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ORIGINAL ARTICLE

A novel eco-friendly approach of combining vermicompost and effective microorganisms sustains wheat (*Triticum aestivum* L.) drought tolerance by modulating photosynthetic performance and nutrient acquisition

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Abstract

The most significant threat to global food security is water scarcity. Despite the fact that vermicompost (an effective organic fertilizer rich in humic substances, macro- and micro-nutrients, earthworm excretions, beneficial soil microbes, plant growth hormones, enzymes) and effective microorganisms (EM; photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, fermenting fungi) have been recognized as powerful strategies for alleviating environmental stresses, their combined effect has not been studied. Herein, as a first investigation, we aimed to enhance wheat's drought tolerance using an eco-friendly approach that combined vermicompost and EM. The study employed twelve treatments in a completely randomized design. The treatments included control, as well as single and combined applications of vermicompost and EM at three different irrigation levels (100%, 70%, and 30% of field capacity). Vermicompost and EM, applied singly or in combination, ameliorated drought-induced reduction in wheat growth and productivity by elevating photosynthetic pigment content, photochemical processes, Calvin cycle enzyme activity, net photosynthetic rate, transpiration rate, stomatal conductance, maximum quantum efficiency of PSII photochemistry, actual photochemical efficiency of PSII, electron transport rate, photochemical quenching coefficient, and effective quantum yield of PSII photochemistry. Additionally, adding vermicompost and/or EM improved wheat drought tolerance by increasing nutrient (nitrogen, phosphorus, potassium, iron, zinc, copper) acquisition, roots' ATP content, H^+-pump activity, and membrane stability index while lowering hydrogen peroxide content, lipid peroxidation, and electrolyte leakage. The new evidence demonstrates that combining vermicompost with EM sustains wheat drought tolerance by regulating photosynthetic efficiency, nutrient acquisition, root H^+-pump activity, and membrane stability. Overall, utilizing vermicompost/EM is a novel approach to improving plant physiological responses and overcoming drought-related challenges.

Keywords Effective microorganisms · Nutrient acquisition · Photosynthetic efficiency · Vermicompost · Water deficit · Wheat (*Triticum aestivum* L.)

Introduction

Drought is the most detrimental stress, significantly reducing plant growth and productivity (Talaat 2023). Previous studies have shown that water scarcity reduces crop yield by increasing oxidative damage, affecting cell membrane stability, depressing carbon assimilation, impeding photochemical processes, and hampering leaf gas exchange (Wasaya et al. 2021; Younas et al. 2021; Ahmad et al. 2022). Furthermore, it inhibits ion absorption and the activity of various enzymes (Baghbani-Arani et al. 2021; Heidarzadeh et al. 2022). Interestingly, plants have an inbuilt defensive system that reduces

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dryness's impacts by slowing growth and increasing antioxidant activity (Ebrahimi et al. 2021; Feizabadi et al. 2021).

Cost-effective and ecologically safe management techniques are required to increase agricultural productivity in water-scarce environments. Vermicompost is an effective supplement that can be used in sustainable agricultural systems to maintain soil fertility and plant nutrition, protect soil biodiversity, and boost crop yields (Parastesh et al. 2019; Choudhary and Machavaram 2023). Applying vermicompost has been shown to improve plant growth by increasing soil microbial activity and enriching the soil with nutrients such as nitrogen, phosphorus, potassium, zinc, copper, manganese, and iron (Rezaei-Chiyaneh et al. 2021; Song et al. 2022; Boruah and Deka 2023; Rehman et al. 2023). Vermicompost also provides a significant amount of humic matter, which prevents soil erosion and nutrient leaching while improving soil moisture retention and acting as an excellent root-growing medium (Ma et al. 2022). Additionally, because of its high content of vitamins and plant growth hormones such as auxins, gibberellins, and cytokinins, vermicompost can promote plant development and biomass (Cai et al. 2018).

Vermicompost has potential uses in water-stressed regions (Ebrahimi et al. 2021; Feizabadi et al. 2021; Jahan et al. 2023; Rehman et al. 2023). It can reduce crop water requirements by 30–40% because of its high-water absorption capacity (Arancon et al. 2005). Moreover, its superior physical characteristics including high porosity, aeration, drainage, and water-holding capacity, can reduce water evaporation by preventing soil crusting (Song et al. 2022). In line with its role as a growth-promoter under water stress, studies by Baghbani-Arani et al. (2021), Younas et al. (2021), and Ahmad et al. (2022) demonstrate that it improves plant nutrient uptake and photosynthetic efficiency. Application of vermicompost also enhanced the plant's antioxidant capacity when water was scarce (Hafez et al. 2021; Younas et al. 2021; Ghaffari et al. 2022; Heidarzadeh et al. 2022). However, little is known regarding vermicompost's impact on wheat plants grown under water stress.

In sustainable agriculture approaches, effective microorganisms (EM; including photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes, and fermenting fungi) are critical to prevent the worsening of environmental stresses (Talaat and Shawky 2017; Abd El-Mageed et al. 2020). Applying EM enhances soil microbial diversity, structure, and fertility while reducing the requirement for chemical fertilizers and pesticides (Talaat et al. 2015; Abd El-Mageed et al. 2022). In addition to producing bioactive substances like amino acids, vitamins, sugars, lactic acid, hormones, and enzymes, EM inhibited soil diseases, accelerated the breakdown of organic materials in the soil, and mitigated the harmful effects of stress on plants (Abd El-Mageed et al. 2022; Abdelkhalik et al. 2023). Numerous studies have shown that EM improves plant stress tolerance by modulating photosynthesis, protein synthesis, nutrient uptake, osmotic balance, and antioxidant activity (Talaat 2015; Abd El-Mageed et al. 2020; Abdelkhalik et al. 2023).

Wheat (*Triticum aestivum* L.) feeds a substantial proportion of the world's population (Shopova et al. 2021; Wasaya et al. 2021). Unfortunately, drought has a deleterious impact on its physiological and biochemical processes (Ahmad et al. 2022; Jahan et al. 2023). Some studies have been undertaken to determine how plants grown under stressful conditions respond to a single application of vermicompost or EM (Abd El-Mageed et al. 2022; Ahmad et al. 2022; Ghaffari et al. 2022; Abdelkhalik et al. 2023). However, no one has investigated the effect of their combined treatment on wheat drought tolerance. We conducted this study, as a first investigation, to fill this gap by evaluating the effect of individual and combination treatments of vermicompost and EM on wheat plants grown under water deficiency. Consequently, it has been hypothesized that these effective amendments (vermicompost and EM) could lessen the damaging effects of water stress on wheat growth and production by modifying photosynthetic activity and preserving ion content through modulation of H^+-pump activity and ATP content. This hypothesis was tested by measuring changes in photosynthetic efficiency, including photosynthetic pigment concentration, photochemical reaction activity, gas exchange capacity, chlorophyll fluorescence system, Calvin cycle enzyme activity, as well as changes in nutrient (nitrogen, phosphorus, potassium, iron, zinc, copper) acquisition, roots' ATP content, roots' H^+-pump activity, lipid peroxidation, hydrogen peroxide content, membrane stability index, and electrolyte leakage, in wheat plants cultivated under both well-watered and water-stressed conditions with vermicompost and/or EM applications. This work provides new evidence that combining EM and vermicompost can mitigate drought-related damage to wheat plants.

Materials and methods

Plant materials and experimental design

In a greenhouse at Cairo University's Faculty of Agriculture's Plant Physiology Department, a pot experiment was carried out under natural light and temperature conditions of 65% relative humidity and 22/16 ± 2 °C (day/night) temperature. The experiment was repeated twice, on November 15th of 2021 and 2022. Wheat (*Triticum aestivum* L. cv. Sakha 95) grains were obtained from Egypt's Wheat Research Department, Agriculture Research Centre, Ministry of Agriculture. The 30 cm diameter and 35 cm height pots were filled with 15 kg of clay loamy soil (37% sand, 28% silt, and 35% clay). Ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_5), and potassium sulfate (48% K_2O) were applied at rates of 2.0, 2.0, and 0.5 g pot^{-1}, respectively. Additionally, 30 days after planting, 2.0 g pot^{-1} of ammonium nitrate was added. Fertilization was carried out as recommended by the Ministry of Agriculture. Each pot contained ten grains, which were later thinned to six after germination.

Before sowing, the pots were separated into three groups. The first group was a control [well-watered (WW; 100% of field capacity)], while the other two were irrigation treatments [water deficit stress (WD1; 70% of field capacity) and water deficit stress (WD2; 30% of field capacity)]. Soil water content (SWC) % was 15.5%, 10.8%, and 4.6%, respectively. Drought conditions were ensured by measuring SWC, which was calculated as previously described by Coombs et al. (1987). SWC $\% = [(FW - DW)/DW] \times 100$, where FW was the fresh weight of a portion of soil taken from the internal area of each pot and DW was the dry weight of the soil portion after for 4 days of oven drying at 85 °C. All irrigation treatments were implemented from grain sowing to grain filling.

Twelve treatments were used, including three levels of irrigation (100%, 70%, and 30% of field capacity) and four treatments [control, as well as individual and combined applications of vermicompost and EM]. The experiment was set up in a completely randomized design, with two factors (water deficit stress and application treatments) and four replicates.

Preparation of vermicompost

For vermicompost production, a 5 m^2 area was considered; then, well-rotted animal manure (rabbit + horse manure) was spread over a 50 m long, 1 m wide, and 40 cm thick area. To lessen the salt, well-rotted animal manure was washed away with water prior to production. After 1 day when the bed moisture reached 65–70%, 8–10 kg of epigeic earthworm species; *Eisenia andrei*, *Eisenia fetida*, *Eudrilus eugeniae*, *Lumbricus rubellus*, and *Perionyx excavatus* were added per ton of animal manure. Daily watering was planned to maintain an appropriate moisture level (60–70%). In addition, an effort was made to keep the ambient temperature at 23 ± 3 °C. The vermicompost was stirred and aerated with a rake every 7–10 days to make sure there was enough air and oxygen inside the vermicompost, which was ready after 3 months. Prior to harvesting, irrigation was turned off. Due to the moisture in the lower layers, the earthworms moved to the lower portion when the top layer dried. Vermicompost was consequently taken out of the top layers. The soil was thoroughly mixed with the vermicompost at a ratio of (1:4; vermicompost:soil) before planting.

Soil and vermicompost analysis

Using a Mettler pH FE28-Standard, the pH of soil and vermicompost was detected at a 1:2.5 ratio (soil/water, m/v). The titration with potassium dichromate was used to measure the organic matter. Total nitrogen was determined using the Kieldahl apparatus, total phosphorus was measured using the sodium hydroxide alkali fusion–molybdenum antimony colorimetric resistance method, total potassium was detected using a flame photometer (Sherwood Flame photometer, Model-410; Sherwood Scientifics, Ltd., Cambridge UK), available nitrogen was determined using the alkaline hydrolysis diffusion method, available phosphorus was measured using the $NaHCO_3$ extraction-molybdenum antimony colorimetric resistance method, and rapidly potassium was detected using the NH_4OAc extraction-flame photometry method (Boruah et al. 2019). Iron, zinc, and copper element analyses were performed using an atomic-absorption spectrophotometer (Unicam 989-AA Spectrometer-UK). The physio-chemical properties of the soil and vermicompost used in the current experiment are shown in Table 1.

