Pak. J. Bot., 57(5): 1615-1630, 2025. DOI: http://dx.doi.org/10.30848/PJB2025-5(28)

ADAPTIVE RESPONSES OF TWO DISTINCT WHEAT VARIETIES TO DROUGHT STRESS THROUGH MICROBIAL-AUGMENTED VERMICOMPOST

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Abstract

Wheat, a vital cereal crop, is predominantly grown in arid and semi-arid regions, making it susceptible to drought stress. The adverse effects of drought on wheat can potentially be mitigated through the application of cellulolytic microbes-enriched vermicompost. To explore this, two field studies were conducted at the Student Research Farm, Agronomy Department, University of Agriculture Faisalabad, during 2020-21 and 2021-22. The research examined the impact of cellulolytic microbes-enriched vermicompost on the agronomic, physiological, and enzymatic antioxidant traits of wheat under varying soil moisture levels. The treatments included: (a) Three soil moisture levels: well-watered (D0, 70% field capacity), moderate drought (D1, 45% field capacity), and severe drought (D2, 30% field capacity). (b) Two wheat varieties: Galaxy-13 (drought-sensitive) and Faisalabad-08 (drought-tolerant). (c) Four microbial enriched vermicompost levels: VT0 (control, no vermicompost), VT1 (6 t/ha wheat straw vermicompost), VT2 (6 t/ha rice straw vermicompost), and VT3 (4 t/ha cow dung vermicompost). The results revealed that the highest crop growth and yield were achieved with 4 t/ha cow dung vermicompost (VT3), followed by rice straw vermicompost (VT2) and wheat straw vermicompost (VT1). The lowest performance was observed in the control (VT0) across both wheat cultivars. Faisalabad-08 consistently outperformed Galaxy-13 in growth and yield under both moderate and severe drought conditions. The findings further highlighted that drought stress significantly reduced agronomic, physiological, biochemical, and growth traits of wheat. However, the application of vermicompost enhanced these characteristics and improved yield, even under water-limited conditions.

Key words: Drought levels, Field experiment, Microbes, Straw, Vermicompost, Wheat cultivars.

Introduction

After corn and rice, wheat (*Triticium aestivum* L.) is amongst the most commonly produced staple crop in a variety of climates. Globally, China ranked first with a reported 134.30 million tons of wheat yield, followed by India, Russia, the United States, and France, with Pakistan ranked seventh among other developing nations (FAO, 2017). It covers an area of approximately 219.52 million hectares worldwide and touches production of almost 733.91 million tons (WAP, 2018). Wheat contributes almost 1.70% to the GDP of Pakistan and its value addition is 8.7% in agriculture sector of country and wheat production was projected approximately 27 million tons on the total cultivated area of 9.20 million hectares (Govt. Pakistan, 2019-20).

The biochemical, physiological, morphological, and yield attributes of plants are negatively influenced by many constraint factors, such as harsh climatic conditions, erratic weather, sowing time, salinity, heavy metals, pest attack and irrigation water availability. Drought is the major restriction factor of all these factors, which is usually correlated with plant yield reduction rather than other constraint factors (Anjum & Tanveer, 2016; Flexas *et al*., 2004).

Water deficit stress arises when the moisture in the plant is limited and thus the plant reabsorbs the water from the rhizosphere, thus reducing its natural functioning under the impact of water scarcity. Plants susceptible to extreme drought stress severely interrupt their several morpho-physiological functions, such as leaf area, photosynthetic content, plant biomass, plant growth and development, yield and yield contributing parameters. In plants, water shortage greatly represses the mechanism of photosynthesis associated with glucose formation. Photochemistry and photosynthetic metabolism are impaired, and CO_2 accessibility is also decreased under restricted water regimes (Lawlor & Cornic, 2002; Akram, 2011; Anjum *et al*., 2017). In the field trial, the absorption of CO_2 by the plant facing water stress conditions normally decreases (Chaves *et al*., 2002; Hussain *et al*., 2018; Mitchell *et al*., 1998). Plants exposed to drought stress conditions considerably stimulate free radical mechanisms that impairs the plant's photosynthesis system, protein biosynthesis, and other metabolites. Plants have an innate mechanism to reduce drought stress injury by restricting their growth, such as shoot and leaves (Chaves, 1991). According to reports, different genotypes of wheat respond differently to water constraint (Tang *et al*., 2002; Khakwani *et al*., 2011).

The production of crop is exaggerated by water deficit stress by modifying many metabolic processes, including reduced rate of carbon absorption, enhanced oxidative damage, and decreased gaseous exchange of leaf (Hussain *et al*., 2018; Anjum *et al*., 2017). It also retards various enzymatic processes, ions uptake, and leaf development due to which crop productivity severely hampers (Sharma & Garg, 2018; Todaka *et al*., 2017). Water deficit stress is a serious issue as it takes hold of several physiological processes of crop growth and production. To eradicate such problem, however, it is highly recommended to incorporate organic amendments such as vermicompost and cultivation of drought-tolerant cultivars (Ji *et al*., 2010).

We have a lot of farm waste in the form of crop residues, tree residues and FYM, if this waste is managed and used wisely, it helps to enhance quality of soil and levels of nutrient, which helps us to fulfil the increasing population's demand of food. Farmers usually use agriculture wastes as fuel and heat due to a shortage of energy and coal, wasting an abundant supply of nutrient organic matter. Organic matter in the form of agricultural wastes is good source of food for insects and microorganism which can create a serious problem for sustainable agriculture. Vermicomposting techniques can effectively be used to manage agriculture wastes like wheat straw, cow dung, rice straw and paper waste *etc* (Jyotsana *et al*., 2010). The process of vermicompost preparation is commonly described as the aerobic degradation and modification of solid organic residues by manipulation of the biological activity of earthworm and other mesophilic microflora (Garg & Gupta, 2009). The vermicompost produced by earthworm activity enriches predominantly with certain immobilized microflora, growth-regulating hormones, vitamins, macro/ micronutrients, and chitinase, lipase, amylase, and protease are examples of degrading enzymes. With the introduction of another antagonistic microflora, these enzymes have already been secreted by earthworm can degrade the organic substrate (Barik *et al*., 2011).

Many studies have focused on *Bacillus subtilis*, which is found in the gut of the *Eisenia fetida* earthworm. All of these antagonistic bacteria secrete enzymes that degrade cellulolytic matter, such as endo-beta-1, 4-glucanase, cellulase, protease and amylase (Amita Paul *et al*., 2017; Farooq *et al*., 2009). Several researchers in cereal crops, wheat and maize have assessed the beneficial effects of vermicompost alone and combined with other organic fertilizers under non-drought, moderate drought, and severe drought stress situations. Previous research has revealed that the use of vermicompost can mitigate water deficit stress due to its high ventilation, porosity, strong water preservation, and drainage capability (Hosseinzadeh *et al*., 2016). Vermicompost's microorganisms improve the roots' ability to absorb water. Vermicompost also enhances soil water preservation and increases fertilizer solution (Hosseinzadeh *et al*., 2016). Vermicompost contains soluble sugars, sorbitol, betaine, amino acids, and further organic acids, in addition ions of phosphorus, nitrogen, calcium, zinc, boron, magnesium, sulphur, and iron (Hosseinzadeh *et al*., 2016; Aslam *et al*., 2022; Aslam *et al*., 2023). Vermicompost is like peat with a fine structure, strong aeration, porosity, microbial activity, drainage, and a high-water holding capability, as well as a high nutrient content that is ideal for improving plant and soil health (Pathma and Natarajan, 2012). The availability of enzymes and hormones is needed to improve plant health and eliminate pathogens. During the vermicomposting process these enzymes and hormones release from earth worm's gut (Gajalakshmi & Abbasi, 2004).

In our earlier research (Ahmad *et al*., 2022; Ahmad *et al*., 2022a; Ahmad *et al*., 2024), we explored the effects of plant-based (wheat straw, rice straw) and animal-based (cow dung) vermicompost, enriched with cellulose-degrading bacteria, on the physiological and biochemical traits of wheat seedlings under water deficit conditions in pot experiments. The primary objective of these experiments was to determine the optimal vermicompost application rate that maximized plant performance, which was subsequently tested in the field in this study. However, the impact of animal and plant based vermicompost, supplemented with cellulolytic microbes, on wheat growth and productivity under drought stress remains unclear. To fill this knowledge gap, this study sought to evaluate whether cellulolytic microbe-enriched vermicompost could alleviate the adverse effects of drought on wheat productivity under field conditions. We hypothesized that this treatment would enhance drought resistance and improve the physiological, biochemical, and yield-related attributes of wheat. Considering the aforementioned details the two-year trial was directed in field having following particular objectives. i). To assess the role of cellulolytic microbes enriched vermi-fertilizer (prepared from wheat straw, cow dung and rice straw) application on wheat for drought tolerance. ii). To estimate the outcome of vermicompost application on agronomic, physiological, and enzymatic antioxidant traits of wheat grown under drought stress conditions.

