	1.3^{efg}	1.8^{hij}	12.1^{c-i}	28.5^{a-e}	81.3^{b-g}	13.7^{ab}
	T + BC	6.7^{c-g}	1.2^{fg}	1.7^{ij}	14.1^{abc}	30.0^{a-e}	84.7^{a-e}	14.5^{ab}
	T + *B* + BC	7.1^{a-g}	1.9^{cd}	2.0^{e-j}	15.0^{a}	30.5^{a-e}	87.5^{ab}	13.6^{ab}
	Control	5.7^{g}	1.8^{de}	2.0^{d-j}	14.5^{ab}	28.4^{a-e}	83.4^{a-f}	13.7^{ab}
Dilkash-2020	*B*	7.7^{a-e}	1.5^{defg}	1.9^{f-j}	10.7^{ij}	27.2^{def}	75.1^{g}	11.3^{a-d}
	T	6.3^{efg}	1.1^{g}	1.9^{g-j}	13.3^{a-e}	29.1^{a-e}	82.8^{a-f}	14.6^{ab}
	T + *B*	7.2^{a-f}	1.2^{fg}	2.1^{d-j}	12.6^{b-i}	29.4^{a-e}	90.3^{a}	13.7^{ab}
	B + BC	7.5^{a-f}	1.5^{defg}	2.5^{c-g}	12.5^{b-i}	29.1^{a-e}	75.1^{g}	13.0^{ab}
	T + BC	8.3^{a}	1.8^{de}	2.1^{d-j}	11.8^{d-i}	27.4^{c-f}	82.8^{a-f}	12.1^{a-d}
	T + *B* + BC	6.9^{bc-g}	1.4^{defg}	2.5^{c-f}	11.8^{d-i}	26.0^{ef}	83.3^{a-f}	14.3^{ab}
	Control	7.2^{a-f}	1.7^{def}	3.0^{abc}	11.1^{g-j}	27.7^{c-f}	80.8^{b-g}	15.3^{a}
Galaxy-2013	*B*	7.2^{a-f}	1.7^{de}	2.2^{d-j}	13.2^{a-f}	32.5^{a}	88.2^{ab}	12.0^{abcd}
	T	8.2^{ab}	2.9^{ab}	3.3^{ab}	12.3^{c-i}	23.7^{f}	81.4^{b-g}	14.9^{ab}
	T + *B*	8.1^{abc}	1.1^{fg}	2.0^{d-j}	12.1^{c-i}	29.5^{a-e}	75.8^{fg}	7.9^{d}
	B + BC	8.3^{a}	1.9^{cd}	2.6^{cde}	12.3^{c-i}	32.2^{ab}	79.0^{c-g}	8.4^{cd}
	T + BC	7.4^{a-f}	1.5^{defg}	2.2^{d-i}	9.5^{jk}	29.4^{a-e}	79.3^{c-g}	11.5^{a-d}
	T + *B* + BC	7.7^{a-e}	1.0^{g}	1.5^{j}	8.4^{k}	29.2^{a-e}	77.2^{efg}	10.7^{bcd}
	Control	7.3^{a-f}	1.4^{defg}	2.1^{d-j}	10.9^{hij}	29.4^{a-e}	76.8^{efg}	12.2^{a-d}
Anaj-2017	*B*	8.2^{ab}	1.4^{defg}	2.3^{d-i}	13.8^{a-d}	31.8^{abc}	86.7^{abc}	14.0^{ab}
	T	7.7^{a-e}	1.3^{efg}	1.9^{g-j}	11.8^{d-i}	29.5^{a-e}	75.3^{g}	10.7^{bcd}
	T + *B*	8.0^{abcd}	2.4^{bc}	2.6^{bcd}	11.8^{d-i}	32.8^{a}	82.5^{a-f}	14.8^{ab}
	B + BC	7.0^{abcd-g}	3.0^{a}	3.5^{a}	11.7^{e-i}	32.5^{a}	80.7^{b-g}	13.7^{ab}
	T + BC	8.3^{a}	2.5^{ab}	3.0^{abc}	11.6^{e-j}	30.7^{a-d}	81.8^{b-g}	12.7^{abc}
	T + *B* + BC	6.7^{c-g}	1.1^{fg}	1.6^{ij}	11.8^{d-i}	32.5^{a}	79.0^{cdefg}	14.0^{ab}
	Control	6.2^{fg}	1.5^{defg}	1.9^{f-j}	11.2^{f-j}	27.7^{b-f}	77.5^{d-g}	13.9^{ab}
P value		0.1576	0.0003	0.0000	0.0001	0.1403	0.0044	0.2642