Effective microorganisms' application

Ten days after sowing, the EM treatment was applied to plants from each irrigation level. It was used in the EM1 formulation developed by Egypt's Ministry of Agriculture and Land Reclamation. EM1 contained 3.3×10^4 Colony Forming Unit (CFU) mL^{-1} of photosynthetic bacteria (*Rhodopseudomonas palustrus* and *Rhodobacter spaeroides*), 1.3×10^7 CFU mL^{-1} of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, and *Streptoccus lactis*), 1.3×10^4 CFU mL^{-1} of yeast (*Saccharomyces cerevisiae*

Table 1 Physio-chemical properties of the soil and vermicompost used in the experiment

Characteristic	Soil	Vermicompost
Moisture content (%)	2.41 ± 0.09	4.33 ± 0.07
pH	7.20 ± 0.15	7.79 ± 0.11
Organic carbon (%)	0.83 ± 0.01	38.37 ± 0.97
Organic matter (%)	2.11 ± 0.06	61.98 ± 1.01
Total nitrogen (%)	0.22 ± 0.03	3.37 ± 0.10
Total phosphorus (%)	0.06 ± 0.00	2.61 ± 0.09
Total potassium (%)	0.41 ± 0.04	3.07 ± 0.11
Available nitrogen (mg Kg^{-1})	33.14 ± 0.66	564.98 ± 10.03
Available phosphorus (mg Kg^{-1})	28.27 ± 1.03	224.49 ± 6.45
Available potassium (mg Kg^{-1})	46.41 ± 1.29	471.48 ± 10.66
Iron (ppm)	5.90 ± 0.09	1399 ± 16.33
Zinc (ppm)	2.00 ± 0.07	150 ± 3.23
Copper (ppm)	1.10 ± 0.04	41 ± 1.14

Values are means ± standard error ($n = 4$)

and *Candida utilis*), 10^5 CFU mL^{-1} actinomycetes (*Streptomyces albus* and *Streptomyces griseus*), and 10^5 CFU mL^{-1} fermenting fungi (*Aspergillus oryzae*, *Penicillium* sp., and *Mucor hiemalis*)]. A 1:1000 (EM:water) dilution of the EM1 stock solution was performed. During irrigation, 7 mL pot^{-1} from this diluted solution was sprayed on the plant and soil surface as recommended by Egypt's Ministry of Agriculture and Land Reclamation.

Plant growth and production measurements

In 70-day-old wheat plants, plant height, leaf number, total leaf area, as well as shoot and root dry weights were measured. The total leaf area was measured using a portable leaf area meter (LI-COR 3000, Lambda Instruments Corporation, Lincoln, Nebraska, USA). After being dried in an oven for 48 h at 70 °C, the shoot and root dry weights were calculated. Four replicates, each with six plants taken from the same pot, were used to collect the data. At maturity, the number of grains, grain yield, and 1000-grain weight were recorded.

In wheat plants that were 70 days old, the photosynthetic pigment concentration, photochemical reaction activity, gas exchange capacity, chlorophyll fluorescence system, Calvin cycle enzyme activity, roots' ATP content, roots' H^+-pump activity, lipid peroxidation, hydrogen peroxide content, membrane stability index, and electrolyte leakage were identified. When the grains reached maturity, they were collected, and the mineral element acquisition was determined. Four replicates, each with six plants taken from the same pot, were used to collect the data.

Photosynthetic pigment measurement

Photosynthetic pigments from fresh leaves were extracted in 80% (v/v) acetone. The concentration of chlorophyll *a*, chlorophyll *b*, and carotenoids was determined spectrophotometrically using a UV-1750 spectrophotometer (Shimadzu, Kyoto, Japan) in accordance with the method of Lichtenthaler and Buschmann (2001).

Measurement of photosynthetic photochemical reaction activity

To isolate the chloroplast, Cerovic and Plesnicar's (1984) method was followed. Tiwari et al. (1998) reported the identification of the PSII-mediated electron transport from H_2O to *p*-benzoquinone. PSI-mediated electron transport was measured in terms of oxygen consumption using 2, 6-dichlorophenol indophenols as the electron donor and methyl viologen as the final acceptor (Allen and Holmes 1986).

Measurement of gas exchange capacity and Chlorophyll fluorescence system

The Li-Cor-6400 (Li-Cor Inc., Lincoln, NE, USA) infrared gas analyzer was used to measure the attached leaves' gas exchange between 8:30 and 11:30 am. The photosynthetic photon flux density was established at 1000 µmol m^{-2} s^{-1}. The greenhouse's air was maintained at ambient conditions in terms of temperature, relative humidity, and CO_2 concentration. Chlorophyll fluorescence was measured according to Pfündel et al. (2008) method in leaves after 30 min of dark adaptation using a Portable Chlorophyll Fluorometer (PAM2500; Heinz Walz, Effeltrich, Germany).

Assay of Calvin cycle enzymes

ELISA kits (Yaji Biotech, Shanghai, China) were used to measure the activity of Calvin cycle enzymes [ribulose diphosphate carboxylase/oxygenase (Rubisco), fructose 1,6-bisphosphatase (FBPase), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and fructose 1,6-bisphosphate aldolase (FBA)] as described by Talaat and Hanafy (2023).

Determination of mineral elements

A transparent solution was produced after 8 h of boiling perchloric acid and hydrogen peroxide with 0.5 g of dried ground grain. The modified micro-Kjeldahl method was used to determine nitrogen concentration (Pregl 1945). By using the vanadomolybdophosphoric method of Kacar (2008), phosphorus concentration was accomplished. A flame photometer (ELE UK) was used to measure the concentration of potassium. An atomic-absorption spectrophotometer (Unicam 989-AA Spectrometer-UK) was used to determine iron, zinc, and copper concentrations.

Determination of roots' ATP content and H^+-pump activity

The manufacturer's instructions for an ATP Colorimetric/Fluorometric Assay Kit (BioVision, Milpitas, CA, USA) were followed in order to extract the ATP as previously described by Stewart and Guinn (1969). Additionally, wheat roots were cut about 2 cm from the tip and washed with deionized water. The plasma and vacuole membranes were isolated using the method described by Yan et al. (2021). The activity of H^+-ATPase and H^+-PPase was measured using Wang and Sze (1985) method. The inorganic phosphate released from ATP or PP hydrolysis was measured using the Ohnishi et al. (1975) method.

Quantification of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)

Following the directions on the H_2O_2 and MDA kits, 0.1 g of fresh wheat leaves were ground in a mortar with 900 µL of buffer to estimate H_2O_2 and MDA, using the procedure described by Nawaz et al. (2018). At 405 nm and 532 nm, respectively, the contents of H_2O_2 and MDA were measured.

Estimation of electrolyte leakage (EL) and membrane stability index (MSI)

The EL was measured according to the method described by Huo et al. (2016). Fresh leaf tissue (0.5 g) was cut into uniformed discs and placed in test tubes with 10 mL deionized water. The EC1 value was recorded after the samples were kept in the dark at 25 °C for 2 h. Following autoclaving and cooling to 25 °C, the samples' EC2 was determined. The formula for calculating EL was EL (%) = $(EC_2/EC_1) \times 100$.

The MSI was determined using Maishanu and Rabe's (2019) method. Wheat leaf samples (0.2 g) were placed in test tubes with 10 mL deionized water and incubated in a water bath for 30 min at 40 °C, after which the EC1 of the solution was determined. The solution EC2 was determined after heating the samples at 100 °C for 10 min. The formula for calculating MSI was MSI (%) = $[1 - EC1/EC2] \times 100$.

Statistical analysis

The obtained data was analyzed using the two-way variance analysis (ANOVA). A completely randomized design with four replications was used. Because the results of the two seasons followed a similar pattern, a combined analysis was performed. To determine the statistical significance of the means at $p < 0.05$, the least significant difference (LSD) test was used. For data analysis, the SAS software (SAS Inc., Cary, NC) was used. The data are presented as means ± standard error (SE).

Results

Effect of vermicompost and/or EM applications on wheat growth and productivity under well-watered and water-stressed conditions

According to the obtained data, wheat growth and productivity dropped significantly ($p < 0.05$) at moderate (70% FC) and severe (30% FC) water deficit conditions compared to well-watered condition (Figs. 1A–H, 2, Table S). In contrast, vermicompost and/or EM applications dramatically improved these attributes under both non-stressed and stressed circumstances. Under well-watered and water-stressed (70% and 30% FC) conditions, the combined treatment significantly improved the shoot height by 48.2%, 62.2%, and 82.4%; leaves number by 61.7%, 74.0%, and 82.5%; total leaf area by 62.1%, 102.5%, and 157.5%; shoot dry weight by 52.4%, 80.4%, and 135.7%; root dry weight by 59.5%, 88.3%, and 192.5%; grains number by 50.1%, 70.7%, and 102.3%; grain yield by 70.5%, 96.0%, and 137.9%; and 1000-grain weight by 13.9%, 14.7%, and 17.7%, respectively, in comparison to the untreated plants.

Effect of vermicompost and/or EM applications on photosynthetic pigment content under well-watered and water-stressed conditions

When compared to the well-watered plants, plants cultivated in a severe (30% FC) water deficit condition showed significantly lower values of chlorophyll *a* (34.5%), chlorophyll *b* (55.0%), carotenoids (59.5%), and total pigments (44.8%). However, vermicompost and/or EM applications greatly raised their values under both stressed and non-stressed circumstances (Fig. 3A–D). Under well-watered and water-stressed (70% and 30% FC) conditions, the combined treatment significantly ($p < 0.05$) elevated the concentration of chlorophyll *a* by 32.5%, 45.9%, and 63.9%; chlorophyll *b* by 47.7%, 74.4%, and 154.4%; carotenoids by 59.5%, 116.7%, and 180.0%; and total pigments by 40.9%, 62.1%, and 100.5%, respectively, relative to the untreated plants.

Effect of vermicompost and/or EM applications on photosynthetic photochemical reaction activity under well-watered and water-stressed conditions

In comparison to the non-stressed plants, plants grown in a severe (30% FC) water deficit condition showed significantly lower PSI (42.0%) and PSII (66.3%) activity. Contrarily, vermicompost and/or EM applications considerably boosted their activity under both stressed and non-stressed circumstances (Fig. 4A, B). Under well-watered and water-stressed (70% and 30% FC) conditions, the combined treatment significantly ($p < 0.05$) increased PSI activity by 37.4%, 58.9%, and 114.5% and PSII activity by 49.5%, 108.3%, and 212.5%, respectively, in comparison to the untreated plants.

Effect of vermicompost and/or EM applications on gas exchange capacity under well-watered and water-stressed conditions

A significant fall in net photosynthetic rate (P_n, 53.8%), stomatal conductance (G_s, 67.3%), and transpiration rate (T_r, 57.2%) values was evident in plants grown under a severe (30% FC) water deficit condition relative to the non-stressed plants. On the contrary, vermicompost and/or EM applications significantly increased these

