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Enhancing Wheat Crop Resilience to Drought Stress through Cellulolytic Microbe-Enriched Cow Dung Vermicompost

Ali Ahmad, Zubair Aslam,* Rana Nadeem Abbas, Korkmaz Bellitürk, Saddam Hussain, Sadam Hussain, Muhammad Ahmad, Usman Zulfiqar,* Ihab Mohamed Moussa, and Mohamed S. Elshikh

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ABSTRACT: Background: Wheat, an important cereal crop, is commonly cultivated in arid and semiarid areas, and therefore, it often experiences water deficit conditions. The consequences of induced stress on wheat can be mitigated through vermicompost amendments. To address drought stress on wheat seedlings, a pot experiment was conducted in the wire-house in which two contrasting wheat cultivars, Faisalabad-08 (drought-tolerant) and Galaxy-13 (drought-sensitive), were exposed to three water level conditions: well-watered [D0, 70% of field capacity (FC)], moderate drought (D1, 45% FC), and severe drought (D2, 30% FC). Four rates of vermicompost, derived from cow dung enriched with cellulolytic microbes, were applied (VT0, control; VT1, 4 t ha^{-1}; VT2, 6 t ha^{-1}; and VT3, 8 t ha^{-1}) to the experiment. Data on various physiological, biochemical, and enzymatic antioxidants were recorded. Results: Our results demonstrated that the drought treatments significantly reduced nutrient accumulation, chlorophyll and SPAD values, and carotenoid content in both cultivars where the maximum reduction was recorded for severe drought stress. Nonetheless, the application of vermicompost significantly improved these traits, and statistically maximum chlorophyll contents, SPAD value, and total carotenoid contents were observed for VT1 in both cultivars under drought treatments. While the lowest chlorophyll and carotenoid contents were recorded for untreated replicated pots. Among the cultivars, Faisalabad-08 exhibited greater resistance to drought, as evidenced by higher values of the aforementioned traits compared to Galaxy-13. Soil-applied vermicompost also showed a positive influence on antioxidant enzyme activities in both wheat cultivars grown under well-watered as well as water-scarce conditions. Conclusions: The findings of this study revealed that drought conditions substantially decreased the enzymatic antioxidants and physiological and biochemical attributes of the wheat crop. However, soil-applied vermicompost, particularly at an optimum rate, had a positive impact on the wheat seedlings under drought conditions. Moving forward, exploring the potential of utilizing cellulolytic microbe-enriched cow dung vermicompost stands as a promising avenue to mitigate the detrimental effects of water stress on wheat. Further research in this direction could offer substantial insights into enhancing wheat resilience and productivity under water stress conditions.

1. INTRODUCTION

Wheat is a major staple crop that fulfills the caloric and protein needs of over 80% of the global population.[1] According to an estimate, about 788.50 million metric tons of wheat are produced worldwide each year. Genetically, wheat is categorized into two major groups: hexaploid bread wheat and tetraploid durum wheat.[2] Bread wheat (*Triticum aestivum* L.) always shared a major part of the cultivated area as compared with durum wheat. Wheat crop is widely grown across diverse agro-climatic conditions, ranging from tropical to subtropical regions, which exposes it to various environmental stresses. Abiotic stresses, also known as environmental stresses, such as extreme temperature, drought, salt-alkalinization, and metal contaminants exert detrimental effects on the growth, development, and physiology as well as biochemical traits in plants.[3−5] However, drought is one of the most important environmental stresses restricting the growth and developmental processes and yield of crop plants.[6]

Drought is a major problem in both arid and semiarid regions. Drought stress and insufficient availability of rhizic water severely hamper crop production in terms of both yield and quality on a global scale. Crop plants are highly susceptible to drought conditions, which severely disrupt several morphophysiological functions including seedling growth and photosynthetic efficiency. This disruption primarily occurs due to the limitation of CO_2 uptake, consequently leading to a potential reduction in seed yield.[7−9] Limited water availability also impairs protein biosynthesis and other metabolites in plants. The genetic traits of drought escape, avoidance, and tolerance play a vital role in enhancing and stabilizing crop productivity. The escape mechanism involves early flowering and accelerated reproductive development, with the aim of minimizing excessive yield losses. Avoidance strategies encompass improved water use efficiency achieved through stomatal closure.[10] In addition, plants have evolved diverse tolerance mechanisms to minimize the detrimental effect of drought by restricting their growth and physiological and

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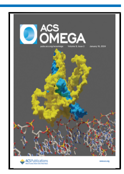

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biochemical mechanisms.[11,12] Previous studies have explored that under moderate drought conditions, the downregulation of the photosynthetic process primarily affects the leaves, resulting in a notable reduction in stomatal conductance. However, under these conditions, the overall photosynthetic efficacy is not markedly influenced. Also, it has been well stated that crop genotypes exhibit variable responses under water-scarce conditions.[13,14] Understanding plant physiological behaviors under drought conditions could pave the way for the advancement of drought-tolerant crop varieties.[15] Under drought conditions, several plant species showed various changes in metabolic activities, along with diminished leaves' relative water content and seedling vigor.[16,17] Wheat seedlings display variable growth rates across different soil field capacities and accumulate a wide range of compatible solutes, such as proline and betaine, which play a critical role in augmenting drought tolerance.[18]

Furthermore, multiple approaches have been used to enhance wheat resistance to drought stress, including the introduction of drought resistance genotypes and the identification and screening of drought-tolerant cultivars using both conventional and nonconventional methods. Variable drought tolerance for various cultivars has been reported in previous published reports.[19] The development of drought-tolerant crop varieties is a crucial strategy for addressing drought stress. However, high cost and extended time requirements have posed significant challenges in advancing the development of drought-tolerant crop varieties. Indeed, different management strategies have gained considerable attention in improving crop performance under drought stress. Among these, soil amendment with vermicompost is a cost-effective and environmentally friendly strategy to bolster crop seedling vigor and production under drought stress.