Data are means of three replicates. Treatments included *Trichoderma* sp. (*T*), *Bacillus* sp. (*B*), their combination (*T* + *B*), and integration with biochar (BC; 1.5 g plant^{-1}), along with a control (sterile water). Genotypes included Dilkash-2020 and Anaj-2017 (resistant), and Galaxy-2013 and Seher-2006 (susceptible). Traits measured were awn length (AL), dry root weight (DRW), fresh root weight (FRW), internode length (IL), peduncle length (PL), plant height (PH), and root length (RL). Different superscript letters within columns indicate significant differences at $P \leq 0.05$ (LSD test), while P-values show overall treatment effects.

The maximum number of tillers (4.67) was recorded in Seher-2006 under *Trichoderma* treatment, while the lowest (2.00) was observed in Galaxy-2013 with T + BC. The highest spike weight (2.93 g) was observed in Anaj-2017 under control conditions, whereas the lowest (1.40 g) was recorded in Dilkash-2020 under control. Grain weight per spike (2.37 g) was highest in Anaj-2017 control plants, while the lowest (1.13 g) was observed in Anaj-2017 under *Bacillus* sp. treatment. The number of grains per spike (46.33) was highest in Anaj-2017 under T + B + BC treatment, while the lowest (23.00) was recorded in Dilkash-2020 control. Yield per plant (7.10 g) was also highest in Anaj-2017 under *Trichoderma* sp. treatment, whereas the lowest yield (2.53 g) was observed in Galaxy-2013 under T + BC treatment. Brown rust severity showed clear genotype- and treatment-dependent patterns, with resistant genotypes (Anaj-2017 and Dilkash-2020) exhibiting lower disease levels under microbial treatments compared to susceptible genotypes.

Table 2. Effect of BCAs and biochar application on yield-related parameters in four wheat genotypes.