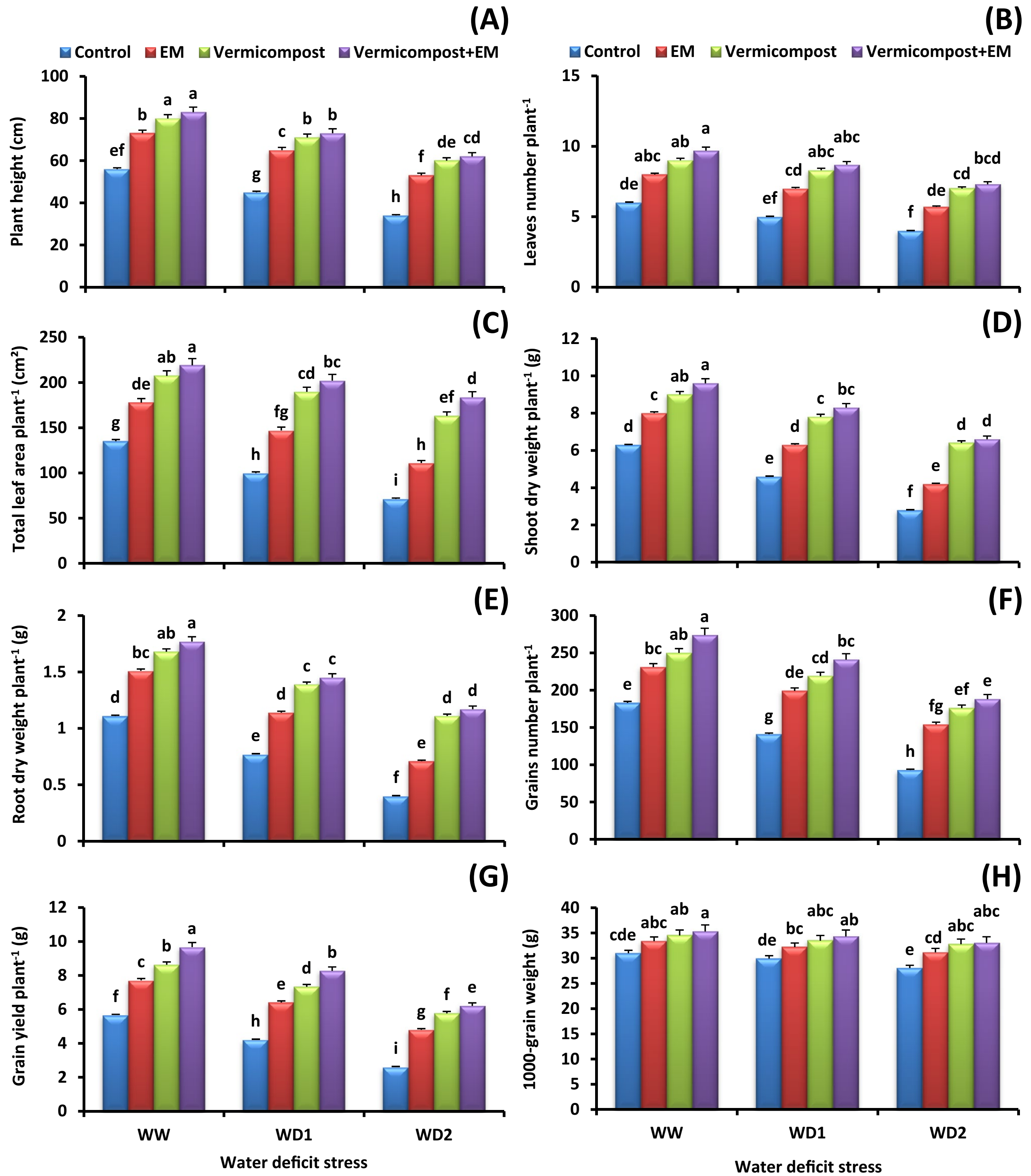

Fig. 1 Effect of effective microorganisms (EM), vermicompost, and their interaction on the: **A** plant height (cm), **B** leaves number plant^{-1}, **C** total leaf area plant^{-1} (cm^2), **D** shoot dry weight plant^{-1} (g), **E** root dry weight plant^{-1} (g), **F** grains number plant^{-1}, **G** grain yield plant^{-1} (g), and **H** 1000-grain weight (g) of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

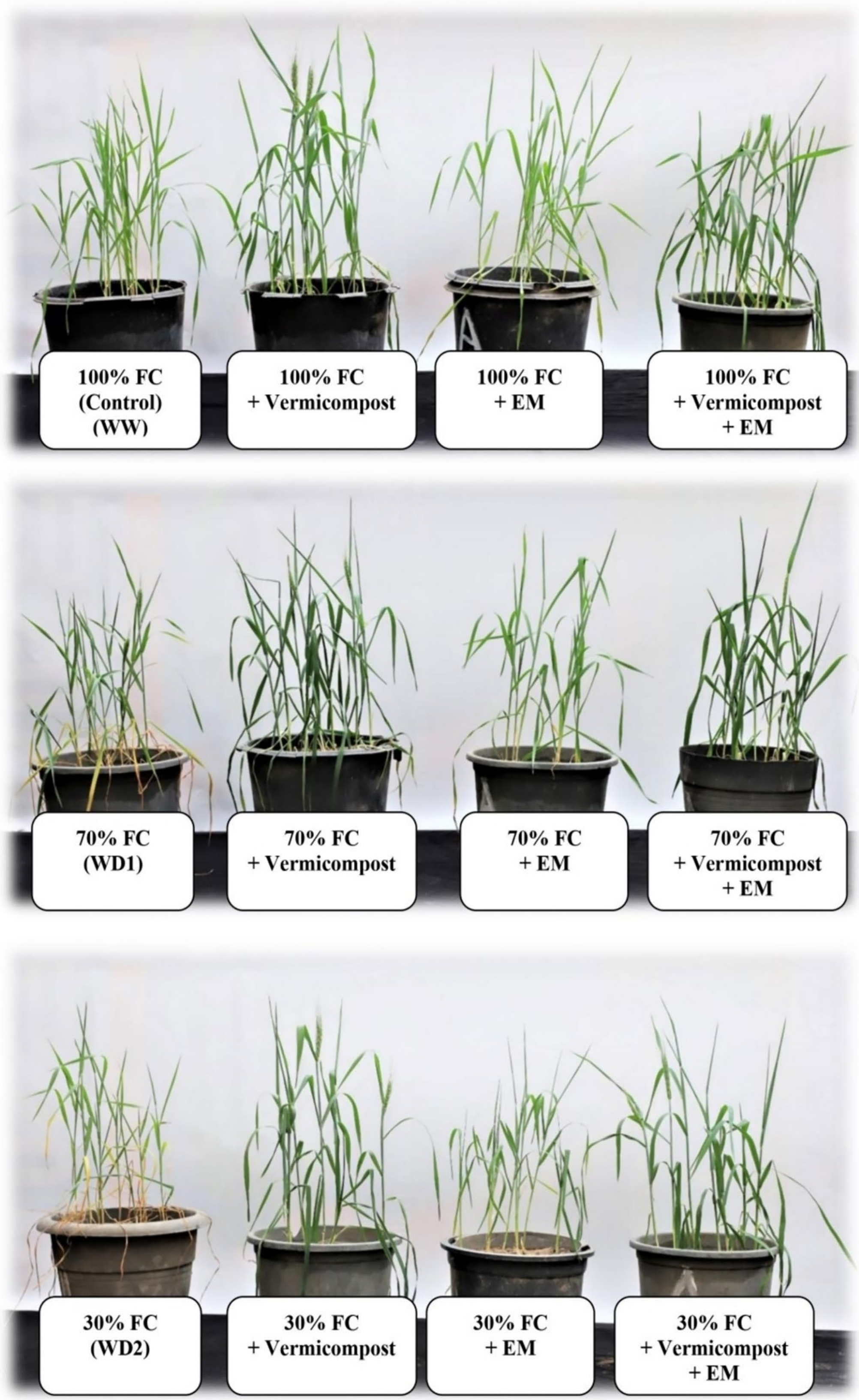

Fig. 2 Effect of effective microorganisms (EM), vermicompost, and their interaction on the growth of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]

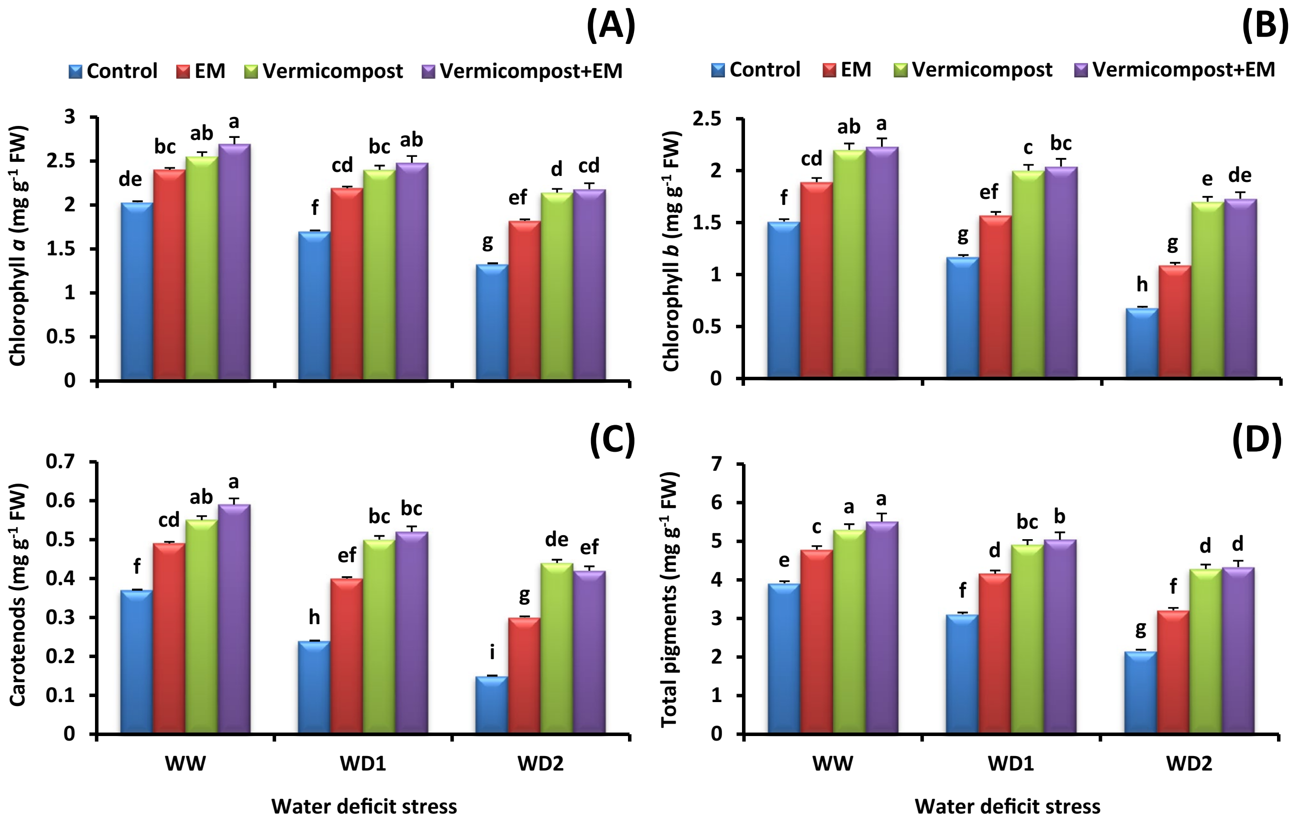

Fig. 3 Effect of effective microorganisms (EM), vermicompost, and their interaction on the concentrations of: **A** chlorophyll *a*, **B** chlorophyll *b*, **C** carotenoids, and **D** total pigments (mg g^{-1} FW) in leaves of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

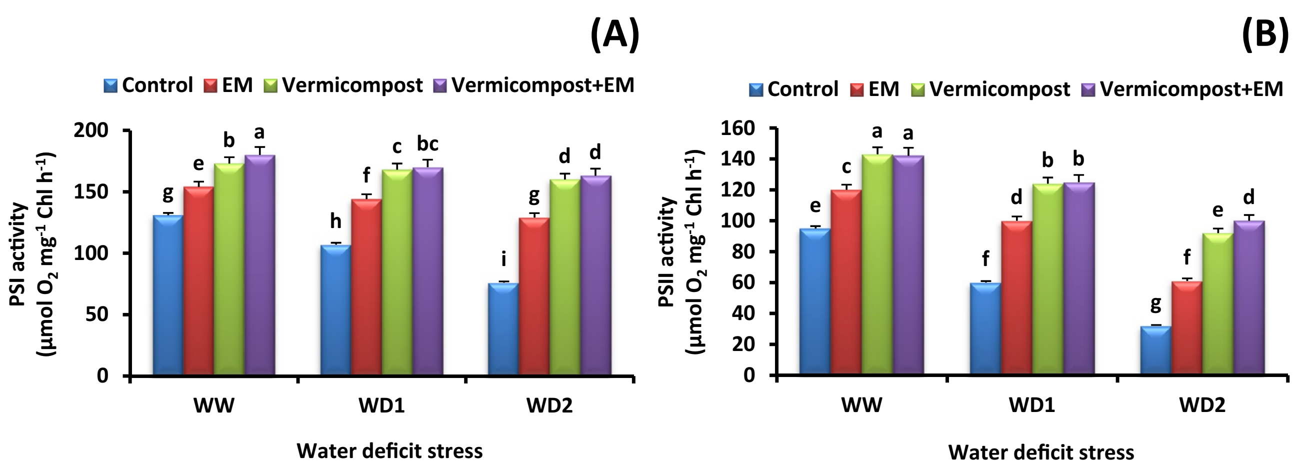

Fig. 4 Effect of effective microorganisms (EM), vermicompost, and their interaction on the: **A** PSI and **B** PSII electron transport activities (µmol O_2 mg^{-1} Chl h^{-1}) in leaves of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