Vermicompost amendments play a critical role in improving drought tolerance in plants, primarily attributed to their simulative effects on protein, enzyme synthesis, and enzyme activity within plants.[20,21] Vermicompost, characterized by its high porosity, water holding capacity, and humic substances, significantly enhances the soil's physical and chemical properties.[22−24] In vermicompost, the presence of microorganisms like mycorrhizal fungi and soluble compatible compounds contributes to improved soil physical properties and water uptake by plant roots.[25] Vermicompost application as an organic fertilizer amplifies nutrient availability, soil water retention, and uptake, ultimately enhancing plant performance under stress conditions.[24] In comparison to chemical and other organic fertilizers, previous studies have clearly demonstrated improved nutrient availability, as well as increased enzyme and microbial activity, following the application of vermicompost.[26,27] In our previous studies,[2,28] we studied the influence of plant-based vermicompost amendments on the physiological and biochemical characteristics of wheat seedlings under water deficit stress. However, the influence of animal-based vermicompost supplemented with a cellulolytic microbe on wheat seedlings under drought stress remains shrouded in uncertainty. To address this knowledge gap, our study aimed to investigate whether the application of cellulolytic microbe-enriched cow dung vermicompost could effectively mitigate the detrimental effects of drought on wheat seedlings. We hypothesized that this treatment would achieve this by increasing nutrient accumulation and enhancing physio-biochemical characteristics in the plants. Our specific objectives in this study were to elucidate the influence of cow manure vermicompost enriched with cellulolytic microbes on physio-biochemical properties and enzymatic antioxidants of wheat plants subjected to drought conditions. Second, we sought to assess the performance of two contrasting wheat cultivars under different moisture levels.

2. MATERIALS AND METHODS

2.1. Experimentation. The research was conducted in a controlled environment; for that, a wire-house located at the Department of Agronomy, University of Agriculture, Faisalabad (latitude = 31°-04′ N, longitude = 73°-06′ E, altitude = 184.4 m) was used. This study was carried out during the first week of October 2020, utilizing pots filled with soil as the experimental setup. The treatments used in this trial are outlined in Table 1. Two distinct wheat varieties, namely, Faisalabad-08 and Galaxy-13 as drought-tolerant and -sensitive, respectively,[19,29] were procured from Ayub Agricultural Research Institute, Faisalabad. These varieties were subjected to both well-watered conditions and drought stress conditions. Fifteen grains of each cultivar were sown in individual pots filled with soil (20 cm height ×20 cm diameter, using 5 kg of soil per pot). Different rates of cellulolytic microbe-enriched vermicompost were applied to each pot based on the assigned treatments: control (no-vermicompost application, VT0), 4 t ha^{-1} (VT1), 6 t ha^{-1} (VT2), and 8 t ha^{-1} (VT3). Later, 10 seedlings were retained in each pot to ensure a uniform crop stand. The soil with 8.28 pH, 1.46 dSm^{-1} Ec, 0.054% nitrogen (N), 36.5 and 325 ppm accessible phosphorus (P) and exchangeable potassium (K), respectively, and 1.08% organic matter was used for pot filling. Nitrogen and phosphorus at 60 and 57 mg kg^{-1} soil, respectively, were applied as the basal application. Sowing was done with uniform soil moisture in all replicated pots. The pots were arranged in a completely randomized design with factorial arrangements, and the experiment consisted of three replications. The vermicompost was applied as soil application at the time of sowing. Drought stress treatments were imposed by maintaining field capacity (FC), and three treatments were as 70% FC (no drought), 45% FC (mild drought), and 30% FC (severe drought). The water stress treatments were started after the first irrigation and were sustained until harvesting. For replicated pots, water losses were compensated by irrigating to sustain the required FC level. A soil moisture meter (TZS-W) was used to monitor the soil moisture content. After 35 days of stress treatments, leaf samples from each replicated pot were harvested to analyze the biochemical, physiological, and enzymatic antioxidant

Table 1. Treatment Details, Materials, and Rates used

treatments
Factor A: Soil Moisture Levels
D0 = 70% of FC (no drought)
D1 = 45% of FC (mild drought)
D2 = 30% of FC (severe drought)
Factor B: Wheat Cultivars
V1 = Faisalabad-08 (drought tolerant)
V2 = Galaxy-13 (drought sensitive)
Factor C: Cellulolytic Microbe-Enriched Cow Dung Vermicompost
VT0 = control
VT1 = 4 t ha^{-1}
VT2 = 6 t ha^{-1}
VT3 = 8 t ha^{-1}

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Table 2. Raw Material and Vermicompost's Physical and Chemical Analysis[a]

samples	moisture (%)	pH	EC	Ash	N	P	K	Ca	Mg	Fe	S	Cd	Ni	Pb	Hg	Cr
cow dung	31.66	7.50	7.81	30.60	0.68	0.27	0.47	1.86	0.40	0.25	0.18	0.48	11.00	25.33	1.38	9.00
cow dung vermicompost	54.33	6.89	3.75	39.00	1.07	0.81	0.97	3.95	0.73	0.36	0.32	0.21	5.00	11.00	0.95	6.00
cow dung vermicompost enriched with the microbial strain	52.66	6.38	7.76	45.33	1.82	1.01	1.90	4.75	0.92	0.52	0.49	0.09	3.66	5.33	0.71	2.50

[a]pH, potential of hydrogen; EC, electrical conductivity (dS/m); Ash (%); N, nitrogen (%); P, phosphorus (%); K, potassium (%); Ca, calcium (%); Mg, magnesium (%); Fe, iron (%); S, sulfur (%); Cd, cadmium (ppm); Ni, nickel (ppm); Pb, lead (ppm); Hg, mercury (ppm); Cr, chromium (ppm).