Varieties	Treatments	SL (cm)	NOT	SW (g)	GWPS (g)	NOGPS	YPP (g)	BR
Seher-2006	*B*	10.0^{b-e}	3.3^{cde}	1.9^{f-i}	1.3^{c-i}	30.3^{c-g}	4.4^{ghi}	1.7^{ab}
	T	9.2^{de}	4.7^{a}	1.6^{hi}	1.2^{f-j}	27.0^{efg}	5.6^{cdefg}	2.0^{ab}
	T + *B*	10.0^{bcde}	3.3^{cde}	1.9^{e-i}	1.2^{e-j}	30.3^{c-g}	4.1^{hi}	2.0^{ab}
	B + BC	9.2^{de}	3.0^{de}	2.7^{a-e}	1.4^{b-g}	43.7^{ab}	4.2^{hi}	1.3^{a}
	T + BC	10.3^{b-e}	3.3^{cde}	2.1^{c-i}	1.3^{e-j}	35.7^{a-f}	4.2^{hi}	1.3^{a}
	T + *B* + BC	9.7^{cde}	3.7^{bcd}	2.0^{defghi}	1.4^{b-h}	33.0^{bc-g}	5.0^{defgh}	1.7^{ab}
	Control	9.3^{de}	3.7^{bcd}	2.9^{ab}	1.1^{h-j}	42.3^{ab}	2.6^{jk}	1.3^{a}
Dilkash-2020	*B*	10.8^{abc}	3.0^{de}	2.0^{d-i}	1.5^{a-d}	37.0^{a-f}	3.6^{ij}	1.0^{a}
	T	9.7^{cde}	4.3^{ab}	2.5^{a-g}	1.3^{c-i}	32.7^{b-g}	5.9^{abcde}	1.3^{a}
	T + *B*	10.7^{a-d}	4.3^{ab}	2.5^{a-g}	1.6^{ab}	41.0^{abc}	6.9^{ab}	1.3^{a}
	B + BC	11.0^{abc}	4.0^{abc}	2.6^{a-f}	1.5^{a-d}	35.0^{ab-f}	6.1^{abcd}	1.0^{a}
	T + BC	10.8^{abc}	4.3^{ab}	1.9^{e-i}	1.4^{b-f}	33.0^{bc-g}	6.2^{abc}	1.3^{a}
	T + *B* + BC	10.7^{a-d}	2.7^{ef}	2.3^{a-h}	1.3^{c-i}	25.7^{fg}	3.6^{ij}	1.3^{a}
	Control	11.2^{abc}	2.7^{ef}	1.4^{i}	1.1^{hij}	23.0^{g}	2.3^{k}	2.7^{b}
Galaxy-2013	*B*	11.4^{ab}	3.3^{cde}	2.3^{a-h}	1.2^{g-j}	32.3^{b-g}	3.9^{hi}	1.3^{a}
	T	11.5^{ab}	4.3^{ab}	2.7^{a-d}	1.1^{hij}	39.0^{abcd}	4.9^{defgh}	1.3^{a}
	T + *B*	10.9^{abc}	3.0^{de}	2.1^{b-i}	1.2^{f-j}	33.0^{b-g}	3.6^{ij}	1.7^{ab}
	B + BC	10.2^{b-e}	3.3^{cde}	2.3^{a-h}	1.3^{d-j}	39.0^{abcd}	4.3^{ghi}	1.3^{a}
	T + BC	12.1^{a}	2.0^{f}	2.3^{a-h}	1.3^{e-j}	36.3^{a-f}	2.5^{jk}	1.3^{a}
	T + *B* + BC	9.0^{e}	3.7^{bcd}	1.8^{ghi}	1.1^{hij}	27.7^{d-g}	4.2^{hi}	2.0^{b}
	Control	9.8^{cde}	3.3^{cde}	2.6^{a-f}	1.1^{j}	34.3^{b-g}	2.1^{k}	1.0^{a}
Anaj-2017	*B*	10.7^{a-d}	4.0^{abc}	2.5^{a-g}	1.1^{hij}	35.7^{a-f}	4.2^{hi}	1.3^{a}
	T	9.0^{e}	4.0^{abc}	2.0^{c-i}	1.8^{a}	30.3^{c-g}	7.1^{a}	1.0^{a}
	T + *B*	10.7^{a-d}	3.0^{de}	2.8^{abc}	1.5^{b-e}	43.7^{ab}	4.4^{ghi}	1.7^{ab}
	B + BC	9.8^{cde}	3.7^{bcd}	2.7^{a-e}	1.6^{abc}	38.3^{a-e}	5.8^{bcdef}	1.7^{ab}
	T + BC	11.2^{abc}	3.3^{cde}	2.4^{a-h}	1.4^{b-g}	38.0^{a-e}	4.6^{fghi}	2.0^{b}
	T + *B* + BC	10.3^{b-e}	3.7^{bcd}	2.7^{a-d}	1.3^{d-j}	46.3^{a}	4.7^{efghi}	1.0^{a}
	Control	10.4^{b-e}	3.0^{de}	2.9^{a}	1.1^{ij}	42.3^{ab}	3.9^{hi}	2.3^{c}
P value		0.1624	0.0000	0.0189	0.0006	0.0262	0.0001	

Data are means of three replicates. Treatments included *Trichoderma* sp. (T), *Bacillus* sp. (B), their combination (T + B), and biochar (BC; 1.5 g plant^{-1}), along with a control. Traits measured were spike length (SL), number of tillers (NOT), spike weight (SW), grain weight per spike (GWPS), number of grains per spike (NOGPS), yield per plant (YPP), and brown rust severity (BR). Different superscript letters indicate significant differences at $P \leq 0.05$ (LSD test), and *P*-values show overall treatment effects.

Correlation Analysis

The correlation matrix revealed strong interrelationships among 14 major traits, including morphological, yield-related, and disease-associated characteristics (Figure 1). Pearson's correlation coefficients (r) and their significance levels (p) were analyzed to pinpoint the key factors influencing yield and rust resistance. A close relationship was observed between spike weight (SW) and the number of grains per spike (NOGPS) ($r = 0.81$, $p < 0.001$), while grain weight per spike (GWPS) was also strongly linked to NOGPS ($r = 0.70$, $p < 0.001$). This indicates that spikes with more grains tend to be heavier and contribute to higher overall yield.

Several yield components showed positive and significant correlations. Yield per plant (YPP) exhibited the strongest association with NOGPS ($r = 0.91$, $p < 0.001$), confirming it as the most critical determinant of total grain yield. YPP also maintained moderate positive relationships with the number of tillers (NOT) ($r = 0.60$, $p = 0.002$) and GWPS ($r = 0.58$, $p = 0.003$), suggesting that these traits jointly enhance productivity. Likewise, SW displayed a moderate positive correlation with NOGPS ($r = 0.64$, $p = 0.001$), implying that spikes with greater weight usually contain more grains.