Table 2 Effect of vermicompost, effective microorganisms (EM), and their interaction on the gas exchange parameters in leaves of wheat plants grown under different water deficit conditions

Water deficit conditions + different applications	Photosynthetic rate (P_n, μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (G_s, mol H_2O m^{-2} s^{-1})	Transpiration rate (T_r, mmol H_2O m^{-2} s^{-1})
Well-watered (WW)	17.3 ± 0.81[c]	0.49 ± 0.02 [cd]	7.01 ± 0.14[ef]
WW + EM	20.6 ± 0.62[b]	0.59 ± 0.03[bc]	8.64 ± 0.22[c]
WW + Vermicompost	21.8 ± 0.79[ab]	0.66 ± 0.04[ab]	9.41 ± 0.18[b]
WW + Vermicompost + EM	23.0 ± 0.92[a]	0.71 ± 0.04[a]	9.77 ± 0.20[a]
Water deficit stress (WD1; 70% of field capacity)	13.6 ± 0.47[d]	0.37 ± 0.02[e]	5.39 ± 0.11 [h]
WD1 + EM	18.5 ± 0.39[c]	0.50 ± 0.03 [cd]	6.84 ± 0.15[f]
WD1 + Vermicompost	20.4 ± 0.60[b]	0.59 ± 0.03[bc]	8.10 ± 0.19[d]
WD1 + Vermicompost + EM	21.1 ± 0.43[b]	0.65 ± 0.04[ab]	8.27 ± 0.28[d]
Water deficit stress (WD2; 30% of field capacity)	8.0 ± 0.28[e]	0.16 ± 0.02[f]	3.00 ± 0.17[i]
WD2 + EM	14.9 ± 0.40[d]	0.39 ± 0.03[de]	5.81 ± 0.19 [g]
WD2 + Vermicompost	18.0 ± 0.25[c]	0.49 ± 0.05 [cd]	7.10 ± 0.18[e]
WD2 + Vermicompost + EM	20.4 ± 0.31[b]	0.56 ± 0.02[bc]	7.14 ± 0.14[e]

Different letters in the same column indicate significant differences ($p < 0.05$) between different treatments according LSD test. Values shown are means ± standard error (SE) of four replicates

values under both non-stressed and stressed conditions (Table 2). Under well-watered and water-stressed (70% and 30% FC) conditions, the combined treatment significantly ($p < 0.05$) enhanced the P_n by 32.9%, 55.1%, and 125.0%; G_s by 44.9%, 75.7%, and 250.0%; and T_r by 39.4%, 53.4%, and 138.0%, respectively, compared to the untreated plants.

Effect of vermicompost and/or EM applications on chlorophyll fluorescence attributes under well-watered and water-stressed conditions

Plants cultivated under a severe (30% FC) water deficit condition had significantly lower maximum quantum efficiency of PSII photochemistry (F_v/F_m, 58.9%), effective quantum yield of PSII photochemistry ($F_v/F_{m'}$, 61.0%), actual photochemical efficiency of PSII (Φ_{PSII}, 65.5%), electron transport rate (ETR, 63.0%), and photochemical quenching coefficient (qP, 50.0%) values than those in the well-watered plants. Drought-induced decrease in these attributes was significantly alleviated by vermicompost and/or EM applications (Table 3). Water deficit conditions greatly increased the non-photochemical quenching coefficients (qN) value, which was mitigated by vermicompost and/or EM treatments (Table 3). Under well-watered and water-stressed (70% and 30% FC) conditions, the combined application significantly ($p < 0.05$) improved the F_v/F_m by 43.0%, 69.9%, and 179.5%; $F_v/F_{m'}$ by 35.1%, 54.2%, and 146.7%; Φ_{PSII} by 44.8%, 82.9%, and 215.0%; ETR by 25.3%, 57.6%, and 172.5%; and qP by 22.1%, 47.3%, and 126.5%, respectively, compared to the untreated plants.

Effect of vermicompost and/or EM applications on photosynthetic enzyme activity under well-watered and water-stressed conditions

When compared to the well-watered plants, plants cultivated in a severe (30% FC) water deficit condition displayed a considerable drop in Rubisco (59.5%), FBPase (54.8%), GAPDH (49.5%), and FBA (41.3%) activity. In contrast, vermicompost and/or EM treatments decreased drought damage and significantly ($p < 0.05$) enhanced their activities (Fig. 5A–D). The combination treatment significantly increased the activity of Rubisco (161.4%), FBPase (118.0%), GAPDH (105.0%), and FBA (72.8%) as compared to the untreated plants at a severe (30% FC) water stress condition.

Effect of vermicompost and/or EM applications on nutrient acquisition under well-watered and water-stressed conditions

Plants cultivated in a severe (30% FC) water deficit condition acquired significantly less nitrogen (52.6%), phosphorus (50.0%), potassium (49.7%), iron (46.8%), zinc (46.4%), and copper (53.0%) than the non-stressed plants. Contrarily, application of vermicompost and/or EM significantly reduced the negative impacts of water shortage on the content of macro- and micro-elements (Fig. 6A–F). When compared to the untreated plants under a severe (30% FC) water stress condition, combined treatment significantly ($p < 0.05$) enhanced the concentration of nitrogen, phosphorus, potassium, iron, zinc, and copper by 120.0%, 150.0%, 138.4%, 104.1%, 97.0%, and 132.7%, respectively.

Table 3 Effect of vermicompost, effective microorganisms (EM), and their interaction on the chlorophyll fluorescence attributes in leaves of wheat plants grown under different water deficit conditions

Water deficit conditions + different applications	Maximum quantum efficiency of PSII photochemistry (F_v/F_m)	Actual photochemical efficiency of PSII (Φ_{PSII})	Effective quantum yield of PSII photochemistry ($F_v/F_{m'}$)	Electron transport rate (ETR)	Photochemical quenching coefficient (qP)	Non-photochemical quenching coefficients (qN)
Well-watered (WW)	1.07 ± 0.05^{e}	0.58 ± 0.03^{c}	0.77 ± 0.06^{bcd}	72.8 ± 0.70^{cd}	0.68 ± 0.04^{cd}	0.34 ± 0.02^{cd}
WW + EM	1.33 ± 0.04^{bc}	0.68 ± 0.04^{bc}	0.89 ± 0.06^{ab}	80.9 ± 0.90^{b}	0.75 ± 0.04^{abc}	0.33 ± 0.02^{cd}
WW + Vermicompost	1.42 ± 0.06^{ab}	0.77 ± 0.04^{ab}	0.98 ± 0.08^{a}	90.0 ± 1.11^{a}	0.83 ± 0.05^{a}	0.30 ± 0.01^{d}
WW + Vermicompost + EM	1.53 ± 0.07^{a}	0.84 ± 0.03^{a}	1.04 ± 0.08^{a}	91.2 ± 0.83^{a}	0.83 ± 0.05^{a}	0.30 ± 0.02^{d}
Water deficit stress (WD1; 70% of field capacity)	0.83 ± 0.05^{f}	0.41 ± 0.01^{e}	0.59 ± 0.05^{de}	51.6 ± 0.64^{e}	0.55 ± 0.07^{e}	0.44 ± 0.03^{b}
WD1 + EM	1.13 ± 0.04^{de}	0.57 ± 0.01^{cd}	0.76 ± 0.07^{bcde}	67.9 ± 0.88^{d}	0.69 ± 0.06^{bcd}	0.37 ± 0.03^{c}
WD1 + Vermicompost	1.27 ± 0.05^{bcd}	0.68 ± 0.04^{bc}	0.86 ± 0.07^{abc}	80.0 ± 1.01^{b}	0.78 ± 0.05^{ab}	0.33 ± 0.04^{cd}
WD1 + Vermicompost + EM	1.41 ± 0.06^{ab}	0.75 ± 0.05^{ab}	0.91 ± 0.06^{ab}	81.3 ± 0.99^{b}	0.81 ± 0.04^{a}	0.32 ± 0.03^{cd}
Water deficit stress (WD2; 30% of field capacity)	0.44 ± 0.05^{g}	0.20 ± 0.01^{f}	0.30 ± 0.02^{f}	26.9 ± 0.35^{f}	0.34 ± 0.02^{f}	0.57 ± 0.04^{a}
WD2 + EM	0.85 ± 0.04^{f}	0.43 ± 0.01^{de}	0.58 ± 0.04^{e}	53.1 ± 0.56^{e}	0.60 ± 0.04^{de}	0.43 ± 0.05^{b}
WD2 + Vermicompost	1.07 ± 0.05^{e}	0.56 ± 0.02^{cd}	0.70 ± 0.04^{cde}	69.9 ± 0.64^{d}	0.70 ± 0.05^{bc}	0.36 ± 0.05^{c}
WD2 + Vermicompost + EM	1.23 ± 0.05^{cd}	0.71 ± 0.03^{b}	0.74 ± 0.05^{bcde}	73.3 ± 0.81^{c}	0.77 ± 0.04^{abc}	0.35 ± 0.04^{cd}

Different letters in the same column indicate significant differences ($p < 0.05$) between different treatments according LSD test. Values shown are means ± standard error (SE) of four replicates

Effect of vermicompost and/or EM applications on roots' H^+-pump activity under well-watered and water-stressed conditions

Water scarcity significantly reduced the ATP content and PM H^+-ATPase activity in wheat roots; however, treatments with vermicompost and/or EM significantly raised their values and lessened the water stress's inhibitory effect (Fig. 7A, B). In compared to the untreated plants, the combined application of vermicompost and EM resulted in a 280.0% increase in ATP content and a 93.8% rise in PM H^+-ATPase activity in the roots of wheat plants grown under a severe (30% FC) water stress condition. However, the vermicompost application gave the best result and significantly improved the PM H^+-ATPase activity by 38.5% and 52.0% when compared to the untreated plants under well-watered and water-stressed (70% FC) conditions, respectively.

Further evidence from our research showed that water scarcity increased VM H^+-ATPase and VM H^+-PPase activities. Additionally, vermicompost and/or EM treatments increased their activities even more under drought conditions (Fig. 7C, D). The activity of VM H^+-ATPase (59.4%) and VM H^+-PPase (52.3%) in wheat roots was significantly ($p < 0.05$) boosted by combination treatment under the severe (30% FC) water stress condition compared to the untreated plants.

Effect of vermicompost and/or EM applications on oxidative stress markers under well-watered and water-stressed conditions

In comparison to the non-stressed plants, plants grown in a severe (30% FC) water deficit condition showed a significant increase in H_2O_2 (70.1%) and MDA (68.9%) values. On the contrary, the application of vermicompost and/or EM significantly decreased their values (Fig. 8A, B). The combination treatment significantly ($p < 0.05$) decreased the H_2O_2 content by 34.1% and 41.7% and MDA content by 36.2% and 43.0% under water-stressed (70% and 30% FC) conditions, respectively, relative to the untreated plants.