Materials and Method

The experiment was conducted at Agronomic Research Area, University of Agriculture, Faisalabad. An optimized amount of wheat straw, rice straw and cow dung vermicompost were applied under field conditions at the time of sowing using randomized complete block design with split-split plot arrangement and were replicated thrice. Seed bed well prepared according to the demand of crop. Wheat crop was sown@ 120 kg ha^{-1} seed rate. Recommended fertilizers were applied @ 110: 100 kg ha^{-1} NP according to soil analysis. All other management practices were kept uniform except the treatments under study. The net plot size was 4.5m × 2.25m. For vermicomposting the epigeic species of earth worms *Eisenia fetida* were used. The active strains of cellulose degrading microbes *i.e.,* C-03, C-18 and C-21 were used for wheat straw, cow dung and rice straw vermicompost respectively. After completing all the practices treatments were applied in their respective plots. The factor wise experimental plan is as under: Factor A: Drought levels (main plot); D_0= 70% of field capacity (no drought), D_1= 45% of field capacity (mild drought), D_2= 30% of field capacity (severe drought). Factor B: Wheat cultivars (sub plot); V_1 = Faisalabad-08 (drought tolerant), V_2 = Galaxy-13 (drought sensitive). Factor C: Cellulolytic microbes and earth worms based vermicompost (sub-sub-plot); VT_0 = Control, VT_1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT_2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT_3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

Meteorological data of experimental site: The weather in respect of temperature (°C), relative humidity (%) and rainfall (mm) of the experimental site for the year 2020-21 and 2021-22 is given in the (Fig. 1).

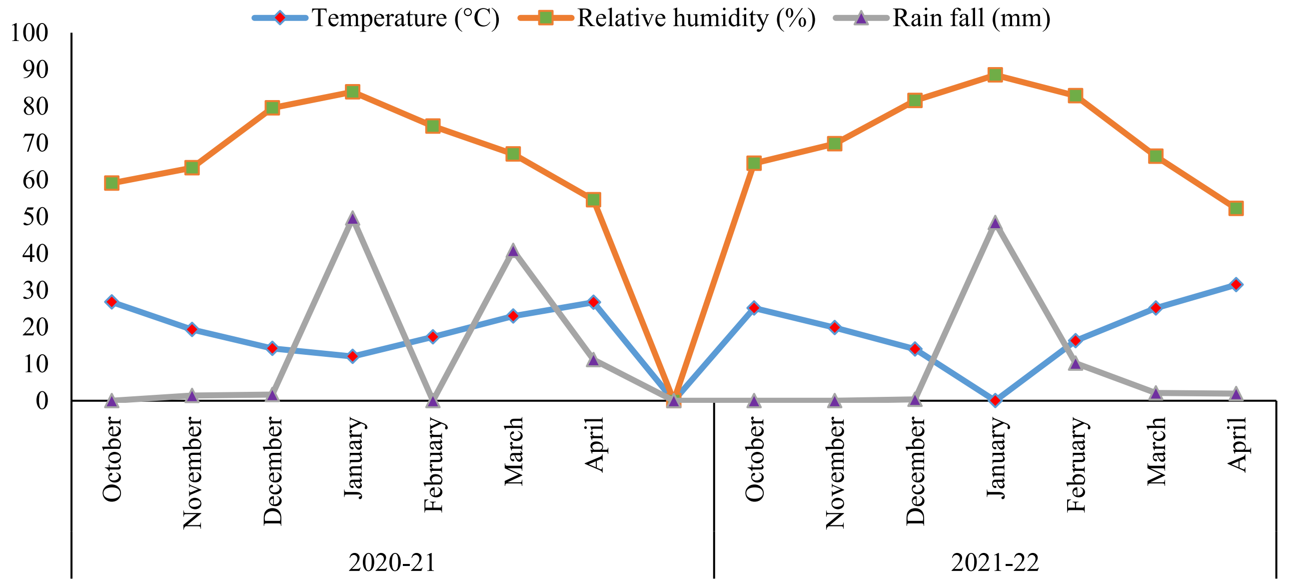

Fig. 1. Meteorological data of the experimental site for the growing seasons 2020-21 and 2021-22.

Data to be recorded: Data on various agronomic, physiological and enzymatic antioxidants traits of the plants grown in the field were recorded using standard procedures that are described as under.

Agronomic parameters

(i). Plant height (cm): Ten plants were picked from each sub-plot to calculate the plant height. Using a meter rod from the soil surface to the top of the plant, height was measured and then the average plant height was determined.

(ii). Number of total tillers m^{-2}: The number of total tillers m^{-2} was reported using a quadrate of 1m^{-2}. In each sub plot, drop it randomly. Tillers were counted and the average was calculated.

(iii). Spike length (cm): Ten plants were picked randomly from each sub-plot and the spike length was determined with the help of foot rod from the beginning of the spike to the top of it and average was calculated.

(iv). Number of spikelets per spike: Ten plants were picked from each subplot, and the number of spikelets from each spike were counted. The average number of spikelets per spike were determined.

(v). Number of grains per spike: Ten plants were picked randomly from each sub-plot. Their spikes were isolated and manually threshed. The number of grains were measured and then averaged from each spike.

(vi). 1000 grain weight (g): Grains were separated during the threshing and 1000 grains were counted. They were weighted on the electronic balance. Weight was measured in grams.

(vii). Biological yield (t ha^{-1}): A sample of plants from 1 m^2 of area was collected from each sub-plot. Weight on the electronic balance and the conversion of biological yield into (t ha^{-1}) was calculated.

(viii). Grain yield (t ha^{-1}): From each sub plot, an area of one m^2 was selected and harvested. Threshed manually and took the weight of the grain by electronic balance, and the yield of the grain was converted to t ha^{-1}.

(ix). Straw weight (t ha^{-1}): Straw yield per plot of each treatment was calculated and expressed in t ha^{-1}.

(x). Harvest index (%): By dividing grain yield over biological yield, the harvest index was determined.

$$\text{HI (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Physiological parameters

(i). Leaf water potential (Ψ_w) [-MPa]: To measure the leaf water potential, at booting stage a fully emerged flag leaf was incised. With water potential apparatus (Chas W. Cook & Sons. Birmingham B 42, ITT England) leaf water potential was determined by adopting the procedure defined by Scholander *et al.*, (1964). With the cut surface popping out of the opening, a single leaf (flag leaf) was sealed in the pressure chamber. Pressure filled gas was exerted to the leaf till the xylem components visible on the cut section. To avoid any evaporative losses sampling was carried out between 6.00 A.M. to 8.00 A.M. The leaves were placed as early as possible in the pressure chamber and separate measurements were taken on three leaves from the control and stress treatments.

(ii). Leaf osmotic potential (Ψs) [-MPa]: The leaf used in water potential measurement was frozen for seven days in the freezer below -20 ^{0}C. Then, following a frozen leaf material thawing, inserting by syringe. The extracted sap was used directly to assess the osmotic potential using an osmometer (Wescor 5500).

(iii). Turgor pressure (Ψp) [MPa]: The difference of water potential and osmotic potential is measured as turgor pressure.

$$(\Psi p) = (\Psi w) - (\Psi s)$$

(iv). Canopy Temperature (°C): It indicates the direct measurement of energy emitted by wheat plants. IRIS, infrared temperature sensors was used to measure it. It gives data on plant metabolic activity *i.e.* plant water status and water use (Pettigrew, 2004; Singh *et al*., 2018).

(v). Photosynthetic rate (An) [µmol m^{-2} s^{-1}]: IRGA, infrared gas analyzer was attached to plant leaves to measure photosynthetic rates (Singh *et al*., 2018; Rosolem *et al*., 2019). In each treatment, five measurements were collected from five different plants and then, their mean was taken.

(vi). Transpiration rate (*E*) [mmol m^{-2} s^{-1}]: Transpiration rate (E) was measured on leaf attached to the plant by using IRGA, infrared gas analyzer (Singh *et al*., 2018; Rosolem *et al*., 2019). Five measurements were recorded from five different plants in each treatment and then their average was taken.