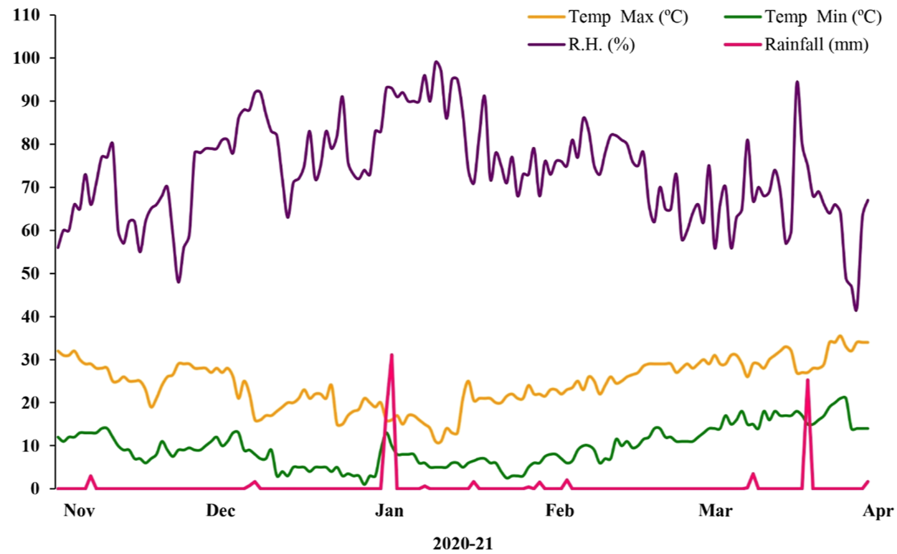

Figure 1. Weather data during 2020−21. Temp: temperature; RH: relative humidity.

activities. In order to compare the oxidative traits and chlorophyll pigments, the rate of vermicompost that yielded significantly higher physiological and biochemical indices was compared against the control.

2.2. Preparation of Vermicompost. The most commonly used earthworm for vermicomposting, *Eisenia fetida* (an epigeic species), was employed as the test species. Animal wastes and cellulosic materials have a substantial role on a global scale, given their abundant availability and their capability to be converted into sugars, chemical feedstock, and fuels.[30] Cellulose-degrading bacteria were utilized as inoculants to enhance the vermicomposting process and improve nutrient availability for crops, aimed at creating the finest vermicompost characterized by improved nutrient accessibility and a thriving microbiota. These bacteria were extracted from the gut of earthworm species, following the proposed procedures of Shankar et al.[31] and Goteti et al.[32] The serial dilution method was utilized to isolate active bacteria, where first the procedure was done at 28 °C and then extracted on half-nutrient agar media. In this study, a total of one hundred strains were used initially, out of which only eight strains were found to be active for degrading cellulosic material. Among the active strains, C-18 was found to be the most effective. Later, a broth medium was used for mass cultivation of this strain which was injected with precomposting material. A fully mature vermicompost was prepared after 60 days, which was applied to wheat after sieving.

The chemical composition such as pH, electric conductivity, nutrient, and heavy metal concentrations of prepared vermicompost and raw material (cow-manure) quantified by following the standard protocols of Ahmad et al.[2] and Aslam et al.[30] is shown in Table 2.

2.3. Meteorological Conditions. Weather data outside of the wire-house were collected from a nearby weather observatory throughout the crop growing season. The mean values for each month are presented in Figure 1.

2.4. Determination of Biochemical Attributes. *2.4.1. Macro- and Micronutrients.* The concentrations of macro- and micronutrients (including N, P, K, calcium, magnesium, iron, zinc, and sulfur) were measured as proposed by Black[33] using digested plant samples with Kjeldahl's apparatus, spectrophotometer, flame photometer, and atomic absorption spectroscopy technique.

2.4.2. Malondialdehyde, Proline, and Soluble Protein Contents. Malondialdehyde contents were assayed according to Heath and Packer.[34] Proline contents were determined as reported previously by Bates et al.[35] by using the top leaf of wheat. The soluble proteins in the shoot were determined by using Bovine Serum Albumin, according to Bradford.[36]

2.5. Physiological Attributes. The following physiological parameters were measured 60 days after sowing.

2.5.1. Chlorophyll Contents and SPAD Values. Leaf extraction was performed according to the procedure of Arnon.[37] First, 0.2 g of fresh leaves were suspended in 80%

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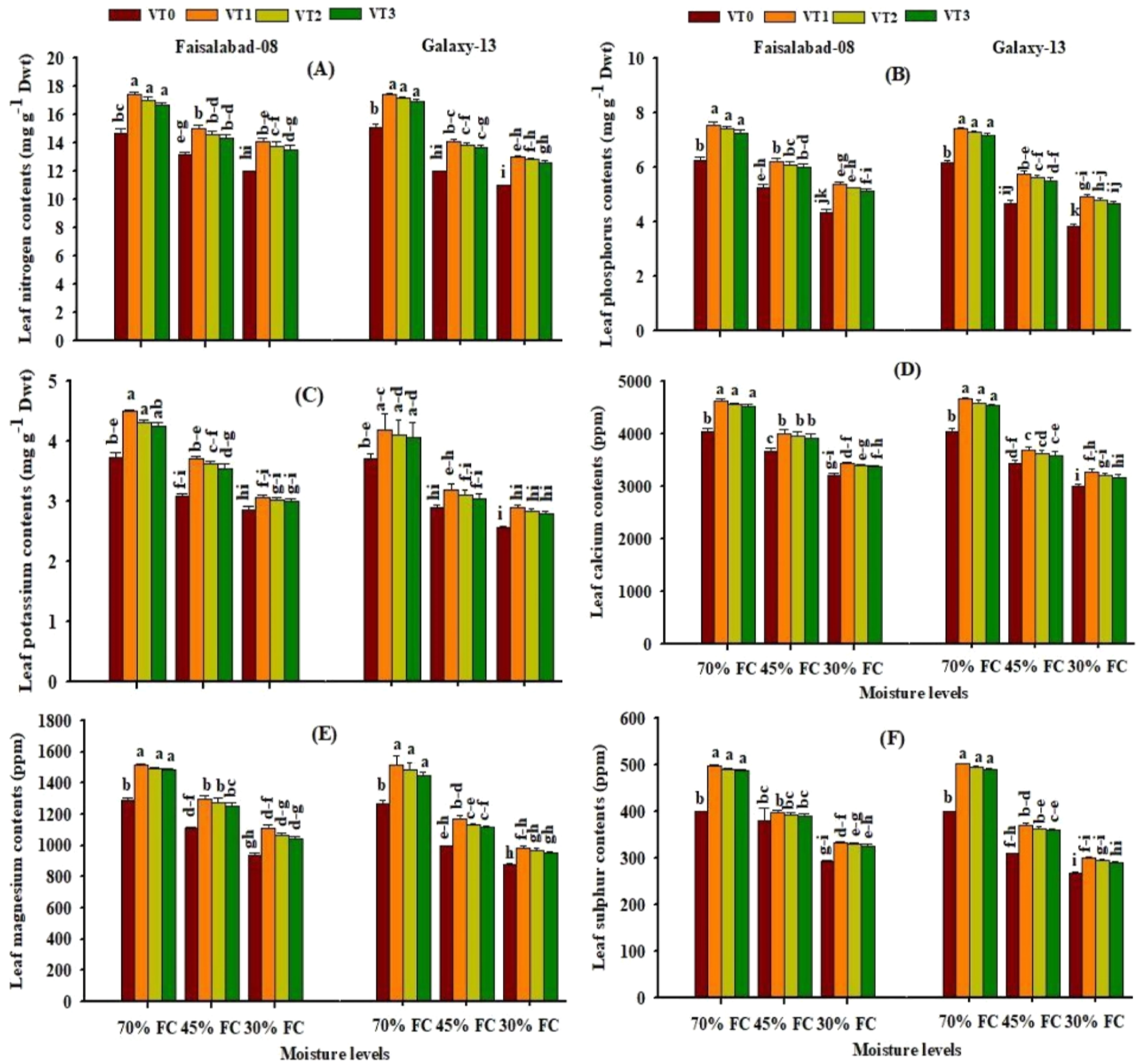