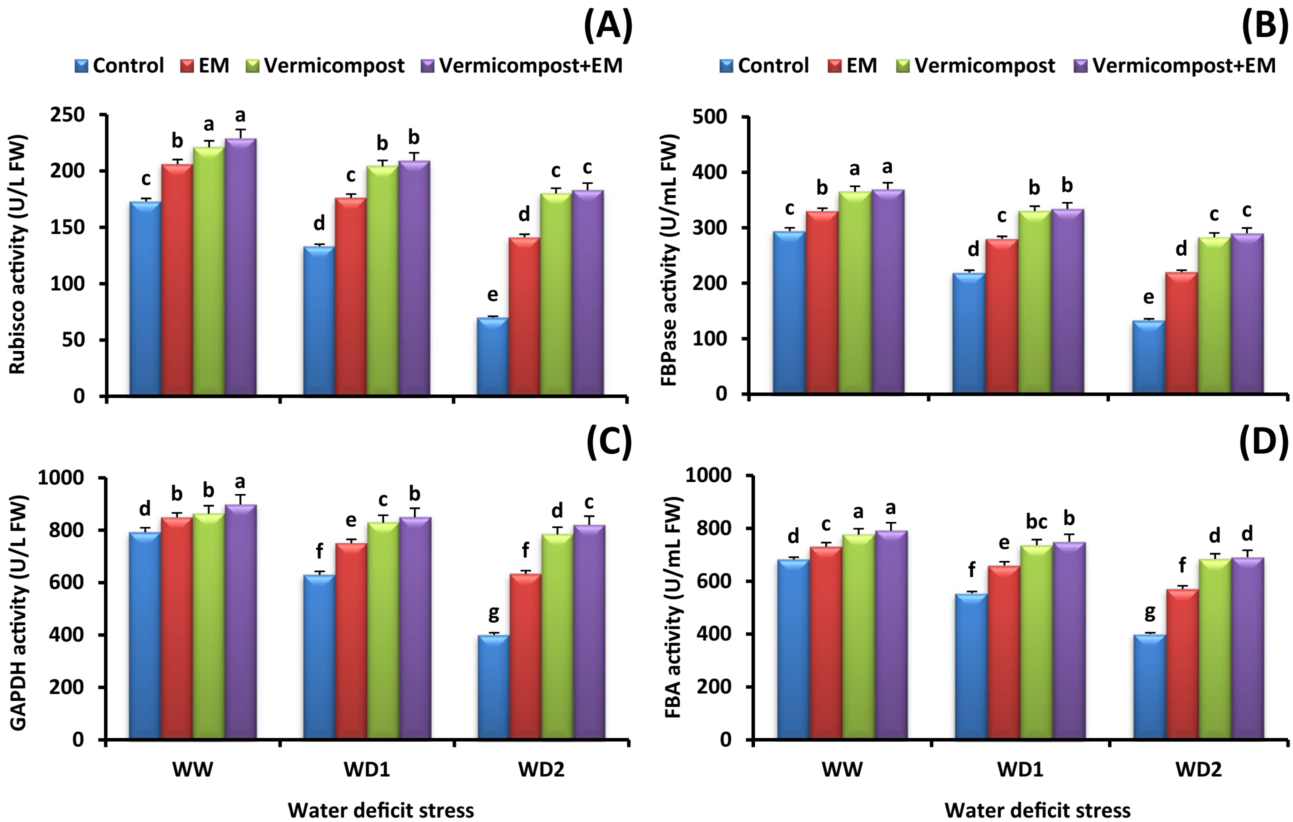

Fig. 5 Effect of effective microorganisms (EM), vermicompost, and their interaction on the activity of: **A** ribulose diphosphate carboxylase/oxygenase (Rubisco), **B** fructose 1,6-bisphosphatase (FBPase), **C** glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and **D** fructose 1,6-bisphosphate aldolase (FBA) in leaves of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

Effect of vermicompost and/or EM applications on cell membrane stability under well-watered and water-stressed conditions

Water deficiency reduced MSI value while increasing EL. In contrast, vermicompost and/or EM applications improved wheat's ability to withstand drought by raising MSI and lowering EL (Fig. 8C, D). Under a severe (30% FC) water stress condition, combined treatment significantly ($p < 0.05$) raised MSI (110.7%) and decreased EL (33.3%) in wheat leaves, compared to the untreated ones.

Notably, using vermicompost alone or in combination with EM improved all of the tested parameters more effectively than using EM alone. Furthermore, under a severe (30% FC) water stress condition, when combined with EM, vermicompost had a greater effect on modulating total leaf area, grain yield, P_n, F_v/F_m, Φ_{PSII}, ETR, phosphorus, potassium, iron, ATP, and MSI values, as well as PSII, GAPDH, and PM H^+-ATPase activities than either vermicompost or EM alone.

Discussion

Drought stress is one of the major global factors affecting wheat production (Chowdhury et al. 2021). Vermicompost (Feizabadi et al. 2021; Ahmad et al. 2022; Ma et al. 2022; Jahan et al. 2023; Rehman et al. 2023) and EM (Talaat et al. 2015; Talaat and Shawky 2017; Abd El-Mageed et al. 2020, 2022; Abdelkhalik et al. 2023) have been found to improve plant growth and productivity, particularly under challenging conditions. Nonetheless, no information on the effect of combining vermicompost with EM on wheat drought tolerance is currently available. The current study demonstrates that combining vermicompost with EM can alleviate the deleterious effects of water deficiency via enhancing photosynthetic machinery, nutrient uptake efficiency, root H^+-pump activity, and cell membrane stability. This study sheds new light on the mechanisms of water stress alleviation in wheat using vermicompost and EM treatments.

Drought is the most serious threat, limiting plant growth and productivity (Todorova et al. 2016; Wasaya et al.

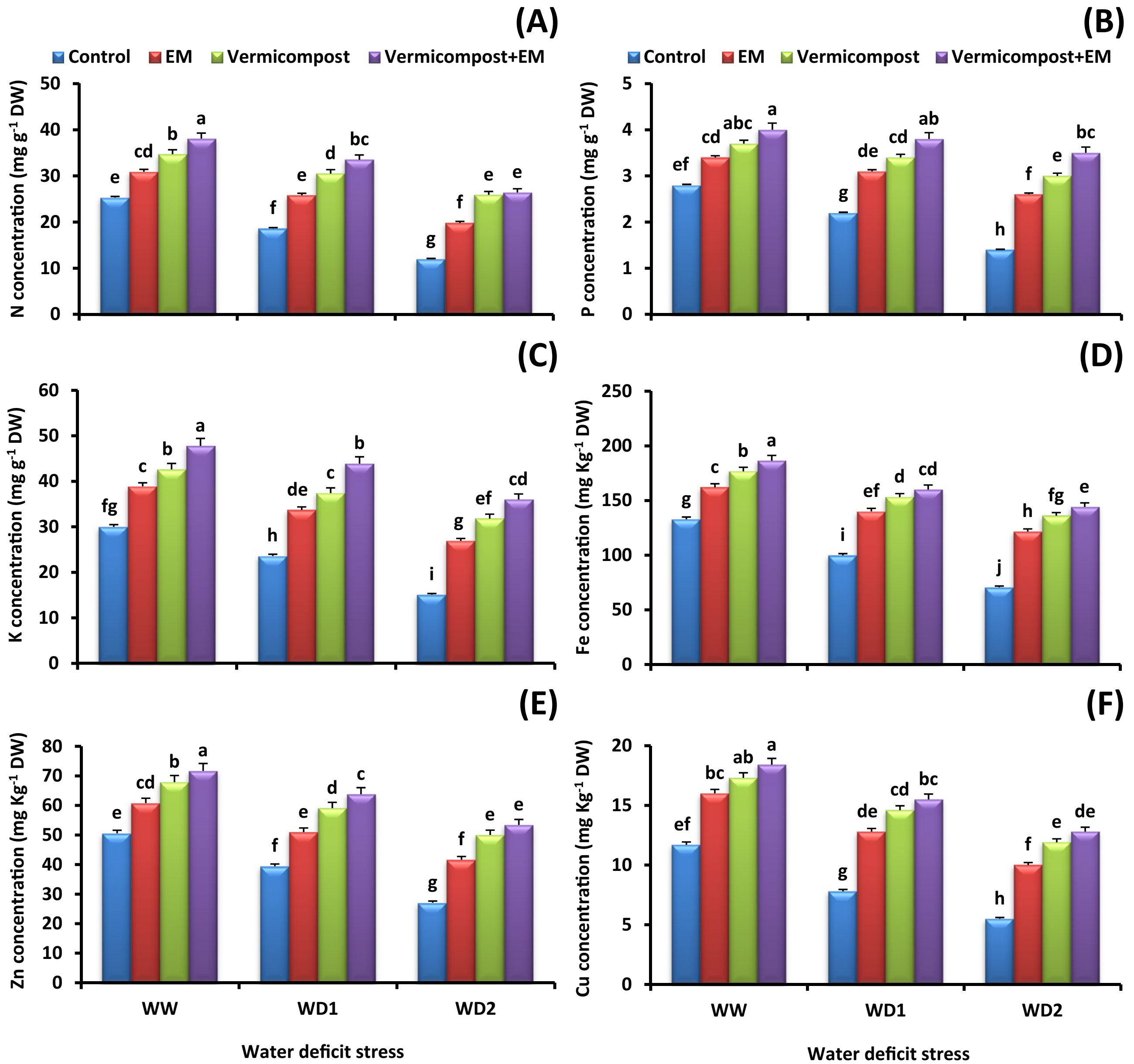

Fig. 6 Effect of effective microorganisms (EM), vermicompost, and their interaction on the concentration of: **A** nitrogen (N), **B** phosphorus (P), **C** potassium (K), **D** iron (Fe), **E** zinc (Zn), and **F** copper (Cu) in grains of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

2021; Talaat 2023). Our findings manifested a significant depression in the growth and productivity of wheat plants grown in water-scarce circumstances. This decline could be explained by the effects of ROS buildup, which can block critical metabolic processes like photosynthesis, mineral uptake, enzyme activity, and damage to important cellular compartments (Talaat and Shawky 2012, 2022; Chowdhury et al. 2021). Our research also showed a considerable reduction in photosynthetic pigment content, photochemical processes, Calvin cycle enzyme activity, photosynthetic rate, acquisition of essential nutrients, along with a significant damage to cell membrane structure that in turn lessens wheat productivity under water-stressed environments. Contrarily, we found that vermicompost and/or EM applications significantly enhanced wheat growth and production under water-scarce conditions. This can be explained by lowering oxidative damage and photosynthetic pigment degradation while improving photosynthetic activity, nutrient acquisition, roots' H^+-pump activity, and roots' ATP content. In agreement with our results, Baghbani-Arani et al. (2021),

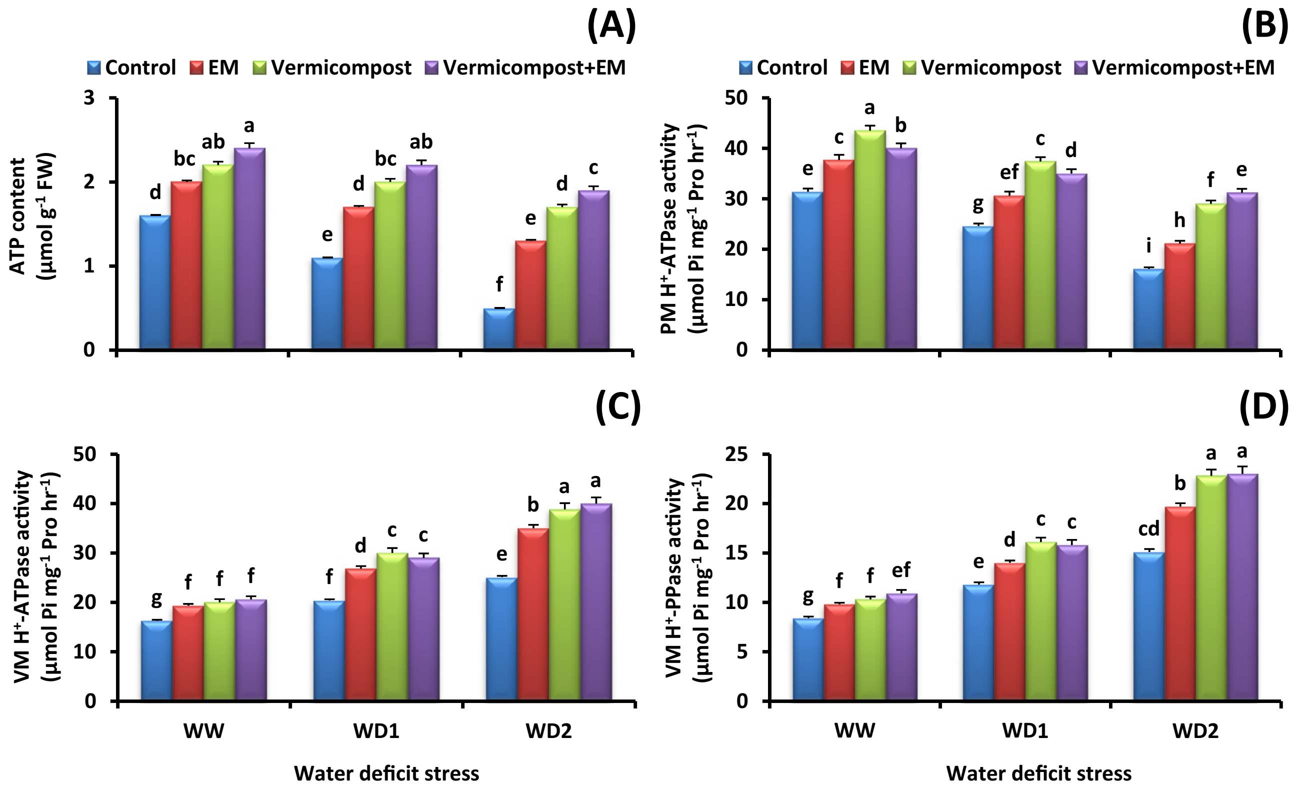

Fig. 7 Effect of effective microorganisms (EM), vermicompost, and their interaction on the: **A** ATP content, **B** plasma membrane (PM) H^+-ATPase activity, **C** vacuole membrane (VM) H^+-ATPase activity, and **D** vacuole membrane (VM) H^+-PPase activity in roots of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