(vii). Stomatal Conductance (*gs*) [µmol m^{-2} s^{-1}]: The stomatal conductance was measured using an open system LCA-4ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). The measurement was carried out with the following adjustments: leaf surface area 6.25 cm^2, ambient CO_2 concentration (Cref) 371µmol mol^{-1}, temperature of leaf chamber (Tch) varying from 25-28ºC, leaf chamber volume gas flow rate (v) 296 mL min^{-1}, leaf chamber molar gas flow rate (U) 400 µmol s^{-1}, ambient pressure (P) 97.95 kPa, PAR (Qleaf) at leaf surface maximum up to 770 µmol m^{-2} s^{-1}. Measurement of stomatal conductance (gs) was made on the youngest fully emerged leaf (normally flag leaf from top) of each plant.

(viii). Sub-Stomatal CO_2 concentration (C_i) [µmol CO_2 mol air^{-1}]: The fully expanded leaves, using a CIRAS-2 (PP system®) portable gas exchange system (CO_2 and H_2O) connected to a gas exchange chamber (Parkinson Leaf Cuvette). The system measured CO_2 concentration (Parkinson *et al*., 1980; Parkinson & Porometry, 1983).

Antioxidant enzymes activities

Enzyme extraction: Using a 50 mM cooled phosphate buffer (pH 7.8) for the extraction of antioxidant enzymes, fresh flag leaves (0.5 g) were ground in the pestle and mortar. Following filtration via cheese cloth, the homogenate was centrifuged at 15000 x g for 20 min at 4°C, and the supernatant was used for enzyme assays.

(i). Superoxide dismutase (SOD) [U mg^{-1} protein]: SOD activity was assayed according to the Giannopolitis & Ries (1977) process; by finding photo chemical reduced of nitroblue tetrazolium (NBT) at 560 nm. SOD activity was calculated by adding 3 mL of total reaction 50 µM NBT solution (NBT dissolved in ethanol), 13 µM riboflavin solution, 13 mM methionine solution, 75 nM EDTA solution, 50 Mm phosphate buffer (pH 7.8). The reaction solutions were put in a chamber under 30 W fluorescent lamps illumination. The reaction initiated when the fluorescent lamps were switched on and stopped 5 minutes later when they were switched off. The blue formazane formed by photoreduction of NBT was measured at 560 nm as an increase in absorbance. The non-leaf extract of reaction mixture was taken as control and placed under the light. The blank solution with the same exact reaction mixture (including enzyme extract) was, however, placed in the dark. A UV-visible spectrophotometer was used to read the absorption of the irradiated solution at 560 nm (IRMECO U2020). One unit of SOD was specified as the amount of enzyme needed to inhibit the rate of reduction of NBT at 560 nm by 50% compared to tubes that do not have the plant extract.

(ii). Peroxidase (POD) [U mg^{-1} protein]: The Peroxidase behavior was determined by using the oxidation of guaiacol and characterized as 0.01 absorbance shift min^{-1} mg^{-1} protein. To form the reaction mixture in 100, 400, 500 µL of enzyme extract, guaiacol (20mM), H_2O_2 respectively and 2 ml Phosphate (50 mM) were combined. The reaction mixture's absorbance at 470 nm was measured every 20 sec for up to 5 minutes. Activity of POD is express in U mg^{-1} protein (Chance & Maehly, 1955).

(iii). Catalase (CAT) [U mg^{-1} protein]: A UV-visible spectrophotometer was used to observe the CAT behavior detected by decomposition and change in absorption due to Hydrogen peroxide every 30 s for 5 min at 240 nm. The reaction mixture for CAT was 900 µL H_2O_2 (5.9 mM) and 2 mL phosphate buffer, respectively (50 mM). The reaction was initiated by adding an extract of 100 µL of enzyme to the reaction mixture. The Catalase activity was expressed as U mg^{-1} protein (Chance & Maehly, 1955).

Statistical analysis

The recorded data regarding physiological, enzymatic antioxidants, yield and yield related agronomic traits of the experiment was statistically evaluated by applying the method of Fisher's analysis of variance (ANOVA). Tukey's HSD test was used ($p \leq 0.05$) to compare significant treatments means using Statistic version 8.1 (Analytical Software ©, 1985-2005) according to Steel *et al*., (1997) and graphical presentation was done by Excel 2016 and Sigma Plot 10.0.

Results

Agronomic parameters: Different moisture levels and cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost application and two contrasting wheat genotypes showed significant effect on plant height, number of total tillers, spike length and spikelets per spike during both years of study. The data regarding the above-mentioned attributes affected by moderate drought (45% FC), severe drought (30% FC) and VT (Table 1, Fig. 2). Less plant height, number of total tillers, spike length and spikelets per spike were calculated in drought prone field as compared to well-watered field due to adverse effect of drought. The highest value was recorded at optimized cellulolytic microbes enriched vermicompost levels cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) in moderate and severe drought fields in both cultivars. At well-watered conditions the plant height, number of total tillers, spike length and spikelets per spike were observed statistically at par with each other in both wheat cultivars. In moderate and severe drought stressed field, Faisalabad-08 produced more agronomic attributes than Galaxy-13. Similar response was observed in both wheat cultivars under different moisture levels and vermicompost application during both years 2020-21 and 2021-22 experiments).

The data referred to the number of grains per spike, 1000-grains weight, biological yield and grain yield are presented in (Table 2, Fig. 3). In drought stress significantly affected the above stated agronomic attributes in wheat plants grown at moderate drought stress (45% FC) and severe drought stress (30% FC) as compared to well-watered conditions (70% FC) in both the wheat cultivars. Cellulolytic microbes enriched vermicompost application significantly increased the number of grains per spike, 1000-grains weight, biological yield and grain yield of both cultivars. Maximum value was recorded at optimized cellulolytic microbes enriched vermicompost levels cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) and it was significantly higher from control at both under drought and well-watered conditions. Comparing the cultivars, Faisalabad-08 performed better than Galaxy-13 under moderate and severe drought conditions while under well-watered conditions both cultivars had statistically at par results during both year studies.

The data related the straw weight and harvest index affected by drought stress and vermicompost is shown in (Table 3, Fig. 4). Less straw weight and harvest index obtained in moderate and severe drought affected field as compared to well-watered conditions due to adverse effect of drought stress during 2020-21 and 2021-22 field trials. The highest value was recorded at optimized cellulolytic microbes enriched vermicompost levels, cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) in drought induced and well-watered fields in both cultivars. When we check at well-watered conditions plot, straw weight and harvest index were observed statistically at par with each other in both wheat cultivars during both year studies. Under drought prone field, Faisalabad-08 formed more straw weight and harvest index than Galaxy-13.

Physiological parameters: Table 4, Fig. 5 indicated that leaf water potential, osmotic potential and turgor potential were less at moderate and severe drought plots in comparison to well-watered plot in both wheat cultivars during 2020-21 and 2021-22. The minimum results were calculated under severe drought conditions. Vermicompost application ameliorated the unpleasant effect of drought and caused a significant increase in leaf water potential, osmotic potential and turgor potential. The maximum value was recorded where cellulolytic microbes enriched vermicompost levels, cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) were used under moderate drought, severe drought and well-watered conditions. Among the cultivars, Faisalabad-08 had higher water potential, turgor potential and osmotic potential than Galaxy-13 observed under drought stress conditions, while, at well-watered conditions both cultivars were showed statistically at par during both year trials.

Canopy temperature in both wheat cultivars in the presence or absence of vermicompost (Table 4, Fig. 5) was also affected more at severe drought conditions. The canopy temperature significantly decreased with the vermicompost application under moderate and severe drought stress. The minimum temperature of canopy was achieved in response to cellulolytic microbes enriched cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) and maximum value was recorded in control (without vermicompost application) in Faisalabad-08 as well as in Galaxy-13. Comparing the cultivars, at moderate and severe drought stress, Faisalabad-08 showed lower canopy temperature than Galaxy-13 and proved to be drought tolerant variety.

The data regarding physiological attributes (photosynthetic rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration) was influenced by drought stress and vermicompost amendment are shown in (Table 5, Fig. 6). Drought stress significantly ($p \leq 0.05$) reduced the rate of photosynthesis, transpiration, stomatal conductance and also sub-stomatal CO_2 concentration in the moderate and severe drought tempted field when we compared with well-watered field. Vermi-fertilization augmented physiological attributes both in moderate drought, severe drought and well-watered field conditions. The maximum value was recorded where cellulolytic microbes enriched cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) was used and minimum value was recorded where no vermi-fertilization was done. Comparing the cultivars, Faisalabad-08 (drought tolerant) had higher photosynthetic rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration than Galaxy-13 (drought sensitive).