Figure 2. Effect of soil-applied cellulolytic microbe-enriched cow dung vermicompost [VT0 (control), VT1 (4 t ha^{-1}), VT2 (6 t ha^{-1}), and VT3 (8 t ha^{-1})] on (A) leaf nitrogen contents (mg g^{-1} Dwt), (B) leaf phosphorus contents (mg g^{-1} Dwt), (C) leaf potassium contents (mg g^{-1} Dwt), (D) leaf calcium contents (ppm), (E) leaf magnesium contents (ppm), and (F) leaf nitrogen contents (ppm) of two wheat cultivars under different drought levels [control (70% FC), moderate drought (45% FC), and severe drought (30% FC)]. Bars with different alphabetical letters differ significantly at 5% probability, according to Tukey's HSD test.

acetone solution at −4 °C overnight. Later, the supernatant, after centrifugation, was used for the measurement of absorbance spectrometrically at 645 and 663 nm wavelength for chlorophyll *a* and *b* content, respectively. The values were calculated using the formulas

$$\text{Chl. } a = [12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)] \times V/1000 \times W$$

$$\text{Chl. } b = [22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)] \times V/1000 \times W$$

In these equations, *V* indicates the volume of the extract in mL, *W* indicates the weight of the fresh leaf tissue in g, and Chl. indicates the chlorophyll.

A SPAD meter (Model SPAD-502; Minolta Corp., Ramsey, N.J.) was used to measure the SPAD values.

2.5.2. Total Carotenoid Content. The extract for determination of the total carotenoids was prepared according to Yang et al.[38] Fresh leaves (0.2 g) were extracted in acetone (80%). After centrifugation, the extracted supernatant was used for recording the absorbance spectrometrically (spectrophotometer, Hitachi-U2001, Tokyo, Japan) at 480 nm wavelength. The values were calculated using the equations

$$\text{total carotenoids} = \frac{A^{\text{car}}}{\text{Em}} \times 100$$

where Em = 2500 and A^{car} = 480 + 0.114 (OD 663) − 0.638 (OD 645).

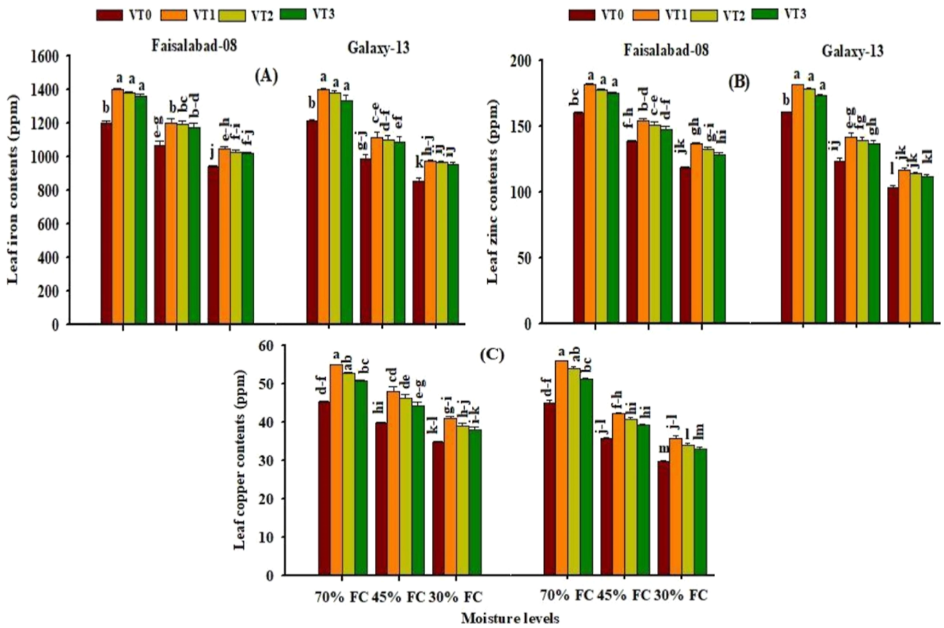

Figure 3. Effect of soil-applied cellulolytic microbe-enriched cow dung vermicompost [VT0 (control), VT1 (4 t ha^{-1}), VT2 (6 t ha^{-1}), and VT3 (8 t ha^{-1})] on (A) leaf iron contents (ppm), (B) leaf zinc contents (ppm), and (C) leaf copper contents (ppm) of two wheat cultivars under different drought levels [control (70% FC), moderate drought (45% FC), and severe drought (30% FC)]. Bars with different alphabetical letters differ significantly at 5% probability, according to Tukey's HSD test.

2.6. Enzymatic Antioxidants. For antioxidant enzyme activities, leaf extraction was performed as reported previously by Chance and Maehly,[39] by using 50 mM phosphate buffer solution (pH 7.8), in which fresh leaves (0.5 g) were grounded. After centrifuging at 15,000*g* for 20 min at 4 °C, the resulting supernatant was used for enzyme assays. Catalase activities were determined using the protocol outlined by Chance and Maehly,[39] with a UV−visible spectrophotometer. Superoxide dismutase activity was determined according to Giannopolitis and Ries.[40] A method described by Putter[41] was followed to determine peroxidase activity.