Ebrahimi et al. (2021), Feizabadi et al. (2021), Hafez et al. (2021), Ahmad et al. (2022), Heidarzadeh et al. (2022), and Jahan et al. (2023) demonstrated that vermicompost significantly boosted plant growth and development under water scarcity. Most likely, improved soil water retention capacity, reduced nutrient leaching, increased nutrient availability, and the presence of phosphate-solubilizing bacteria, nitrogen-fixing bacteria, zinc-solubilizing bacteria, actinomycetes, humus, and growth hormones like cytokinins, auxins, and gibberellins in vermicompost are the causes of these restorations in vermicompost-treated plants (Mengistu et al. 2017; Cai et al. 2018; Rezaei-Chiyaneh et al. 2021; Ma et al. 2022; Song et al. 2022). Additionally, in this study, EM application greatly increased wheat growth and production. This result is consistent with those of Talaat (2015) and Abd El-Mageed et al. (2022), who found that EM stimulate plant growth and crop productivity under stressful conditions. Actually, under challenging conditions, EM can significantly increase crop production by producing biologically active molecules (such as lactic acid, vitamins, amino acids, esters, sugars, enzymes, hormones, etc.), improving soil physical and chemical properties, accelerating organic waste decomposition, activating beneficial soil microorganisms, increasing nutrient availability, and boosting photosynthetic capacity (Talaat et al. 2015; Talaat and Shawky 2017; Abd El-Mageed et al. 2020; Abdelkhalik et al. 2023).

Water scarcity is regarded as a negative environmental stress that impairs photosynthesis (Ahmad et al. 2022; Talaat 2023). Our findings indicate that water stress reduces photosynthetic efficiency by negatively affecting the photosynthetic pigment concentration, photochemical reaction activity, gas exchange capacity, Calvin cycle enzyme activity, and chlorophyll fluorescence system. Our results are consistent with those of Talaat (2021), Ghaffari et al. (2022), and Talaat and Hanafy (2023) who found that stressful conditions impair photosynthesis by reducing electron transfer between the two photosystems, photosynthetic pigment level, gas exchange and chlorophyll fluorescence properties, and photosynthetic enzyme activity. Conversely, under water-stressed conditions, our results showed a considerable improvement in photosynthetic metrics in plants treated with vermicompost and/or EM. These findings are consistent with those reported by Abd El-Mageed et al. (2020), Song et al. (2022), Abdelkhalik et al. (2023), and Rehman et al. (2023). This improvement may have been caused by enhanced nutrient and water absorption (Feizabadi et al.

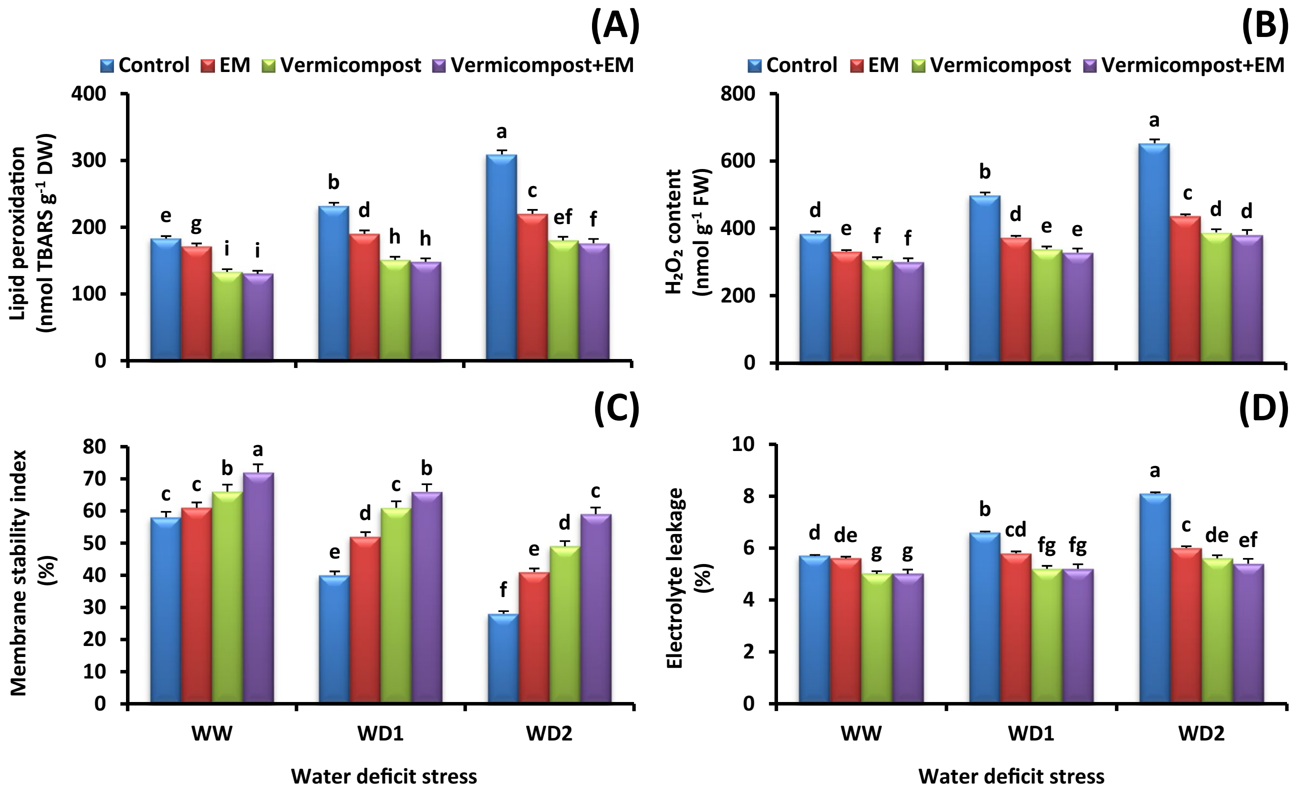

Fig. 8 Effect of effective microorganisms (EM), vermicompost, and their interaction on the: **A** lipid peroxidation, **B** hydrogen peroxide (H_2O_2) content, **C** membrane stability index (%), and **D** electrolyte leakage (%) in leaves of wheat plants grown under three different irrigation levels [100% (WW), 70% (WD1), and 30% (WD2) of field capacity]. Bars represent ± standard error of the mean (n = 4) and the alphabets show significant difference (LSD's test, $p < 0.05$) among treatments

2021; Wasaya et al. 2021; Younas et al. 2021; Ahmad et al. 2022; Abdelkhalik et al. 2023; Jahan et al. 2023) as well as the preservation of antioxidant capacity, membrane integrity, and osmolytes level (Talaat 2015; Abd El-Mageed et al. 2020; Jahan et al. 2023; Rehman et al. 2023).

Plant nutrient uptake is often disrupted by water deficit (Talaat and Shawky 2017; Ahmad et al. 2022). Our data indicated a significant decrease in grain nutrient acquisition due to water scarcity. Likewise, investigations by Baghbani-Arani et al. (2021) and Heidarzadeh et al. (2022) showed that nutrient availability decreased during drought stress. Conversely, when water was scarce, our data demonstrated a significant improvement in grain nutrient acquisition in plants treated with vermicompost and/or EM. Actually, a number of factors are thought to have contributed to vermicompost-mediated improved wheat nutrient content under drought conditions, including altered soil physical properties, which reduces nutrient leaching (Song et al. 2022), enhanced soil porosity and water retention, which increases nutrient availability (Coulibaly et al. 2018; Ma et al. 2022), improved inorganic fertilizer effectiveness, which enhances nutrient uptake (Rezaei-Chiyaneh et al. 2021; Song et al. 2022), reduced oxidative damage (Ahmad et al. 2022), and the presence of necessary macro- and micro-nutrients, beneficial microorganisms, and other bioactive compounds in vermicompost (Cai et al. 2018; Ma et al. 2022). Additionally, our study demonstrated a significant increase in grain nutrient acquisition with EM treatment under water-deficit conditions. This improvement is most likely the result of increased organic matter breakdown and mineralization, which releases more nutrients into the soil for plant absorption (Talaat and Shawky 2017; Naik et al. 2020); improved tissue water content and cell membrane integrity, which increases plant nutrient acquisition (Talaat 2015; Abd El-Mageed et al. 2022); and stimulated root growth, which increases the potential for nutrient uptake (Talaat et al. 2015; Abd El-Mageed et al. 2020; Abdelkhalik et al. 2023). Remarkably, by increasing nutrient uptake, the application of vermicompost and/or EM significantly increased grain yield in terms of both quantity and quality.

Ion transfer is greatly controlled by the roots' ATP content and H^+-pump activity (Talaat and Hanafy 2023). Previous studies have shown that increased root VM H^+-ATPase and VM H^+-PPase activity confers drought tolerance (Vigani et al. 2019; Cheng et al. 2021). This implies that they could be able to maintain cell turgor