Enzymatic antioxidants: Interactive effect of drought and vermicompost on catalase activity, superoxide dismutase and the peroxidase was recorded statistically significant during both years 2020-21 and 2021-22. The data about enzymatic antioxidants were influenced by moderate drought, severe drought and vermicompost are shown in (Table 6, Fig. 7). According to data more catalase, superoxide dismutase and also peroxidase activity was observed in drought stressed field as we compared to well-watered conditions. As drought stress enhanced the enzymatic antioxidants started to increase, but according to data record their increase was less to the requirement of the crop. The highest value was recorded when cellulolytic microbes enriched cow dung VT (4 t ha^{-1}) followed by rice straw VT (6 t ha^{-1}) and also wheat straw VT (6 t ha^{-1}) was used, and minimum value was recorded where no vermi-fertilization was applied in drought involved field in both cultivars. Under well-watered conditions the antioxidants enzymes were not influenced by vermicompost levels. In moderate and severe drought field, Faisalabad-08 generated more catalase, superoxide dismutase and peroxidase than Galaxy-13 whereas in well-watered field both cultivars produced similar antioxidant enzymes.

Table 1. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the agronomic parameters of wheat cultivars under different drought levels.

SOV	DF	2020-21				2021-22			
		Plant height (cm)	Number of total tillers (m^{-2})	Spike length (cm)	Spikelet's per spike	Plant height (cm)	Number of total tillers (m^{-2})	Spike length (cm)	Spikelet's per spike
Drought stress (DS)	2	1554.04**	55311.80**	102.12**	305.54**	1050.29**	24293.80**	81.70**	382.59**
Vermicompost (VT)	3	156.24**	3252.00**	20.64**	131.94**	213.75**	2344.20**	22.30**	69.97**
Wheat (W)	1	120.12**	806.70**	8.68**	20.05**	100.35**	654.00**	6.87**	36.12**
DS × VT	6	0.26^{ns}	428.80**	0.48**	0.26**	0.70^{ns}	200.80**	0.03^{ns}	0.67^{ns}
DS × W	2	44.04**	327.90**	2.85**	9.84**	15.85**	232.60**	3.04**	4.62**
VT × W	3	0.01^{ns}	1.60^{ns}	0.07^{ns}	0.01^{ns}	0.09^{ns}	0.70^{ns}	0.01^{ns}	0.19^{ns}
DS × VT × W	6	0.04^{ns}	3.90^{ns}	0.04^{ns}	0.08^{ns}	0.14^{ns}	2.10^{ns}	0.04^{ns}	0.03^{ns}
Error	46	1.78	52.70	0.14	0.43	1.37	16.30	0.23	0.43

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

Table 2. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the agronomic parameters of wheat cultivars under different drought levels.

SOV	DF	2020-21				2021-22			
		Grains per spike	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Grains per spike	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})
Drought stress (DS)	2	762.09**	1013.54**	71.45**	20.01**	1438.43**	927.04**	63.72**	15.48**
Vermicompost (VT)	3	143.82**	133.87**	4.61**	1.43**	148.38**	121.38**	3.54**	1.22**
Wheat (W)	1	33.34**	23.35**	2.42**	0.58**	100.35**	70.01**	2.16**	0.59**
DS × VT	6	0.02^{ns}	0.23^{ns}	0.14^{ns}	0.05**	1.25^{ns}	0.30^{ns}	0.15^{ns}	0.08**
DS × W	2	27.26**	18.35**	1.21**	0.18**	22.93**	28.76**	0.68*	0.15**
VT × W	3	0.08^{ns}	0.01^{ns}	0.02^{ns}	0.00^{ns}	0.53^{ns}	0.05^{ns}	0.00^{ns}	0.00^{ns}
DS × VT × W	6	0.22^{ns}	0.07^{ns}	0.01^{ns}	0.01^{ns}	0.34^{ns}	0.13^{ns}	0.00^{ns}	0.00^{ns}
Error	46	0.72	0.89	0.10	0.00	1.05	1.15	0.13	0.00

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

Table 3. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the agronomic parameters of wheat cultivars under different drought levels.

SOV	DF	2020-21		2021-22	
		Straw weight (t ha^{-1})	Harvest index (%)	Straw weight (t ha^{-1})	Harvest index (%)
Drought stress (DS)	2	16.07**	231.03**	16.45**	146.91**
Vermicompost (VT)	3	0.90**	21.92**	0.60**	17.67**
Wheat (W)	1	0.62**	6.33**	0.49*	8.86**
DS × VT	6	0.05^{ns}	0.82^{ns}	0.02^{ns}	0.81^{ns}
DS × W	2	0.50**	1.01^{ns}	0.19^{ns}	2.49^{ns}
VT × W	3	0.02^{ns}	0.17^{ns}	0.00^{ns}	0.08^{ns}
DS × VT ×W	6	0.00^{ns}	0.44^{ns}	0.00^{ns}	0.00^{ns}
Error	46	0.07	0.44	0.10	0.54

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

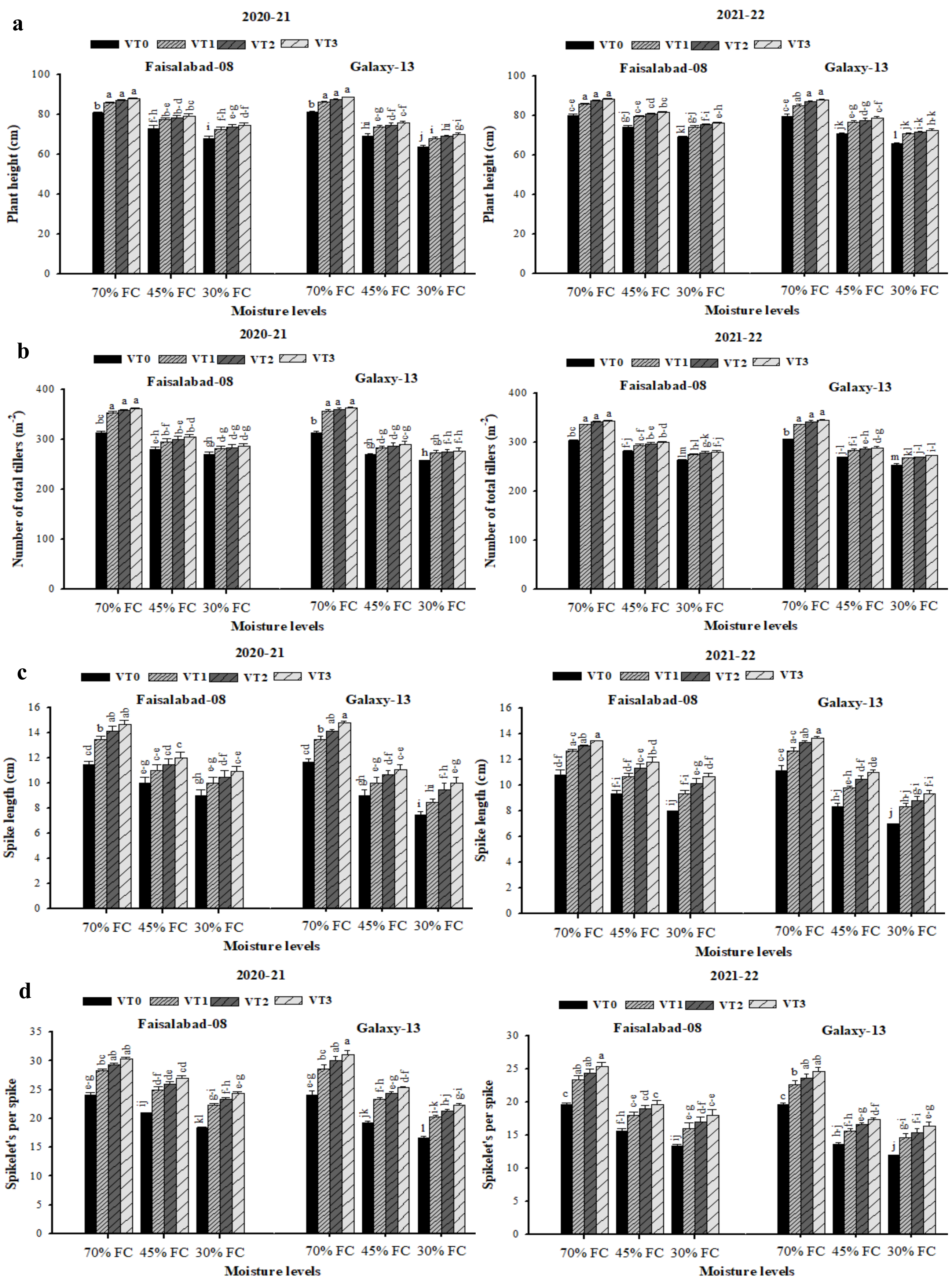

Fig. 2. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on the plant height (a), number of total tillers (b), spike length (c) and spikelets per spike (d) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT_0 = Control, VT_1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT_2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT_3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