2.6.1. Ascorbate Peroxidase Activity. The extract for ascorbate peroxidase determination was prepared as reported previously.[42] Fresh leaves (0.1 g) were homogenized using a mortar and pestle in 2 mg of oxalic acid and Na EDTA extraction solution. After centrifugation, 1 mL of supernatant was separated, to which 5 mL of 20 lg mL^{-1} 2, 6-dichlorophenol-indophenol (DCPIP) dye was added to develop the color in solution. Next, the absorbance was taken at 520 nm wavelength, spectrometrically.

2.7. Statistics. The collected data on physiological and biochemical attributes were analyzed using Fisher's analysis of variance (ANOVA) technique. Treatment means were compared by using Tukey's honest significant difference test using Statistix version 8.1 (Analytical Software, 1985−2005).[43] The graphical presentation was made by Sigmaplot software (Sigmaplot 10.0), and the correlation matrix was generated by R-software version 4.0.3 (R Studio, Boston MA, USA).

3. RESULTS

3.1. Nutrient Accumulation and Biochemical Traits. The concentration of macronutrients in leaves was significantly affected under drought stress where a significant reduction ($p \leq 0.05$) was observed under severe and moderate drought stress for both cultivars (Table S1). Among the drought treatments, the most substantial reduction was recorded under severe drought conditions, followed by moderate drought stress and well-watered conditions. Nonetheless, soil-applied cellulolytic microbe-enriched cow dung vermicompost significantly increased the concentration of macronutrients in leaves under well-watered as well as drought conditions (Figure 2). Among the application rates, vermicompost at 4 t ha^{-1} (VT1) recorded significantly higher values of macronutrients under well-watered and drought conditions. Under severe drought, VT1 increased the N content by 17.35 and 18.18%, P by 24.22 and 27.53%, K by 7.34 and 12.89%, Ca by 7.29 and 8.89%, Mg by 18.50 and 11.73%, and S by 13.89 and 11.80% in Faisalabad-08 and Galaxy-13, respectively, compared with the control without vermicompost application. Among the studied cultivars, Faisalabad-08 showed better performance as it recorded significantly higher values of macronutrients under drought conditions as compared with Galaxy-13 (Table S1, Figure 2).

A significant reduction was also observed for micronutrient concentrations (Fe, Zn, and Cu) in leaves of both cultivars subjected to drought stress (Table S2, Figure 3). The application of soil-applied vermicompost considerably ($p \leq 0.05$) improved the concentrations of these nutrients in both cultivars under water deficit and control conditions (well-watered). A significant increase in these traits was recorded when vermicompost was applied at 4 t ha^{-1} (VT1). Under severe drought, VT1 increased Fe content by 10.96 and 13.61%, Zn by 15.82 and 12.91%, and Cu by 18.29 and 20.22% in Faisalabad-08 and Galaxy-13, respectively, compared with the control in which no vermicompost was applied. Among the studied cultivars, Faisalabad-08 showed significantly higher values of these traits than Galaxy-13 under drought conditions; however, the results were statistically at par under well-watered conditions.

The impact of drought treatments was evident in the increased levels of malondialdehyde (MDA) content in the leaves of both wheat cultivars. However, it is worth noting that

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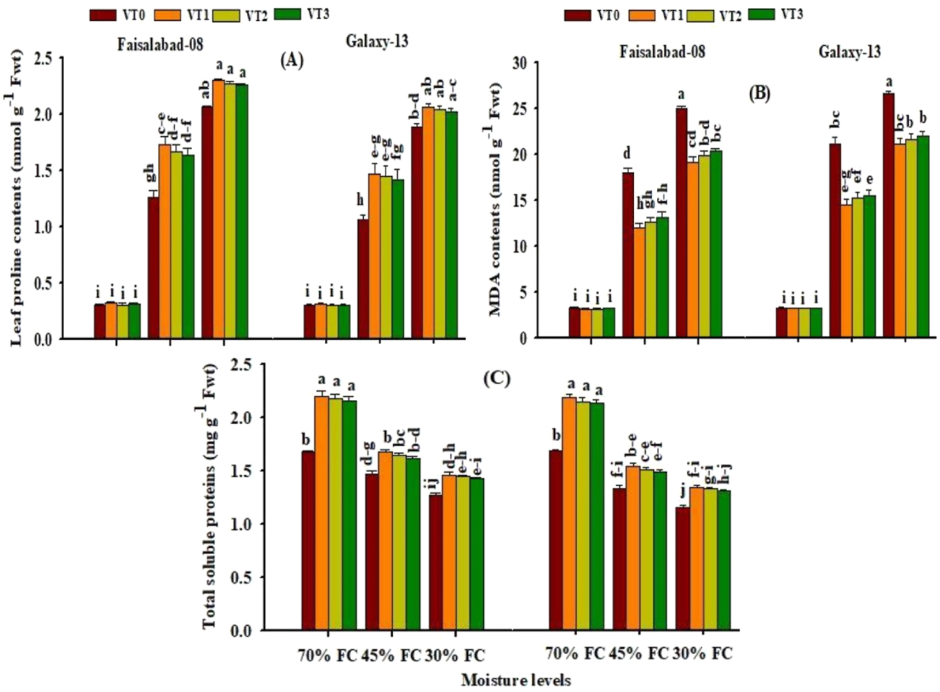

Figure 4. Effect of soil-applied cellulolytic microbe-enriched cow dung vermicompost [VT0 (control), VT1 (4 t ha^{-1}), VT2 (6 t ha^{-1}), and VT3 (8 t ha^{-1})] on (A) leaf proline contents (mmol g^{-1} Fwt), (B) MDA contents (mmol g^{-1} Fwt), and (C) total soluble proteins (mg g^{-1} Fwt) in two wheat cultivars under different drought levels [control (70% FC), moderate drought (45% FC), and severe drought (30% FC)]. Bars with different alphabetical letters differ significantly at 5% probability, according to Tukey's HSD test.

vermicompost amendments had a significant mitigating effect on MDA content under drought conditions. The most substantial reduction in the MDA content was observed with the VT1 treatment, followed by VT2, VT3, and VT0. An opposite trend was recorded for proline and protein contents, where cellulolytic microbe-enriched cow dung vermicompost, particularly at 4 t ha^{-1}, improved the values of these traits of both cultivars under drought conditions (Table S3, Figure 4). Under severe drought, VT1 increased proline content by 11.65 and 9.57% and protein content by 15.87 and 16.52% in Faisalabad-08 and Galaxy-13, respectively, compared with the control.