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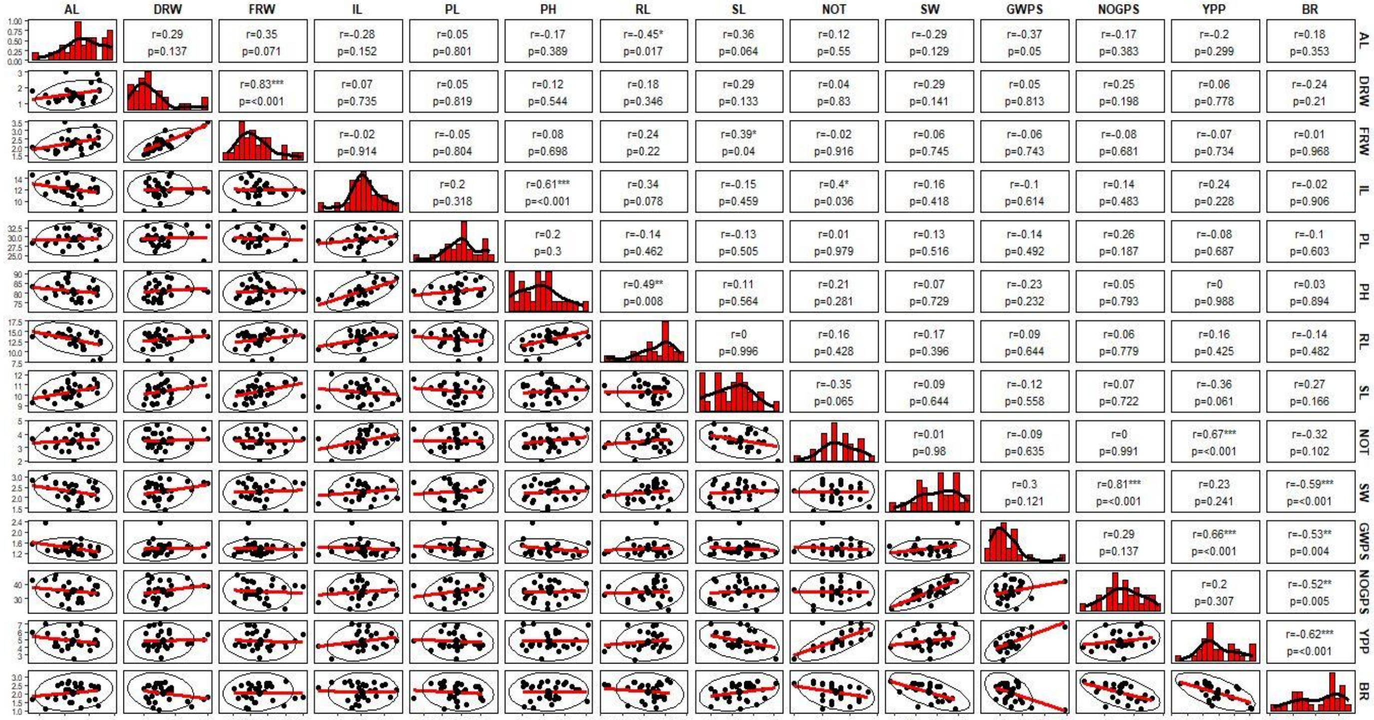

Figure 1. The correlation matrix for key agronomic traits of the samples is shown in the above figure. The diagonal panels display histograms illustrating the distribution of each trait. The upper triangle presents Pearson correlation coefficients (r), ranging from −1 to +1, with significant levels (*p < 0.05), (**p < 0.01), (*** p < 0.001). The lower triangle includes scatter plots visualizing trait pair distributions with regression lines. (Correlation plot was generated using the GGally package in R (v4.5.1, https://cran.r-project.org/bin/windows/base/).