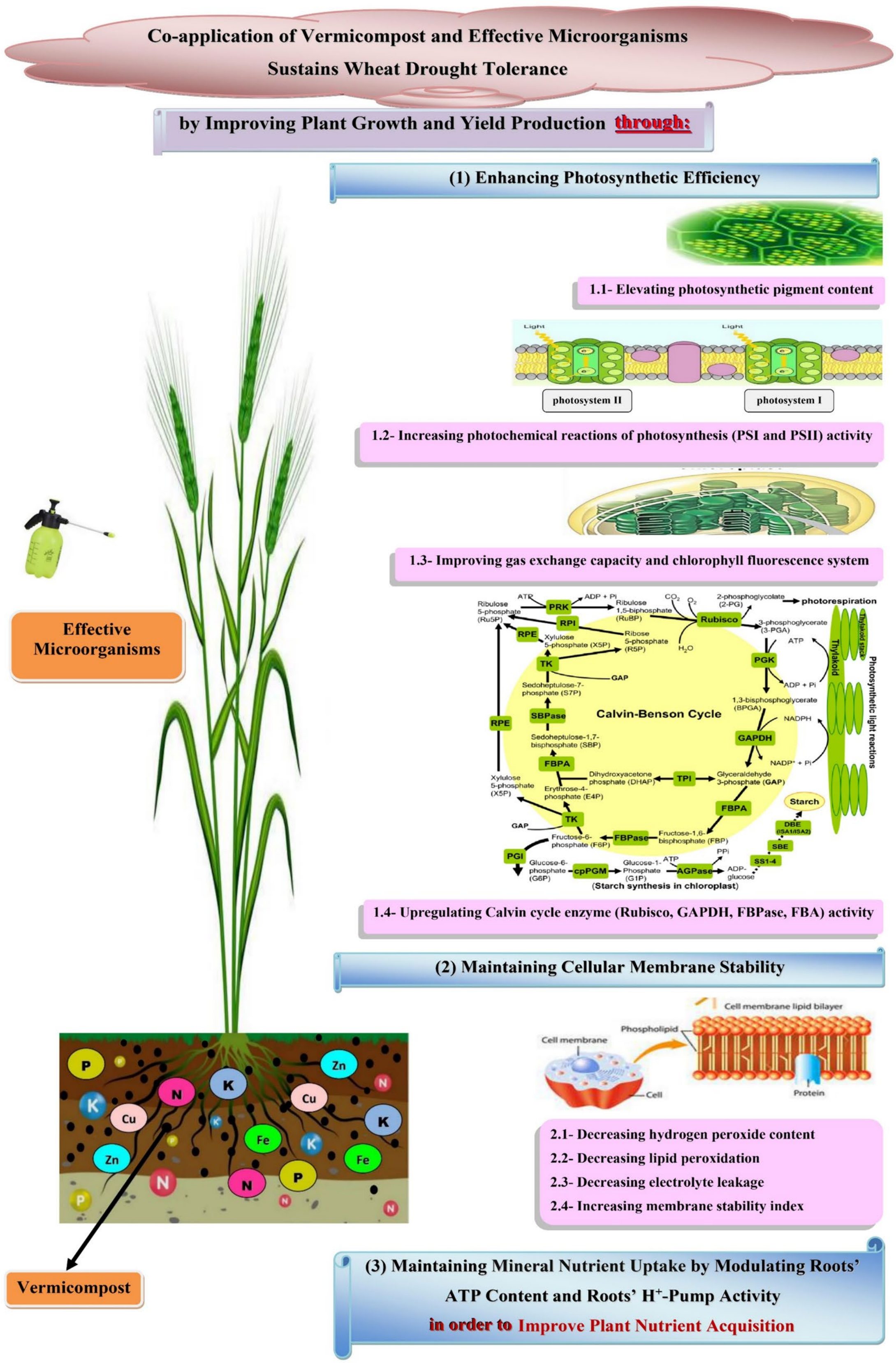

Fig. 9 Co-application of vermicompost and effective microorganisms sustains wheat drought tolerance by enhancing photosynthetic efficiency, maintaining cellular membrane stability, and improving mineral nutrient uptake via modulating roots' ATP content and roots' H^+-pump activity

in plant tissues by generating a proton gradient across the vacuolar membrane (Gaxiola et al. 2016). Furthermore, a correlation between enhanced auxin levels and increased VM H^+-PPase activity has been demonstrated. Auxin promotes cell division during organogenesis and results in longer root systems, improving plant performance and nutrient uptake under drought environments (Park et al. 2005). According to the "acid" growth theory, plant growth requires acidity in the cell wall space, which is caused by H^+ effluxes of PM H^+-ATPase (Staal et al. 2011). Surprisingly, the findings of this study revealed that adding vermicompost and/or EM significantly increased the ATP content and PM H^+-ATPase, VM H^+-ATPase, and VM H^+-PPase activities in wheat roots. This could support the idea that these two amendments could help to maintain plasma membrane polarization, improving nutrient ion uptake and plant performance in water-stressed environments. Moreover, our hypothesis agrees with the result of Canellas et al. (2019), who found that vermicompost increases ion channels activity like H^+/ATPase, improving nutritional ion exchange at the root-soil interface.

Our findings also revealed the presence of oxidative damage in wheat-stressed plants, as evidenced by increased H_2O_2, MDA, and EL levels and reduced MSI. Similar results are reported by Chowdhury et al. (2021), Ahmad et al. (2022), and Talaat et al. (2023). Interestingly, stressed plants exhibited a significant decrease in H_2O_2 and MDA accumulation when treated with vermicompost and/or EM. Decreased MDA and H_2O_2 generation may account for the observed higher MSI and lower EL values. These findings align with those of Talaat (2015), Hosseinzadeh et al. (2018), Abd El-Mageed et al. (2020), Ghaffari et al. (2022), and Abdelkhalik et al. (2023), who observed that stressed-plants treated with vermicompost or EM exhibited a rise in MSI level and a fall in MDA, H_2O_2, and EL values. Actually, the enhanced carotenoids content in wheat leaves of stressed-treated plants is assumed to be responsible for the higher antioxidant capacity. In this respect, Abid et al. (2018) stated that carotenoid is a significant non-enzymatic molecule that, through lowering ROS accumulation, can improve wheat drought tolerance. Moreover, vermicompost is a highly effective antioxidant that can scavenge and capture ROS (Aslam et al. 2021). Furthermore, antioxidant enzyme activity can be increased by the presence of Zn, Fe, Mn, and Cu in vermicompost (Roy et al. 2010). Similarly, EM supplementation increased the activity of antioxidant enzymes, strengthening the ROS scavenging system as a potent H_2O_2 detoxification mechanism (Talaat 2015; Abd El-Mageed et al. 2022). Remarkably, the application of vermicompost, EM, and their combination can preserve normal membrane function and decrease the adverse effects of water-scarce conditions by avoiding EL, stabilizing membranes, and keeping ROS levels within normal ranges.

Our study concludes that combining vermicompost and EM treatments can result in wheat plants with a favorable biochemical profile and a higher yield under water scarcity condition. This combined application improved wheat growth and development, yield quantity and quality, and water stress tolerance by triggering the machinery of photosynthesis, ROS burst, and nutrient absorption (Fig. 9).

Conclusion

Developing an economical, environmentally friendly, and effective water deficit management technique is one of the most important challenges. The current study's findings clearly demonstrate that using co-application of vermicompost and EM reduces the detrimental effects of water scarcity on wheat growth and productivity by enhancing photosynthetic efficiency through improving photosynthetic pigment concentration, gas exchange capacity, photochemical reaction activity, chlorophyll fluorescence system, and Calvin cycle enzyme activity. Furthermore, by inhibiting the generation of H_2O_2 and MDA, the co-application treatment modifies the structure of cellular membranes and reduces oxidative damage. Moreover, this combined treatment improved nutrient acquisition by activating the roots' H^+-pump and increasing the ATP content. In line with the goals of sustainable agriculture, our findings provide novel insight on the use of vermicompost and EM in combination to improve crop stress tolerance.

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Author contributions NBT, SAMA conceived and conceptualized the research. NBT designed and performed the experiment, generated and analyzed the data, and wrote the manuscript. SAMA prepared the vermicompost. All the authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article or supplementary information file.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abd El-Mageed TA, Rady MM, Taha RS, Abd El Azeam S, Simpson CR, Semida WM (2020) Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of *Capsicum annuum* under salt stress. Sci Hortic 261:108930. https://doi.org/10.1016/j.scienta.2019.108930

Abd El-Mageed TA, Gyushi MAH, Hemida KA, El-Saadony MT, Abd El-Mageed SA, Abdalla H, AbuQamar SF, El-Tarabily KA, Abdelkhalik A (2022) Coapplication of effective microorganisms and nanomagnesium boosts the agronomic, physio-biochemical, osmolytes, and antioxidants defenses against salt stress in *Ipomoea batatas*. Front Plant Sci 13:883274. https://doi.org/10.3389/fpls.2022.883274

Abdelkhalik A, Abd El-Mageed TA, Mohamed IAA, Semida WM, Al-Elwany OAAI, Ibrahim IM, Hemida KA, El-Saadony MT, AbuQamar SF, El-Tarabily KA, Gyushi MAH (2023) Soil application of effective microorganisms and nitrogen alleviates salt stress in hot pepper (*Capsicum annum* L.) plants. Front Plant Sci 13:1079260. https://doi.org/10.3389/fpls.2022.1079260

Abid M, Ali S, Qi LK, Zahoor R, Tian Z, Jiang D et al (2018) Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Sci Rep 8:4615. https://doi.org/10.1038/s41598-018-21441-7

Ahmad A, Aslam Z, Belliturk K, Hussain S, Bibi I (2022) Soil application of cellulolytic microbe–enriched vermicompost modulated the morpho-physiological and biochemical responses of wheat cultivars under different moisture regimes. J Soil Sci Plant Nutr 22:4153–4167. https://doi.org/10.1007/s42729-022-01014-4

Allen JF, Holmes NG (1986) Electron transport and redox titrations. In: Hipkins MF, Baker NR (eds) Photosynthesis—energy transduction—a practical approach. IRL Press, Oxford, pp 103–141

Arancon NQ, Edwards CA, Bierman P, Metzger JD, Lucht C (2005) Effects of vermicomposts produced from cattle manure, food waste and paper waste on the growth and yield of peppers in the field. Pedobiologia 49:297–306. https://doi.org/10.1016/j.pedobi.2005.02.001

Aslam Z, Ahmad A, Ibrahim M, Iqbal N, Idrees M, Ali A, Ahmad I, Belliturk K, Nawaz M, Aslam M, Ramzan HN (2021) Microbial enrichment of vermicompost through earthworm *Eisenia fetida* (Savigny, 1926) for agricultural waste management and development of useful organic fertilizer. Pak J Agric Sci 58(3):851–861. https://doi.org/10.21162/PAKJAS/21.1378

Baghbani-Arani A, Modarres-Sanavy SAM, Poureisa M (2021) Improvement the soil physicochemical properties and fenugreek growth using zeolite and vermicompost under water deficit conditions. J Soil Sci Plant Nutr 21:1213–1228. https://doi.org/10.1007/s42729-021-00434-y

Boruah T, Deka H (2023) Comparative investigation on synergistic changes in enzyme activities during vermicomposting of cereal grain processing industry sludge employing three epigeic earthworm species. Environ Sci Pollut Res 30:123324–123334. https://doi.org/10.1007/s11356-023-31043-0

Boruah T, Barman A, Kalita P, Lahkar J, Deka H (2019) Vermicomposting of citronella bagasse and paper mill sludge mixture employing *Eisenia fetida*. Bioresour Technol 294:122147. https://doi.org/10.1016/j.biortech.2019.122147

Cai L, Gong X, Sun X, Li S, Yu X (2018) Comparison of chemical and microbiological changes during the aerobic composting and vermicomposting of green waste. PLoS ONE 13:e0207494. https://doi.org/10.1371/journal.pone.0207494

Canellas LP, Olivares FL, Canellas NOA, Mazzei P, Piccolo A (2019) Humic acids increase the maize seedlings exudation yield. Chem Biol Technol Agric 6:3. https://doi.org/10.1186/s40538-018-0139-7

Cerovic ZG, Plesnicar M (1984) An improved procedure for the isolation of intact chloroplasts of high photosynthetic activity. Biochem J 223:543–545. https://doi.org/10.1042/bj2230543

Cheng H-Q, Zou Y-N, Wu Q-S, Kuca K (2021) Arbuscular mycorrhizal fungi alleviate drought stress in trifoliate orange by regulating H^+-ATPase activity and gene expression. Front Plant Sci 12:659694. https://doi.org/10.3389/fpls.2021.659694

Choudhary V, Machavaram R (2023) A comprehensive review of sustainable soil organic growing media for mat-type paddy seedling nurseries under Indian agronomical condition. J Soil Sci Plant Nutr 23:1515–1534. https://doi.org/10.1007/s42729-023-01153-2

Chowdhury MK, Hasan MA, Bahadur MM, Islam M, Hakim M, Iqbal MA, Javed T, Raza A, Shabbir R, Sorour S, Elsanafawy NE (2021) Evaluation of drought tolerance of some wheat (*Triticum aestivum* L.) genotypes through phenology, growth and physiological indices. Agronomy 11(9):1792. https://doi.org/10.3390/agronomy11091792

Coombs J, Hall DO, Long SP, Scurlock JMO (1987) Techniques in bioproductivity and photosynthesis. Pergamon, Oxford

Coulibaly SS, Edoukou FE, Kouassi KI, Barsan N, Nedeff V, Bi Zoro IA (2018) Vermicompost utilization: a way to food security in rural area. Heliyon 4:1–24. https://doi.org/10.1016/j.heliyon.2018.e01104

Ebrahimi M, Souri MK, Mousavi A, Sahebani N (2021) Biochar and vermicompost improve growth and physiological traits of eggplant (*Solanum melongena* L.) under deficit irrigation. Chem Biol Technol Agric 8:1–14. https://doi.org/10.1186/s40538-021-00216-9