3.2. Physiological Traits. Drought treatments, both moderate and severe drought, significantly decreased the chlorophyll *a*, *b*, total chlorophyll, SPAD value, and total carotenoid content in both cultivars with a maximum reduction under severe drought stress (Table S4, Figure 5). Nonetheless, the application of cellulolytic microbe-enriched cow dung vermicompost improved these traits in both cultivars under water-scarce and control (well-watered) treatments, and a significant increase was recorded for VT1 (4 t ha^{-1}) followed by VT2, VT3, and VT0 (Figure 5). Under severe drought, VT1 increased chlorophyll *a* content by 10.90 and 13.33%, chlorophyll *b* by 14.28 and 15.62%, total chlorophyll by 11.91 and 14.01%, SPAD value by 13.55 and 9.89%, and total carotenoids content by 12.12 and 13.11% in Faisalabad-08 and Galaxy-13, respectively, compared with the control. Between both cultivars, Faisalabad-08 showed significantly higher values of these traits compared to Galaxy-13 under drought conditions; however, there was a nonsignificant difference under well-watered conditions.

3.3. Antioxidant Enzymes. Superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase activities in both cultivars were altered significantly under drought conditions (Table S5, Figure 6). Antioxidant enzyme activities increased under drought conditions; however, cellulolytic microbe-enriched cow dung vermicompost further increased the activities in both cultivars, where the maximum increase was recorded for VT1. As compared with the control, VT1 increased superoxide dismutase activity by 11.40 and 10.80%, catalase by 18.26 and 17.92%, peroxidase activity by 17.57 and 15.46%, and ascorbate peroxidase by 31.07 and 31.96% in Faisalabad-08 and Galaxy-13, respectively. Under moderate and severe drought, Faisalabad-08 outperformed Galaxy-13.

3.4. Correlation Analysis. Pearson's correlation analysis was performed to determine the relationship between physiological, enzymatic antioxidants, and biochemical characteristics when both wheat cultivars were grown under varying moisture conditions and treated with cow dung vermicompost containing cellulolytic microbes (Figure 7). The buildup of nitrogen, phosphorus, and potassium was favorably associated with wheat cultivars' seedling physiological characteristics. Positive associations were found between the enzymatic antioxidant's catalase, superoxide dismutase, and peroxidase and the membrane stability index or oxidative damage of both wheat cultivars.

4. DISCUSSION

In this study, nutrient uptake by wheat seedlings was significantly decreased under drought stress. Similar results were reported previously by Reddy et al.[44] and Puangbut et al.[45] who stated that decreased nutrient concentrations under drought stress were associated with reduced transpiration rates. Drought stress, both moderate and severe, reduced the uptake of both macro- and micronutrients such as N, P, K, Ca, Mg, Fe, S, Zn, and Cu in wheat leaves. The reduction in nutrient uptake levels observed in this study aligns with previous findings in *Dalbergia sissoo* seedlings.[46] The maximum

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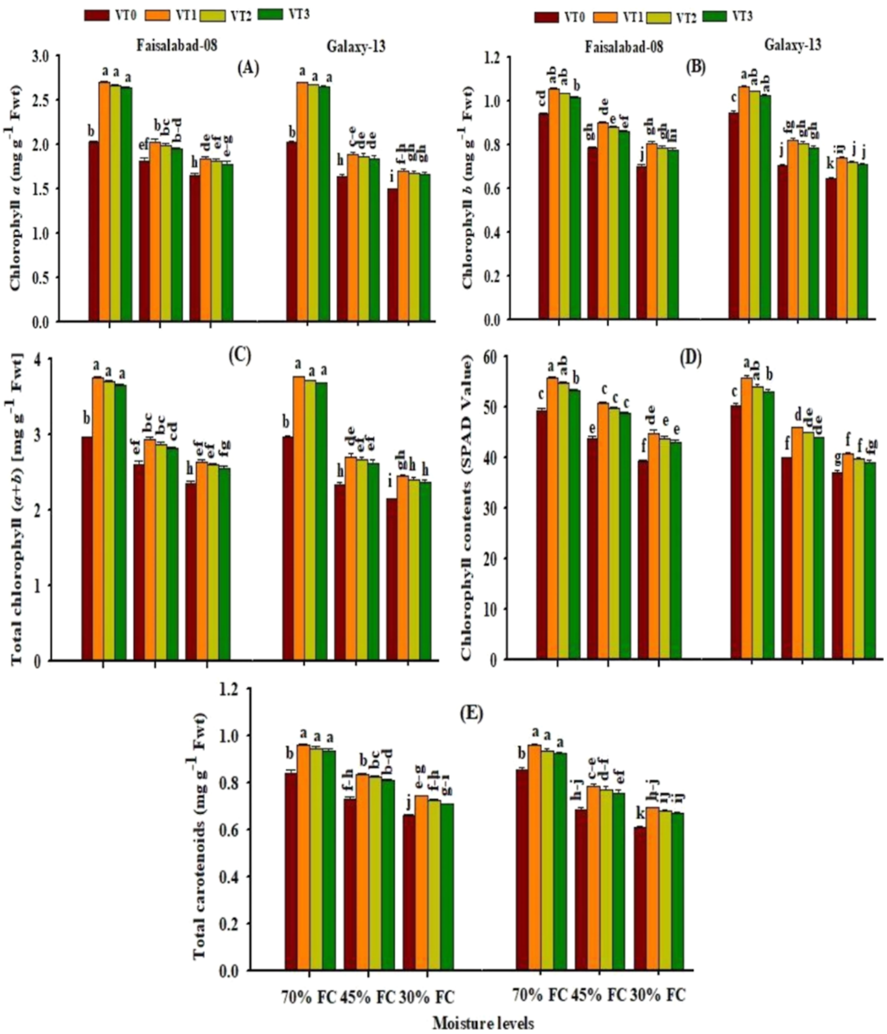