Among morphological traits, plant height (PH) and peduncle length (PL) were moderately associated (*r* = 0.64, *p* = 0.001), indicating that taller plants tend to develop longer peduncles. A strong relationship was also evident between dry root weight (DRW) and fresh root weight (FRW) (*r* = 0.83, *p* < 0.001). Notably, the disease-related trait, BR, showed a significant negative correlation with grain weight (GW) (*r* = -0.63, *p* = 0.001), suggesting that higher rust severity adversely affects grain filling. BR also had moderate negative associations with NOT (*r* = -0.58, *p* = 0.003) and YPP (*r* = -0.58, *p* = 0.003). This indicates that yield decreases under disease pressure.

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Cluster and Biplot Analysis Based on Principal Component Analysis

Principal component analysis (PCA) was performed to examine multivariate relationships among 14 traits in wheat varieties and treatments. The first two principal components, PC1 (20.9%) and PC2 (18.1%), together explained 39% of the total variation and were used for both genotype (Figure 2) and treatment (Figure 3) clustering.

In Figure 2, Anaj-2017 was associated with yield-related traits (YPP, SW, NOGPS, FRW, DRW), while Galaxy-2013 was linked with spike length (SL) and plant height (PH). Seher-2006 showed comparatively moderate trait associations.

In Figure 3, treatments showed partial separation with noticeable overlap, indicating variable responses. *Trichoderma* sp. (T) was associated with several growth and yield traits, whereas *Bacillus* sp. (B) treatments showed greater variability. Combined treatments formed relatively compact clusters, while control treatments were generally positioned toward the negative side of PC1.

PCA biplots (Figures 4 and 5) were generated separately to illustrate trait associations with genotypes and treatments. In Figure 4, the first two principal components (PC1 = 21% and PC2 = 18%) explained 39% of the total variation. Yield-related traits (YPP, GWPS, SW, and NOGPS) were positively associated and aligned with Anaj-2017, while spike-related traits (SL and AL) were oriented toward Galaxy-2013. Root traits (FRW and DRW) showed a close association, indicating a strong correlation. In Figure 5, PC1 (37.4%) and PC2 (21.2%) together explained 58.6% of the total variability. Yield-related traits were aligned with *Trichoderma* sp. treatments, whereas control and biochar-only treatments were associated with higher brown rust (BR).

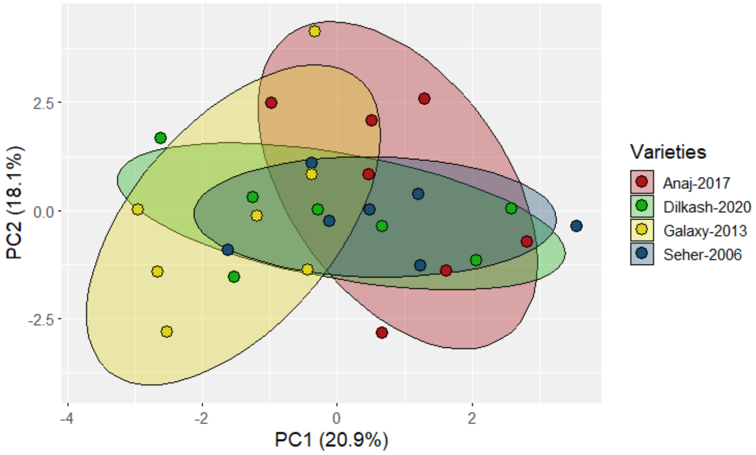

Figure 2. Cluster analysis of wheat varieties performance based on PCA. The plot shows the distribution of wheat varieties in PC1 and PC2 space and is color-coded by treatment. Ellipses represent 95% confidence intervals for the variety groups in which dots represent individual samples.

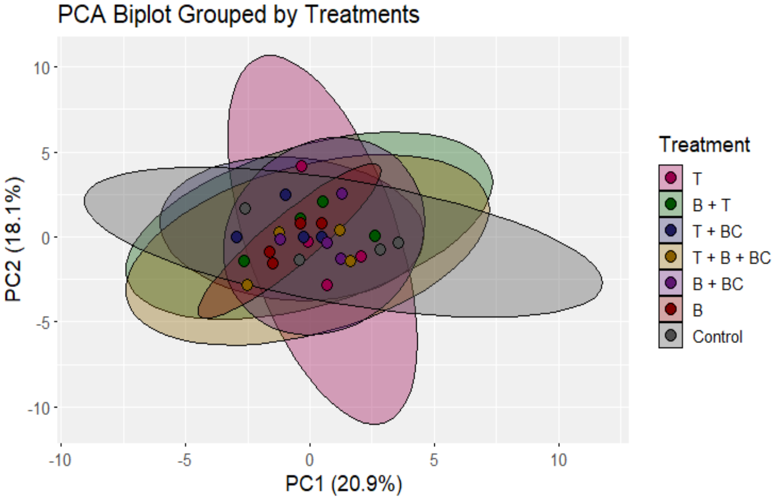

Figure 3. Cluster analysis of treatments showing sample distribution along PC1 (20.9%) and PC2 (18.1%), explaining 39% of total variance. Ellipses indicate 95% confidence intervals.