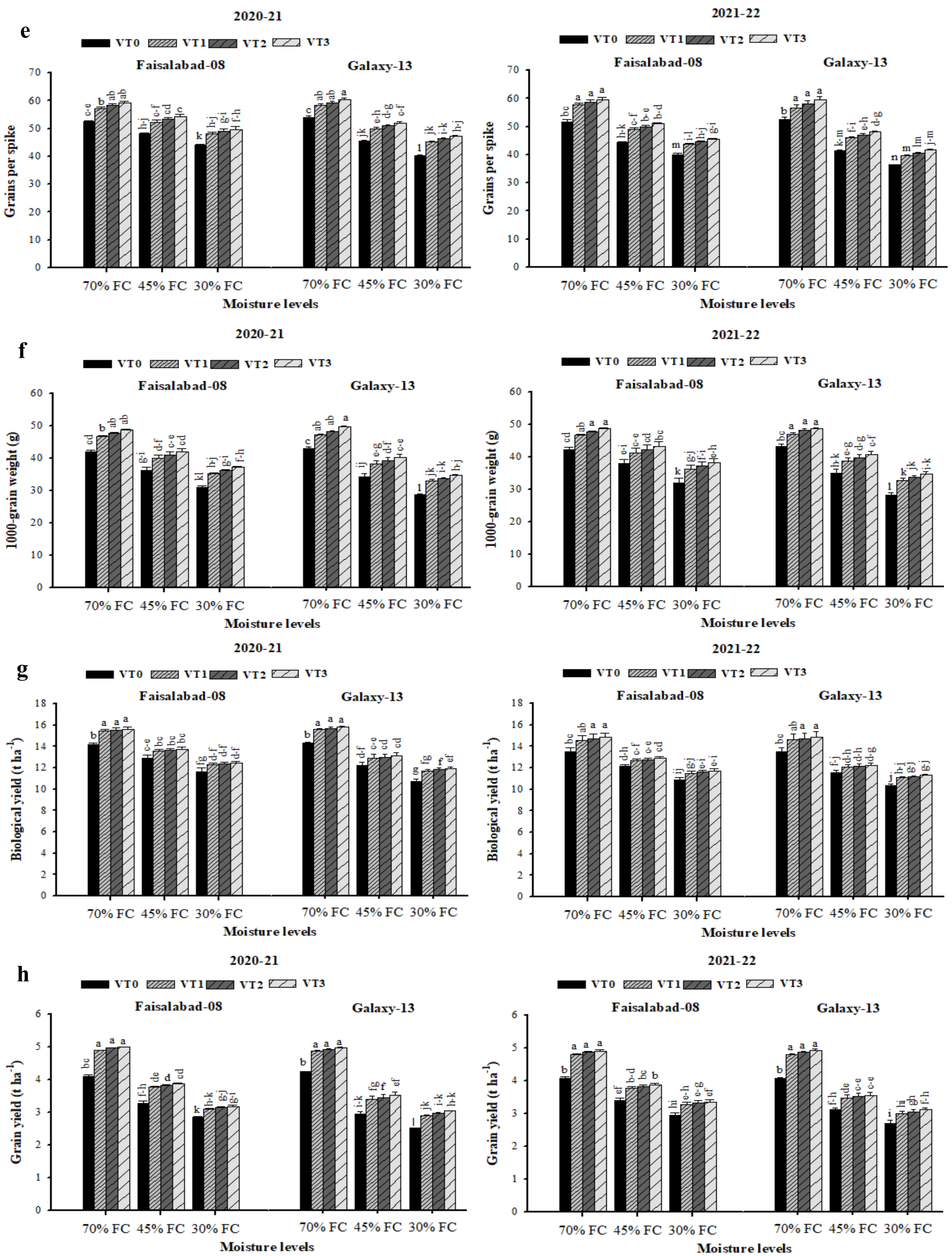

Fig. 3. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on the grains per spike (e), 1000-grain weight (f), biological yield (g) and grain yield (h) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT0 = Control, VT1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

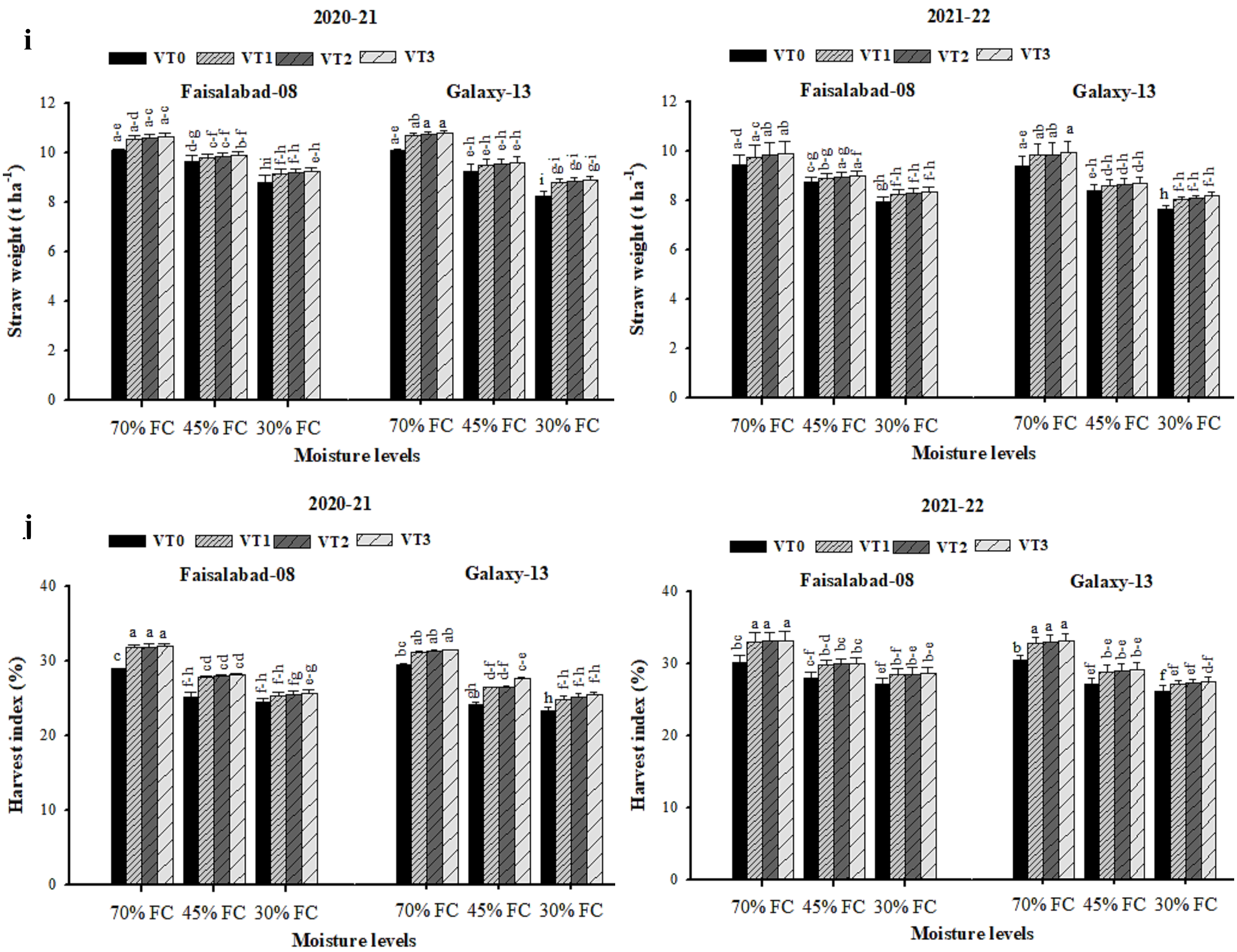

Fig. 4. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on the straw weight (i) and harvest index (j) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT0 = Control, VT1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

Discussion

Reduced agricultural yields in semiarid and arid regions are mostly attributable to drought stress (Taheri-Asghari *et al*., 2009). Wheat is a prevalent crop in this region, although it has been badly affected by the drought. In fields treated to mild water deficit (45% FC) and severe water shortage (30% FC), yields of both cultivars and their components were found to be decreased, as shown by the present field experiment. Dryness and low tissue water potential may reduce crop development and yield by interfering with a number of biochemical and physiological processes, including turgor pressure, photosynthesis, enzymatic activities, and transpiration (Nayyar & Gupta, 2006; Patade *et al*., 2011; Ansari *et al*., 2017). Multiple researchers had demonstrated that vermicompost has aid plant growth regardless of abiotic (environmental) and biotic (plant disease and insect) obstacles (Jat *et al*., 2006; Hosseinzadeh *et al*., 2016). There is evidence that vermicompost may reduce drought stress by promoting plant growth in arid areas (Hafez *et al*., 2021; Aslam *et al*., 2023). Fertilizing fields with cellulolytic microbe-enriched cow dung VT (4 t ha^{-1}), followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}) significantly increased yield of grain and biological yield in both drought-stressed and adequately irrigated fields, demonstrating the vermicompost's beneficial effects. A multitude of characteristics, including height of plant, per unit area tillers, spikelet length, per spike spikelet, and weight of grain, affected yield. When these parameters are increased, the biological output may be substantially increased. Similarly to how the total quantity of grain harvested is the outcome of the cumulative behavior of the yield components, it is also a measure of the efficiency and effectiveness of a technological package. When vermicompost treatment was optimized in both drought-stricken regions and farms with sufficient irrigation, yield and contributing factors rose dramatically. Aslam *et al*., (2019) found that increasing the rate of VT feeding in the field increased the wheat plants' dry matter production, providing increased weight to the data provided here. The addition of vermicompost increased wheat output in two ways: the percentage of ripe grains and the quantity of grains collected per unit of land.