Figure 5. Effect of soil-applied cellulolytic microbe-enriched cow dung vermicompost [VT0 (control), VT1 (4 t ha^{-1}), VT2 (6 t ha^{-1}), and VT3 (8 t ha^{-1})] on (A) chlorophyll *a* (mg g^{-1} Fwt), (B) chlorophyll *b* (mg g^{-1} Fwt), (C) total chlorophyll (*a* + *b*) (mg g^{-1} Fwt), (D) chlorophyll contents (SPAD value), and (E) total carotenoids in two wheat cultivars under different drought levels [control (70% FC), moderate drought (45% FC), and severe drought (30% FC)]. Bars with different alphabetical letters differ significantly at 5% probability, according to Tukey's HSD test.

achievable productivity of a genotype, when cultivated under optimal fertilizer, water conditions, and in a pest- and disease-free environment, is regarded as its production potential.[47] Soil moisture plays a significant role in influencing the transportation of nutrients to the roots as well as their absorption and eventual concentration within the plants. According to Ghanbari et al.,[48] both plant growth and nutrient uptake were diminished under dry conditions. Drought influences the compositions and concentrations of soil solutions due to the decreased soil water retention capacity, which, in turn, affects the rate of diffusion of various plant nutrients.

Total carotenoid and chlorophyll concentrations in wheat leaves (chl. *a* and *b*) are used as indicators of a plant's resistance to drought stress. A high constancy index in these concentrations indicates minimal impact from stress and enhances the availability of chlorophyll.[49] Water-scarce conditions increase the generation of reactive oxygen species (ROS) in plants and accelerate the degradation of chlorophyll.[50] It appears that vermifertilizer incorporates essential micronutrients such as iron, which function as prosthetic groups in hemoproteins, such as CAT, POD, and SOD. These enzymes play a role in scavenging ROS within plants. Vermicompost contains higher nutrient content compared to other organic fertilizers, including N, P, K, Ca, Mg, and micronutrients like Fe, Zn, Cu, and Mn.[51] The reduction of chlorophyll due to drought could potentially influence N metabolism and the production of nitrogenous compounds, such as proline, as part of osmotic regulation.[52] Because glutamate, a precursor to chlorophyll and proline, is inhibited by proline deficiency, chlorophyll synthesis may be reduced.[52] Vermicompost seems to play a role in regulating both water availability and essential nutrients, such as K and N, which are crucial for sustaining proper osmotic pressure within plants. Positive effects were discovered between vermicompost application and total soluble proteins under drought conditions. Wheat plants treated with vermicompost exhibited higher protein content under stressful situations compared to wheat plants that were not treated with vermicompost.[53] The application of cellulolytic bacteria-enriched cow dung vermicompost at 4 t ha^{-1} significantly increased the protein content in both wheat cultivars under moderate and severe drought conditions. Under well-watered conditions, both cultivars exhibited statistically similar protein content. However, in response to drought stress, both cultivars

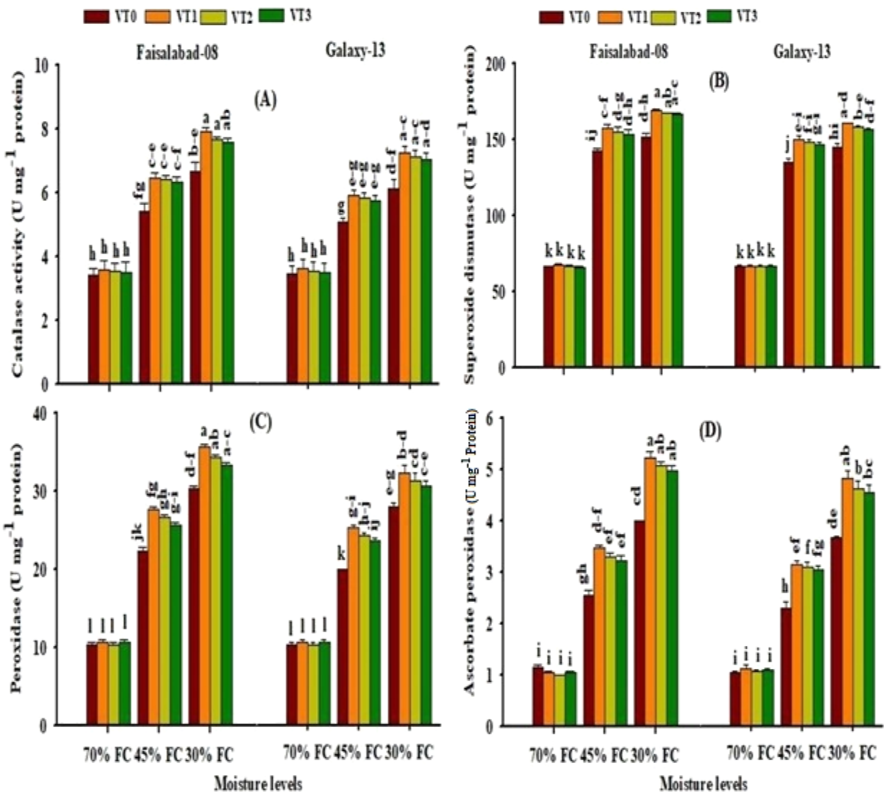

Figure 6. Effect of soil-applied cellulolytic microbe-enriched cow dung vermicompost [VT0 (control), VT1 (4 t ha^{-1}), VT2 (6 t ha^{-1}), and VT3 (8 t ha^{-1})] on (A) catalase (U mg^{-1} protein), (B) superoxide dismutase (U mg^{-1} protein), (C) peroxidase (U mg^{-1} protein), and (D) ascorbate peroxidase (U mg^{-1} protein) activity in two wheat cultivars under different drought levels [control (70% FC), moderate drought (45% FC), and severe drought (30% FC)]. Bars with different alphabetical letters differ significantly at 5% probability, according to Tukey's HSD test.