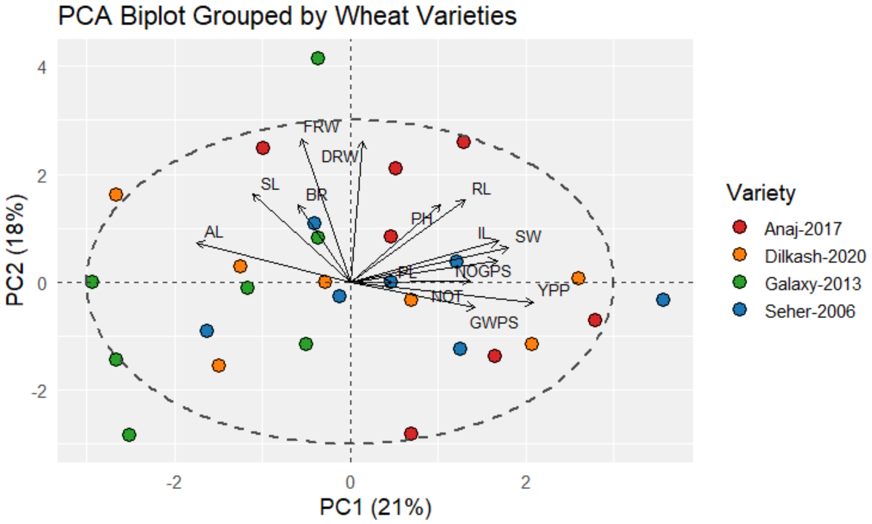

Figure 4. PCA plot: illustrating the distribution of wheat genotypes across the first two principal components (PC1 accounting for 21% and PC2 accounting for 18%, together explaining 39% of the total variance), with each sample color-coded by genotype.

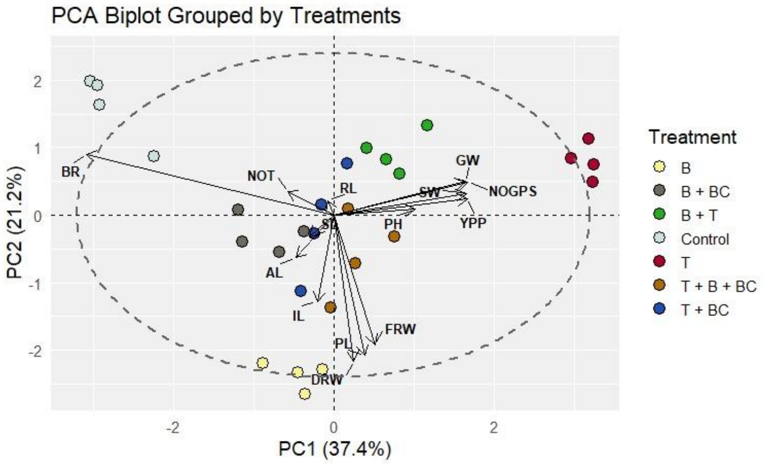

Figure 5. PCA biplot showing the spatial distribution of wheat samples based on treatment groups, plotted across the first two principal components (PC1 = 37.4%, PC2 = 21.2%, cumulatively explaining 58.6% of total variability).

DISCUSSION

Wheat (*Triticum aestivum* L.) is an important cereal crop cultivated in more than 120 countries. *Puccinia triticina* (an obligate foliar pathogen), the causal agent of brown rust disease of wheat, causes significant yield losses (Alemu *et al*., 2019). In this study, seed priming with BCAs, especially *Trichoderma* sp. and *Bacillus* sp., with or without biochar integration, significantly promoted wheat growth and reduced brown rust severity. Results of the current study revealed that seed priming with *Trichoderma* sp. significantly improved several yield-associated traits of wheat, including grain weight per spike (GWPS), number of grains per spike (NOGPS), and yield per plant (YPP). Among the genotypes tested, Anaj-2017 had the strongest and most consistent response to this treatment (Table 2, Fig. 4). These observations are consistent with previous reports linking treatments to improved nutrient uptake, rhizosphere colonization, and induced systemic resistance (Shoresh *et al*., 2010; Jabran *et al*., 2024; Fatima *et al*., 2024).