Table 4. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the physiological parameters of wheat cultivars under different drought levels.

SOV	DF	2020-21				2021-22			
		Leaf water potential (Ψ_w) [-MPa]	Leaf osmotic potential (Ψs) [-MPa]	Turgor potential (Ψp) [MPa]	Canopy temperature (°C)	Leaf water potential (Ψ_w) [-MPa]	Leaf osmotic potential (Ψs) [-MPa]	Turgor potential (Ψp) [MPa]	Canopy temperature (°C)
Drought stress (DS)	2	0.33**	0.07**	0.09**	49.29**	0.31**	0.07**	0.08**	36.84**
Vermicompost (VT)	3	0.01**	0.00**	0.00**	14.64**	0.01**	0.00**	0.00**	16.44**
Wheat (W)	1	0.02**	0.00**	0.00**	4.01**	0.01**	0.00**	0.00**	6.72**
DS × VT	6	0.00^{ns}	4.16**	3.70^{ns}	0.19^{ns}	0.37^{ns}	2.54^{ns}	0.00^{ns}	0.06^{ns}
DS × W	2	0.00**	5.01**	8.66**	4.26**	0.00**	0.00**	0.00**	2.26**
VT × W	3	0.00^{ns}	1.85^{ns}	5.55^{ns}	0.05^{ns}	1.38^{ns}	3.70^{ns}	0.00^{ns}	0.05^{ns}
DS × VT × W	6	0.00^{ns}	3.24^{ns}	1.11^{ns}	0.02^{ns}	5.55^{ns}	7.87^{ns}	0.00^{ns}	0.04^{ns}
Error	46	0.00	2.20	1.13	0.25	2.73	2.03	0.00	0.24

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

Table 5. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the physiological parameters of wheat cultivars under different drought levels.

SOV	DF	2020-21				2021-22			
		Photosynthetic rate (*An*) (µmol Co_2 m^{-2} s^{-1})	Transpiration rate (*E*) (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (*gs*) (µmol m^{-2} s^{-1})	Sub-stomatal CO_2 concentration (C_i) [µmol CO_2 mol air^{-1}]	Photosynthetic rate (*An*) (µmol Co_2 m^{-2} s^{-1})	Transpiration rate (*E*) (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (*gs*) (µmol m^{-2} s^{-1})	Sub-stomatal CO_2 concentration (C_i) [µmol CO_2 mol air^{-1}]
Drought stress (DS)	2	86.46**	25.80**	17.64	20087.20**	49.71**	19.50**	13.49**	185442.40**
Vermicompost (VT)	3	11.21**	4.39**	1.92	1481.60**	7.98**	2.14**	1.82**	1413.10**
Wheat (W)	1	3.73**	1.47**	1.17	470.20**	3.42**	0.61**	0.86**	1241.70**
DS × VT	6	0.06^{ns}	0.04*	0.00	97.60**	0.11^{ns}	0.11*	0.02**	57.20**
DS × W	2	1.87**	0.76**	0.57	210.90**	1.68**	0.29**	0.34**	281.80**
VT × W	3	0.03^{ns}	0.00^{ns}	0.00	0.30^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	7.10^{ns}
DS × VT × W	6	0.02^{ns}	0.00^{ns}	0.00	1.70^{ns}	0.01^{ns}	0.01^{ns}	0.00^{ns}	2.70^{ns}
Error	46	0.10	0.01	0.00	8.30	0.05	0.03	0.00	4.20

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

Table 6. Mean sum of squares regarding the effect of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost on the enzymatic antioxidants of wheat cultivars under different drought levels.

SOV	DF	2020-21			2021-22		
		Catalase activity (U mg^{-1} protein)	Superoxide dismutase (U mg^{-1} protein)	Peroxidase (U mg^{-1} protein)	Catalase activity (U mg^{-1} protein)	Superoxide dismutase (U mg^{-1} protein)	Peroxidase (U mg^{-1} protein)
Drought stress (DS)	2	63.75**	58966.80**	2605.85**	52.95**	61535.30**	2433.75**
Vermicompost (VT)	3	1.59**	454.50**	56.91**	0.92**	326.60**	54.00**
Wheat (W)	1	1.62**	415.70**	32.00**	1.00**	648.00**	42.78**
DS×VT	6	0.37**	94.10**	15.64**	0.17**	66.20**	10.85**
DS×W	2	0.45**	101.00**	13.54**	0.41**	216.10**	8.53**
VT×W	3	0.01^{ns}	1.10^{ns}	0.11^{ns}	0.00^{ns}	0.30^{ns}	0.24^{ns}
DS×VT×W	6	0.00^{ns}	0.80^{ns}	0.15^{ns}	0.00^{ns}	0.80^{ns}	0.09^{ns}
Error	46	0.01	32.20	0.62	0.01	3.00	0.47