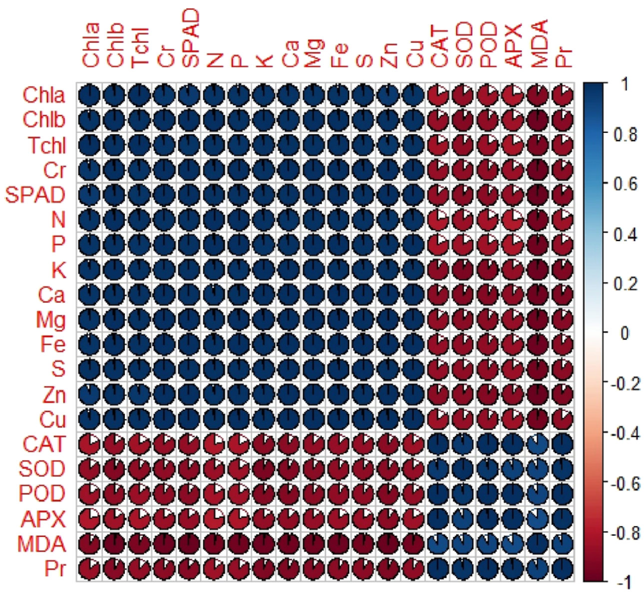

Figure 7. Correlation among biochemical, enzymatic antioxidants, and nutritional traits on two contrasting wheat cultivars under three different moisture levels. Chl *a*; chlorophyll *a*, Chl *b*; chlorophyll *b*, Tchl; total chlorophyll contents (*a* + *b*), Cr; carotenoids contents, SPAD; SPAD value, N; nitrogen contents, P; phosphorus contents, K; potassium contents, Ca; calcium contents, Mg; magnesium contents, Fe; iron contents, S; sulfur contents, Zn; zinc contents, Cu; copper contents, CAT; catalase, SOD; superoxide dismutase, POD; peroxidase, APX; ascorbate peroxidase, MDA; malondialdehyde contents, and Pr; total soluble proteins.

demonstrated distinct reactions in the presence or absence of vermicompost. It is important to note that under moderate and severe drought conditions, the protein content of Faisalabad-08 was much higher than that of Galaxy-13. The increased levels of soluble proteins observed in drought-stressed plants treated with vermicompost were associated with reduced N loss and enhanced N uptake.[54] Drought stress, also known as oxidative stress,[55] can trigger an upsurge in the production and accumulation of ROS such as superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen.[56,57] However, it is also stated that drought stress increased antioxidant activity (CAT, SOD, POD, and APX) compared to that under well-watered conditions. The concentrations of these enzymes increased proportionally with the intensity of the drought. However, their levels remained insufficient to meet the plant's requirements, and drought stress suppressed their activity as well. Consequently, this led to inadequate defense mechanisms in both cultivars due to the heightened formation of ROS. Vermicompost significantly alleviates the impact of drought stress by increasing the activities of SOD, POD, CAT, and APX in wheat seedlings.[58] The application of cow manure vermicompost enriched with cellulolytic bacteria at 4 t ha^{-1} potentially promoted the scavenging mechanisms associated with enhanced activities of SOD, POD, CAT, and APX in both cultivars. Under drought stress, there was a significant ($p \leq 0.05$) and positive relationship between the vermicompost application and the activities of SOD, POD, CAT, and APX.

Based on the findings of the present study, the enhanced absorption of nutrients such as N, P, K, Ca, Zn, Mg, Fe, and Cu into the plant, improved plant hydration, and reduced ROS generation in the presence of VT could contribute to the

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reinforcement of wheat's defensive mechanism against stress. According to Keles,[59] the application of vermicompost led to a reduction in MDA levels in wheat seedlings. Furthermore, vermicompost supplements to wheat resulted in enhanced activities of enzymatic antioxidants while concurrently reducing MDA levels under water stress.[60] ROS are responsible for alterations in lipid peroxidation. According to Garcia et al.,[61] antioxidants stand as the most effective agents in detoxifying these substances and minimizing cellular damage. Wheat demonstrates heightened antioxidant activity, leading to reduced lipid peroxidation.[62] Among the pivotal defense mechanisms, SOD holds great significance as it detoxifies superoxide by converting it to H_2O_2.[21,63] The oxidative damage caused by drought stress was ameliorated through the addition of vermicompost, as evidenced by a significant increase in SOD, POD, CAT, and APX and reduction in MDA levels.[62]

Moreover, in this work, Faisalabad-08 outperformed Galaxy-13 under drought stress. Similar results were reported previously, where authors reported that Faisalabad-08 displays greater drought resistance than Galaxy-13.[19,29] The application of vermicompost enhances the biochemical, physiological, and enzymatic antioxidant performance in wheat under drought stress. The utilization of vermicompost leads to enhancement in water status, photosynthetic activity, the plant's defense system, and membrane stability. However, further investigation is required, especially under field conditions, to validate the outcomes observed in pot trials.

5. CONCLUSIONS

The soil application of vermicompost successfully mitigated the detrimental effects of drought on wheat seedlings by stabilizing the membrane and increasing the activities of the antioxidant enzymes. Vermicompost applied at an optimum quantity@4 t ha^{-1} proved to be a more effective treatment as it exhibited significantly higher concentrations of macro- and micronutrients in wheat leaves, membrane stability, chlorophyll content, and antioxidant activities in both cultivars under drought and well-watered conditions. Vermicompost derived from cow dung and enriched with cellulolytic microbes emerged as a valuable nutritional source, offering benefits under both normal and water-scarce conditions. This makes it a recommended choice for farmers, particularly in drought-prone areas. Among both cultivars, Faisalabad-08 showed comparatively more resistance against drought stress.

ASSOCIATED CONTENT

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04402.

> Mean sum of squares of macro- and micronutrients, malondialdehyde, proline, total proteins, chlorophyll contents, SPAD values, carotenoid contents, and antioxidant enzyme activities (PDF)

AUTHOR INFORMATION

Corresponding Authors

Zubair Aslam – *Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan*; Email: zauaf@hotmail.com

Usman Zulfiqar – *Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*; orcid.org/0000-0003-3820-1476; Email: usman.zulfiqar@iub.edu.pk

Authors

Ali Ahmad – *Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan*

Rana Nadeem Abbas – *Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan*

Korkmaz Bellitürk – *Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Tekirdağ Namık Kemal University, Süleymanpaşa, Tekirdağ 59030, Turkey*

Saddam Hussain – *Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan*

Sadam Hussain – *College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100, China*

Muhammad Ahmad – *Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan*

Ihab Mohamed Moussa – *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*

Mohamed S. Elshikh – *Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04402

Author Contributions

Conceptualization: A.A., Z.A., and R.N.A.; writing—original draft preparation: A.A., S.H., M.A., and U.Z.; writing—review and editing: S.H., U.Z., I.M.M., M.S.E., Z.A., and M.A. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

In this study, experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation.

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