At the genotypic level, Anaj-2017 also recorded the highest fresh and dry root weights (FRW and DRW) under the *Bacillus* sp. + biochar treatment group, which suggests superior root growth and nutrient intake ability. In contrast, Galaxy-2013 had the lowest values under the combination of *Trichoderma* sp. + *Bacillus* sp. + biochar treatment. These results are consistent with Buensanteai *et al*. (2014), who reported improved shoot and root biomass in chili plants under combined microbial treatments, although such responses may vary depending on crop species and environmental conditions. Dilkash-2020 displayed the highest recorded plant height (90.27 cm) under the *Trichoderma* sp. + *Bacillus* sp. treatment. This indicates that utilizing biological control agents can contribute to increased plant growth potential and improved photosynthetic activity, as noted by Rawat *et al*. (2018). The longest length of spikes was found in Galaxy-2013 under *Trichoderma* + biochar, and the maximum tillers were generated from Seher-2006 using *Trichoderma* alone. These findings support the conclusions of Afzal *et al*. (2023), who found spikes and tillers were improved by *T. harzianum* inoculation of wheat.

The highest brown rust symptoms were found in the rust-susceptible genotype Galaxy-2013 under control conditions, while seed priming with *Trichoderma* sp. greatly reduced disease infection. These findings agree with the results by El-Mougy *et al*. (2021) and Silva *et al*. (2012), which indicated effective suppression of foliar diseases by *T. harzianum*, potential achieved through their early colonization and production of antifungal metabolites. Biological yield differed significantly among genotypes and by treatment (Table 2). Anaj-2017 produced the highest biological yield (7.07 g/plant) among the treatments when grown with *Trichoderma* sp., which supports its adaptability and strong response to microbial inoculation. In contrast, control plants consistently produced the lowest yields, highlighting the pronounced benefits of BCA-based priming on growth and productivity.

Although biochar supplementation showed positive effects on plant growth and rust suppression, its performance was not consistently superior to treatments without biochar. The inconsistency in results may be attributed to the timing of the treatments, as biochar was mixed into the soil just before sowing and may not have had enough time to interact effectively with soil microbes. Similar findings have been reported by Thies and Rillig (2012), who noted that biochar often requires an incubation period to stabilize in the soil matrix and interact effectively with the native microbiome. The moderate response of wheat to biochar likely reflects the transitional phase rather than a lack of biological activity. Overall, the effectiveness of these treatments was genotype-dependent, with Anaj-2017 and Dilkash-2020 showing the greatest responsiveness. The results indicate a potential role of BCAs in wheat disease management; however, their effectiveness may vary depending on genotype and environmental conditions. This study has several limitations, as it was conducted at a single site and season. So, results may vary under different soil and climates. Only short-term effects of BCAs and biochar were tested, leaving scope for long-term studies and additional materials. Moreover, detailed physicochemical characterization of biochar was not performed. Future studies should validate these findings across diverse environments and incorporate detailed biochar characterization to support broader application in sustainable agriculture.

CONCLUSION

This study demonstrated that seed priming with BCAs, especially *Trichoderma* sp., with or without biochar, significantly enhanced the growth and yield, and reduced brown rust infection. The efficacy of these treatments was highly influenced by genotype, with Anaj-2017 and Dilkash-2020 showing the highest responsiveness. These findings suggest potential benefits of combining microbial inoculants with organic amendments in promoting plant health and disease resistance; however, these effects were not consistently superior to individual treatments. Further work should focus on exploring genotype-specific responses and microbial interactions with biochar integration as soil amendment under diverse agro-climatic conditions.

AUTHOR CONTRIBUTIONS

Rabia Irshad conducted the main research and wrote the methodology and results. Ameer Hamza Aslam helped in data collection and analysis using R Studio. Malaika Tariq assisted with data collection. Ikhlas Shafique helped in conducting the experiment and data collection. Muhammad Usman assisted in statistical analysis. Muhammad Naveed provided the BCA cultures and reviewed the manuscript. Amjad Abbas helped with experimental design and supervised the study.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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