Feizabadi A, Noormohammadi G, Fatehi F (2021) Changes in growth, physiology, and fatty acid profile of rapeseed cultivars treated with vermicompost under drought stress. J Soil Sci Plant Nutr 21:200–208. https://doi.org/10.1007/s42729-020-00353-4

Gaxiola RA, Regmi K, Paez-Valencia J, Pizzio G, Zhang S (2016) Plant H^+-PPases: reversible enzymes with contrasting functions dependent on membrane environment. Mol Plant 9:317–319

Ghaffari H, Tadayon MR, Bahador M, Razmjoo J (2022) Biochemical and yield response of sugar beet to drought stress and foliar application of vermicompost tea. Plant Stress 5:100087. https://doi.org/10.1016/j.stress.2022.100087

Hafez EM, Omara AED, Alhumaydhi FA, El-Esawi MA (2021) Minimizing hazard impacts of soil salinity and water stress on wheat plants by soil application of vermicompost and biochar. Physiol Plant 172(2):587–602. https://doi.org/10.1111/ppl.13261

Heidarzadeh A, Modarres-Sanavy SAM, Mokhtassi-Bidgoli A (2022) Changes in yield and essential oil compositions of *Dracocephalum kotschyi* Boiss in response to azocompost, vermicompost, nitroxin, and urea under water deficit stress. J Soil Sci Plant Nutr 22:896–913. https://doi.org/10.1007/s42729-021-00700-z

Hosseinzadeh SR, Amiri H, Ismaili A (2018) Evaluation of photosynthesis, physiological, and biochemical responses of chickpea (*Cicer arietinum* L. cv. Pirouz) under water deficit stress and use

of vermicompost fertilizer. J Integr Agric 17:2426–2437. https://doi.org/10.1016/S2095-3119(17)61874-4
Huo Y, Wang M, Wei Y, Xia Z (2016) Overexpression of the maize psbA gene enhances drought tolerance through regulating antioxidant system, photosynthetic capability, and stress defense gene expression in tobacco. Front Plant Sci 6:1223. https://doi.org/10.3389/fpls.2015.01223
Jahan S, Ahmad F, Rasul F, Amir R, Shahzad S (2023) Physicochemical analysis of vermicompost-perlite based activated biochar and its influence on wheat (*Triticum aestivum* L.) growth under water stress. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-023-01258-8
Kacar B (2008) Inal, A. Plant analysis. Nobel publication No: 1241. Appl Sci 63:879
Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr Protoc Food Anal Chem 1:F4. 3.1-F4. 3.8. https://doi.org/10.1002/0471142913.faf0403s01
Ma H, Zhao S, Hou J, Feyissa T, Duan Z, Pan Z, Zhang K, Zhang W (2022) Vermicompost improves physicochemical properties of growing medium and promotes plant growth: a meta-analysis. J Soil Sci Plant Nutr 22:3745–3755. https://doi.org/10.1007/s42729-022-00924-7
Maishanu H, Rabe A (2019) Cell membrane stability and relative water content of *Cymbopogon citratus* (Lemon grass). Annu Res Rev Biol 33:1–7. https://doi.org/10.9734/ARRB/2019/v33i230114
Mengistu T, Gebrekidan H, KibretKW OK, Shimelis B, Yadav H (2017) The integrated use of excreta-based vermicompost and inorganic NP fertilizer on tomato (*Solanum lycopersicum* L) fruit yield quality and soil fertility. Int J Recycl OrgWaste Agric 6:63–77. https://doi.org/10.1007/s40093-017-0153-y
Naik K, Mishra S, Srichandan H, Singh PK, Choudhary A (2020) Microbial formulation and growth of cereals, pulses, oilseeds and vegetable crops. Sustain Environ Res 30:10. https://doi.org/10.1186/s42834-020-00051-x
Nawaz MA, Jiao Y, Chen C, Shireen F, Zheng Z, Imtia M, Bie Z, Huang Y (2018) Melatonin pretreatment improves vanadium stress tolerance of watermelon on seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression. J Plant Physiol 220:115–127. https://doi.org/10.1016/j.jplph.2017.11.003
Ohnishi T, Gall RS, Mayer ML (1975) An improved assay of inorganic phosphate in the presence of extralabile phosphate compounds: application to the ATPase assay in the presence of phosphocreatine. Anal Biochem 69:261–267
Parastesh F, Alikhani HA, Etesami H (2019) Vermicompost enriched with phosphate-solubilizing bacteria provides plant with enough phosphorus in a sequential cropping under calcareous soil conditions. J Clean Prod 221:27–37. https://doi.org/10.1016/j.jclepro.2019.02.234
Park S, Li J, Pittman JK, Berkowitz GA, Yang H, Undurraga S, Morris J, Hirschi KD, Gaxiola RA (2005) Up-regulation of a H^+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. Proc Natl Acad Sci 102:18830–18835. https://doi.org/10.1073/pnas.0509512102
Pfündel E, Klughammer C, Schreiber U (2008) Monitoring the effects of reduced PS II antenna size on quantum yields of photosystems I and II using the Dual-PAM-100 measuring system. PAM Appl Notes 1:21–24
Pregl F (1945) Quantitative organic micro analysis, 4th edn. A. Churchill Ltd., London
Rehman S, De Castro F, Aprile A, Benedetti M, Fanizzi FP (2023) Vermicompost: enhancing plant growth and combating abiotic and biotic stress. Agronomy 13:1134. https://doi.org/10.3390/agronomy13041134
Rezaei-Chiyaneh E, Amani Machiani M, Javanmard A, Mahdavikia H, Maggi F, Morshedloo MR (2021) Vermicompost application in different intercropping patterns improves the mineral nutrient uptake and essential oil compositions of sweet basil (*Ocimum basilicum* L.). J Soil Sci Plant Nutr 21:450–466. https://doi.org/10.1007/s42729-020-00373-0
Roy S, Arunachalam K, Dutta BK, Arunachalam A (2010) Effect of organic amendments of soil on growth and productivity of three common crops viz. *Zea mays*, *Phaseolus vulgaris* and *Abelmoschus esculentus*. Appl Soil Ecol 45:78–84. https://doi.org/10.1016/j.apsoil.2010.02.004
Shopova E, Katerova Z, Brankova L, Dimitrova L, Sergiev I, Todorova D, Talaat NB (2021) Modulation of physiological stress response of *Triticum aestivum* L. to glyphosate by brassinosteroid application. Life 11(11):1156. https://doi.org/10.3390/life11111156
Song XL, Li H, Song J, Chen W, Shi L (2022) Biochar/vermicompost promotes Hybrid Pennisetum plant growth and soil enzyme activity in saline soils. Plant Physiol Biochem 183:96–110. https://doi.org/10.1016/j.plaphy.2022.05.008
Staal M, De-Cnodder T, Simon D, Vandenbussche F, Van der Straeten D, Verbelen J, Elzenga T, Vissenberg K (2011) Apoplastic alkalinization is instrumental for the inhibition of cell elongation in the Arabidopsis root by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid. Plant Physiol 155:2049–2055. https://doi.org/10.1104/pp.110.168476
Stewart JM, Guinn G (1969) Chilling injury and changes in adenosine triphosphate of cotton seedlings. Plant Physiol 44:605–608. https://doi.org/10.1104/pp.44.4.605
Talaat NB (2015) Effective microorganisms improve growth performance and modulate the ROS-scavenging system in common bean (*Phaseolus vulgaris* L.) plants exposed to salinity stress. J Plant Growth Regul 34:35–46. https://doi.org/10.1007/s00344-014-9440-2
Talaat NB (2021) Co-application of melatonin and salicylic acid counteracts salt stress-induced damage in wheat (*Triticum aestivum* L.) photosynthetic machinery. J Soil Sci Plant Nutr 21:2893–2906. https://doi.org/10.1007/s42729-021-00576-z
Talaat NB (2023) Drought stress alleviator melatonin reconfigures water-stressed barley (*Hordeum vulgare* L.) plants' photosynthetic efficiency, antioxidant capacity, and endogenous phytohormone profile. Int J Mol Sci 24:16228. https://doi.org/10.3390/ijms242216228
Talaat NB, Hanafy AMA (2023) Spermine-salicylic acid interplay restrains salt toxicity in wheat (*Triticum aestivum* L.). Plants 12:352. https://doi.org/10.3390/plants12020352
Talaat NB, Shawky BT (2012) Influence of arbuscular mycorrhizae on root colonization, growth and productivity of two wheat cultivars under salt stress. Arch Agron Soil Sci 58:85–100. https://doi.org/10.1080/03650340.2010.506481
Talaat NB, Shawky BT (2017) Microbe-mediated induced abiotic stress tolerance responses in plants. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives, Volume 2 Microbial interactions and agro-ecological impacts. Springer, Singapore, pp 101–133
Talaat NB, Shawky BT (2022) Synergistic effects of salicylic acid and melatonin on modulating ion homeostasis in salt-stressed wheat (*Triticum aestivum* L.) plants by enhancing root H+-pump activity. Plants 11:416. https://doi.org/10.3390/plants11030416
Talaat NB, Ghoniem AE, Abdelhamid MT, Shawky BT (2015) Effective microorganisms improve growth performance, alter nutrients acquisition and induce compatible solutes accumulation in common bean (*Phaseolus vulgaris* L.) plants subjected to salinity stress. Plant Growth Regul 75:281–295. https://doi.org/10.1007/s10725-014-9952-6
Talaat NB, Mahmoud AWM, Hanafy AMA (2023) Co-application of salicylic acid and spermine alleviates salt stress toxicity in wheat:

growth, nutrient acquisition, osmolytes accumulation, and antioxidant response. Acta Physiol Plant 45:1. https://doi.org/10.1007/s11738-02203485-5

Tiwari BS, Bose A, Ghosh B (1998) Photosynthesis in rice under salt stress. Photosynthetica 34:303–306. https://doi.org/10.1023/A:1006857027398

Todorova D, Talaat NB, Katerova Z, Alexieva V, Shawky BT (2016) Polyamines and brassinosteroids in drought stress responses and tolerance in plants. In: Ahmad P (ed) Water stress and crop plants: a sustainable approach, vol 2. Wiley, Oxford, pp 608–627

Vigani G, Rolli E, Marasco R, Dell'Orto M, Michoud G, Soussi A, Raddadi N, Borin S, Sorlini C, Zocchi G, Daffonchio D (2019) Root bacterial endophytes confer drought resistance and enhance expression and activity of a vacuolar H^+-pumping pyrophosphatase in pepper plants. Environ Microbiol 21(9):3212–3228. https://doi.org/10.1111/1462-2920.14272

Wang Y, Sze H (1985) Similarities and differences between the tonoplast-type and the mitochondrial H+-ATPases of oat roots. J Biol Chem 260:10434–10443

Wasaya A, Manzoor S, Yasir TA, Sarwar N, Mubeen K, Ismail IA, Raza A, Rehman A, Hossain A, El-Sabagh A (2021) Evaluation of fourteen bread wheat (*Triticum aestivum* L.) genotypes by observing gas exchange parameters, relative water and chlorophyll content, and yield attributes under drought stress. Sustainability 13(9):4799. https://doi.org/10.3390/su13094799

Yan F, Wei H, Ding Y, Li W, Chen L, Ding C, Tang S, Jiang Y, Liu Z, Li G (2021) Melatonin enhances Na^+/K^+ homeostasis in rice seedlings under salt stress through increasing the root H^+-pump activity and Na^+/K^+ transporters sensitivity to ROS/RNS. Environ Exp Bot 182:104328. https://doi.org/10.1016/j.envexpbot.2020.104328

Younas M, Zou H, Laraib T, Abbas W, Akhtar MW, Aslam MN, Amrao L, Hayat S, Hamid TA, Hameed A, Kachelo GA, Elseehy MM, El-Shehawi AM, Zua ATK, Arif M (2021) The influence of vermicomposting on photosynthetic activity and productivity of maize (*Zea mays* L.) crop under semi-arid climate. PlosOne 16(8):e0256450. https://doi.org/10.1371/journal.pone.0256450