**Significant at 0.01 level of significance; *Significant at 0.05 level of significance, ns, non-significant

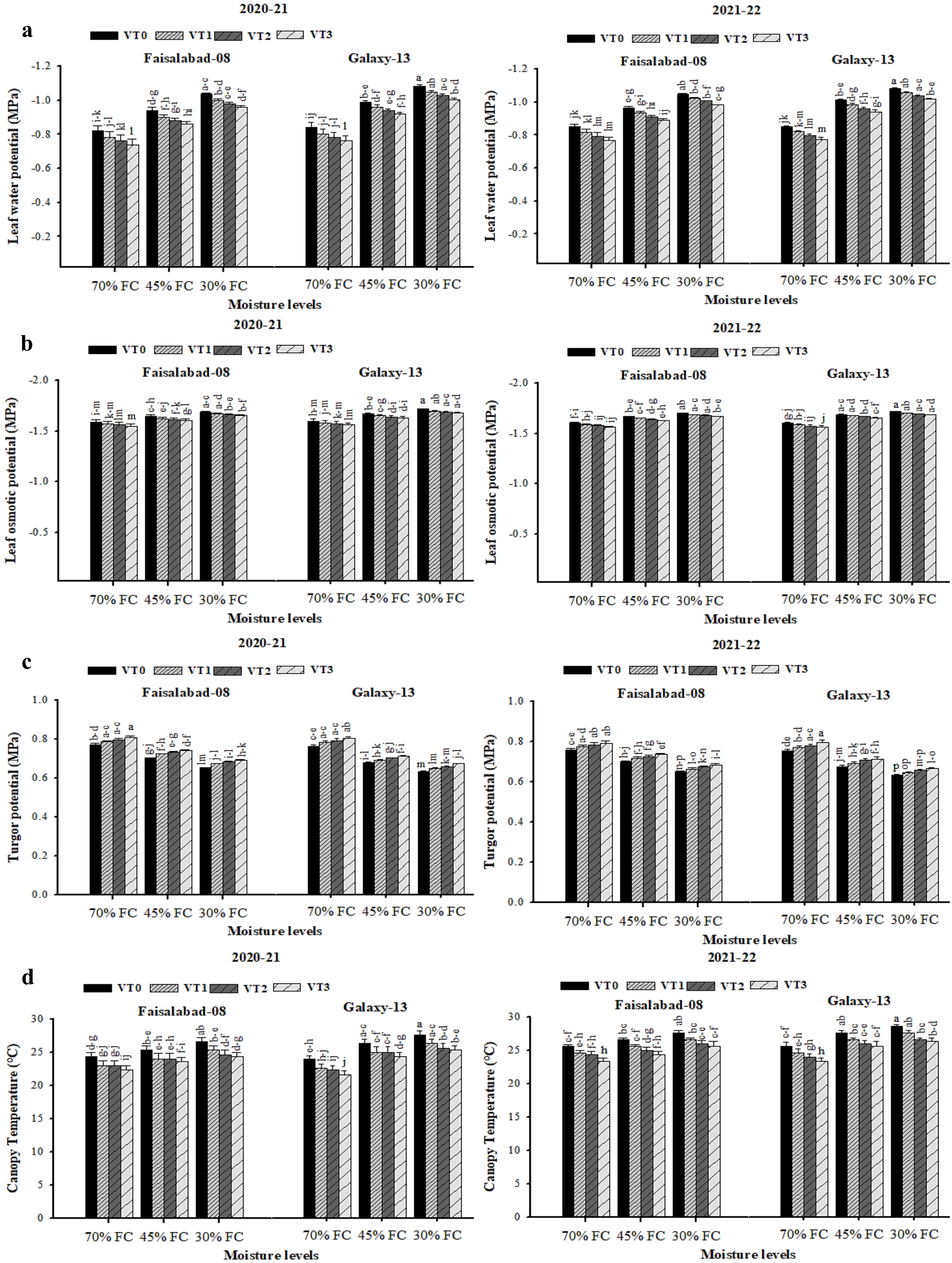

Fig. 5. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on the leaf water potential (a), osmotic potential (b), turgor potential (c) and canopy temperature (d) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT0 = Control, VT1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

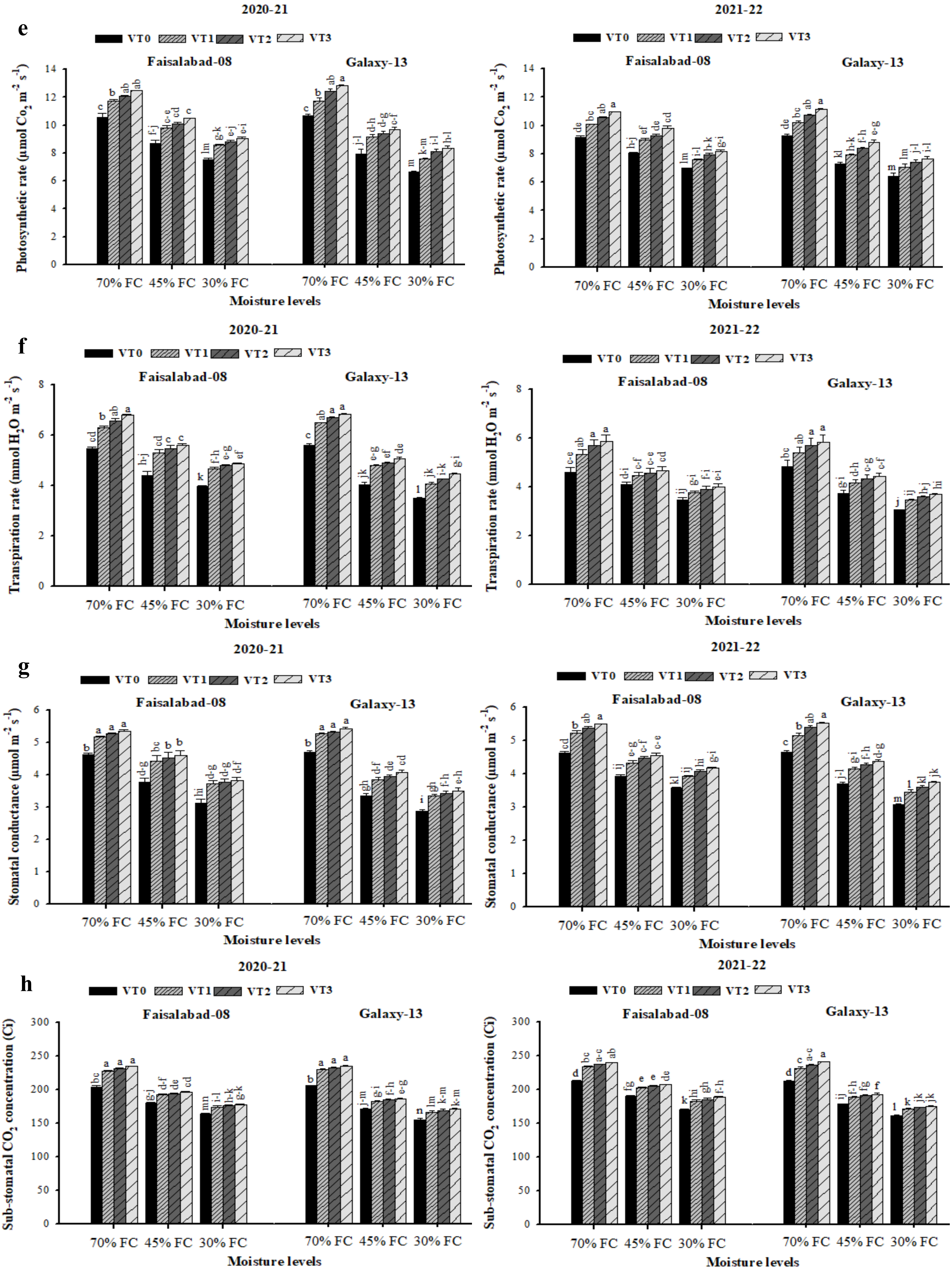

Fig. 6. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on photosynthetic rate (e), transpiration rate (f), stomatal conductance (g) and sub stomatal CO_2 concentration (h) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT0 = Control, VT1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

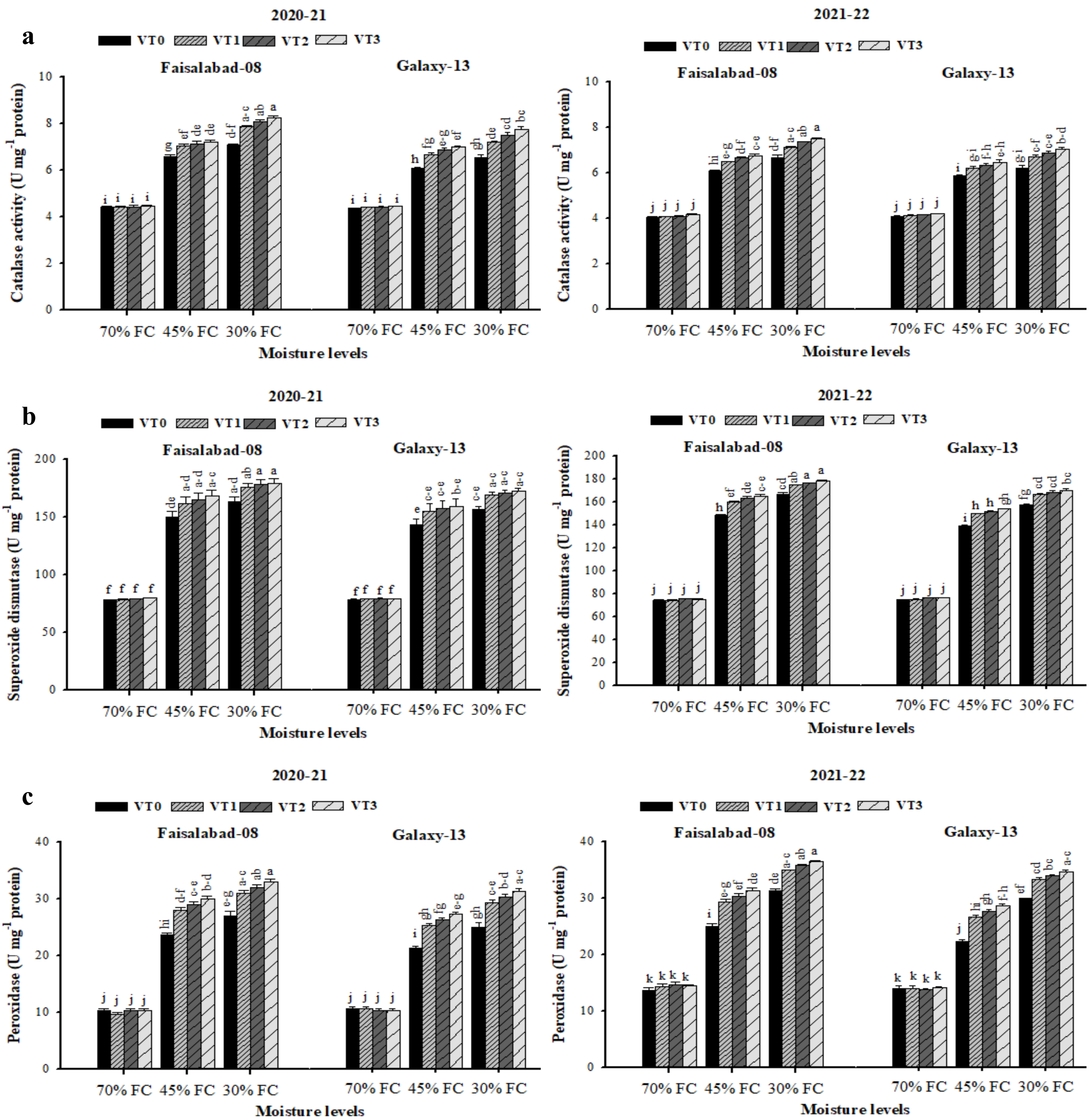

Fig. 7. Effects of soil applied cellulolytic microbes enriched wheat straw, rice straw and cow dung vermicompost (VT) on the catalase activity (a), superoxide dismutase (b) and peroxidase (c) of two wheat cultivars Faisalabad-08 and Galaxy-13 under different drought levels, well-watered condition (70% field capacity-FC), moderate drought (45% FC) and severe drought (30% FC). Bars with different small letters show significant differences at 5% probability level according to Tukey's HSD test. VT0 = Control, VT1 = 6 t/ha wheat straw vermicompost enriched with cellulose degrading microbes, VT2 = 6 t/ha rice straw vermicompost enriched with cellulose degrading microbes, VT3 = 4 t/ha cow dung vermicompost enriched with cellulose degrading microbes.

Since vermicompost treatment in wheat boosted relative growth rate, Hafez *et al*., (2021) hypothesized that the constructive impacts of vermi-fertilization on yield of wheat may be attributable to the plants' higher hydration and photosynthetic rates. Observations indicate that increases in photosynthetic or net assimilation rates increased water efficiency. It was revealed that Faisalabad-08 (a drought-resistant cultivar) and Galaxy-13 (a drought-sensitive cultivar) had the highest grain yield, harvest index, height of plant, biological yield, tillers number, grains number, and spikelets spike^{-1} in well-watered fields, moderate drought conditions, and severe drought conditions, respectively by addition of vermicompost. Vermicompost facilitates the uptake and transfer of essential minerals for plant development and energy generation (Sinclair & Vadez, 2002; Tara, 2003; Kmeťová & Kováčik, 2014).

Both wheat cultivars exhibited substantial increases in photosynthetic rate, osmotic potential, water potential of leaf, transpiration rate, leaf turgor potential, sub-stomatal CO_2 concentration and stomatal conductance after treatment of vermicompost in 2020-21 and 2021-22. Galaxy-13 has shown less dramatic physiological responses to mild and severe drought stress than Faisalabad-08. In reaction to water stress, plants shut their stomata, lowering transpiration, cellular CO_2 concentrations, and net photosynthesis, according to a considerable body of research (Flexas & Medrano 2008; Mssacci *et al*., 2008). The reduction in photosynthesis seen under situations of water stress has been connected to the inhibition of a variety of metabolic processes, including ATP production and Rubisco activity (Mssacci *et al*., 2008). In dry environments, two kinds of components impede photosynthesis. The first group consists of causes of stomatal closure, which lowers CO_2 absorption from leaves and subsequent transport to chloroplasts, hence decreasing photosynthesis (Pagter *et al*., 2005). Non-stomatal limiting variables include the suppression of synthesis ribulose-1, 5-bisphosphate, decline in transfer photosynthetic electron to PSII, and the decrease in chlorophyll content (Pagter *et al*., 2005). The need for NADPC to accept electrons decreases under situations of water scarcity, absence of oxidation, and use of NADPH molecules from the light reaction in photosynthesis. Through the ETC pathway, molecules of oxygen form free radicals such as superoxide, hydroxyl and hydrogen peroxide (Sairam & Saxena, 2000). ROS cause oxidation of liposomal lipids, modification of structural proteins, oxidation and inactivation of sulfhydryl groups (-SH), bleaching of pigments like chlorophyll and carotenoids, and strikes on photosystems (Sairam & Saxena, 2000). These factors may explain why photosynthesis is slowed in arid settings. Iron is added to soil through vermicompost (Davatgar *et al*., 2009). Because negatively charged groups like phenolic acid and carboxylic acid are present, vermicompost's humic compounds have a high potential for absorbing metals (Matos & Arruda, 2003). The iron-containing prosthetic group facilitates the activation of antioxidant enzymes, such as catalase, superoxide dismutase and peroxidase, which may be important for removing ROS in plants, according to Flexas & Medrano (2008). Vermicompost's high porosity, high ventilation capacity, efficient drainage, and water storage all lead to decreased stomatal closure and an increase in photosynthesis-required CO_2 (Arancon *et al*., 2004; Ahmad *et al*., 2025).

Inadequate water supply may result in oxidative damage. Vermicompost treatments and their combinations exhibited significant ($p \leq 0.05$) changes in the levels of antioxidant enzymes, including peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD). Vermicompost increased the POD, SOD, and CAT proportions of leaves under water deficiency stress comparing the control (70% FC). The leading SOD enzyme activity was found in plants having treatment of cow dung VT (4 t ha^{-1}) containing cellulolytic bacteria, followed by rice straw VT (6 t ha^{-1}) and wheat straw VT (6 t ha^{-1}). Utilizing vermicompost may improve a plant's ability to absorb cations like potassium and calcium. Andersen *et al.* (1992) revealed that K protects plants by reducing transpiration, while Ca acts as an enzyme activator. Boosted activities of SOD, POD and CAT enhanced membrane integrity and lowered lipid peroxidation by reducing ROS levels. As a result of drought stress, rice plants treated with vermicompost showed increased levels of the three antioxidant enzymes, namely catalase (CAT), peroxide oxidase (POD), and superoxide dismutase (SOD) (Garcia *et al*., 2014; Wang *et al*., 2017).

A mycorrhizal relationship with plant roots, an increase in soil-dwelling bacteria as nitrogen stabilizers, fungal spores, and actinomycetes, and a host of other helpful microbes are further advantages of vermicompost (Kale *et al*., 1992). For instance, mycorrhizal fungi may store carbohydrates from a plant's roots and use them later in their metabolism. Sugars may be provided as appropriate osmolytes to manage osmotic pressure in the roots and increase water stress resistance (Huerta *et al*., 2010). Around a plant, the incorporating of natural material in the form of vermicompost increases microbial activity and the soil's capacity to create CO_2 (Marinari *et al*., 2000). According to the findings of this field investigation, vermicompost may help wheat withstand to drought. Vermicompost treated with cellulolytic bacteria significantly improved enzymatic antioxidants, physiological features, yield contributing factors, and yield when applied to both wheat cultivars under well-watered and dry field situations.

Conclusion

Topsoil used cellulolytic microbes augmented vermicompost made from wheat straw, rice straw, and cow dung improved growth, stabilized nutrients, increased the actions of SOD, POD, and CAT contents, and lessened the problems caused by moisture deficits at yield and yield-related traits of wheat. Ultimately, during a drought, these changes increased wheat yield. The most widely accepted, efficient, cost-effective, and environment friendly treatments are cellulolytic microbe-enriched cow dung VT (4 t ha^{-1}), rice straw VT (6 t ha^{-1}), and wheat straw VT (6 t ha^{-1}). This is because it has shown stimulatory effects in yield enhancement of both wheat cultivars (Faisalabad-08 and Galaxy-13) under drought. It is advised that farmers incorporate vermicompost into their modern crop production techniques, particularly in drought-prone areas, as it can serve as a valuable nutrient source for all field crops in both normal and water-scarce conditions. Vermicompost is made from wheat straw, rice straw, and cow dung by earth worms and cellulolytic microbes. Due to its high nutrient content, vermicompost increases the availability of both macro- and micronutrients and serves as a backup source of nutrients for biofortification.

Acknowledgement

The results presented in this paper are a part of PhD studies of Dr. Ali Ahmad. The authors gratefully acknowledge HEC for providing financial support to carry out this research work under HEC projects "NRPU-HEC project no. 7527/Punjab/ NRPU/R&D/ HEC/2017_ Vermicomposting: A resourceful organic fertilizer to improve agriculture production and soil health and second project "Vermicomposting: An agricultural waste management technology," Pak-Turk Researchers Mobility Grant Program Phase-II, vide letter No. (Ph-II MG-9)/PAKTURK/R&D/HEC/2018." and PARB project# 18-550, "Developing agricultural waste management system to produce different kinds of organic fertilizers for sustainable agriculture".

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(Received for publication 22 December 2023)