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ORIGINAL PAPER

Co-application of Humic Acid, Potassium Dihydrogen Phosphate, and Melatonin to Ameliorate the Effects of Drought Stress on Barley (*Hordeum vulgare* L.)

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Abstract
Drought has an adverse impact on the production and growth of cereals globally. Barley is one of the significant cereals, full of nutrients, and commonly utilized as human feed and forage. Due to drought stress, cereals' cultivation declined day by day, and ultimate yield does not fulfill the required demand. The current research investigated the consequences of drought stress on morpho-physiological, biochemical, and yield characters of barley plants and a comparison of different treatments and their ameliorating capacity towards drought stress. A pot trial was held in a completely randomized design (CRD) in a triplicate manner to investigate the role of humic acid, potassium dihydrogen phosphate, and melatonin foliar application in ameliorating the drought stress. Two barley varieties (Sultan-17 and Jau-17) were selected for this experiment. The treatments applied were as follows: humic acid (HA; 400 mg L^{-1}), potassium dihydrogen phosphate (KH_2PO_4; 20 mg L^{-1}), and melatonin (MT; 116 mg L^{-1}) with two water levels, i.e., control and drought stress. Under control and drought stress, different growth parameters, i.e., fresh weight of root and shoot, dry weight of root and shoot, leaf area, and physiological and biochemical attributes, i.e., chl *a*, chl *b*, antioxidant enzymes, and total soluble proteins, were studied. Moreover, relative water content, phenolics, proline, MDA content, hydrogen peroxide, relative membrane permeability, mineral contents, and yield parameters were also analyzed in this research. Drought severely affects a majority of parameters that were studied. Results indicated that foliar application of all three treatments improved plant growth, antioxidant enzymes, total soluble proteins, total chlorophyll, mineral contents, and productivity parameters, while foliar application of all three treatments alleviated malondialdehyde content, hydrogen peroxide, and relative membrane permeability value studied under drought stress and non-drought stress. The maximum yield was noticed in Jau-17 plants by HA treatment in either control or drought stress. The order of effectiveness of treatments in this study was HA > MT > KH_2PO_4. All three treatments (HA, KH_2PO_4, and MT) examined in this study had a potential role in combating against drought stress and all treatments also elevated plant growth and yield under control.

Keywords Antioxidant enzymes · Drought stress · Foliar application · Humic acid · Melatonin · Potassium dihydrogen phosphate · ROS species

1 Introduction

Plants are vulnerable to different abiotic stresses and world climate alteration typically results in frequent drought stress circumstances over wide regions on global scale (Adnan et al. 2020). In the future, the most critical threat to global food safety is drought (Hanafy and Sadak 2023; Hasnain et al. 2023). Drought is one of the most serious ambient stresses influencing plant fertility (Brodersen et al. 2019). *Hordeum vulgare* L. (barley) is the fourth leading cereal crop followed by *Zea mays* L. (sugar corn), *Triticum aestivum* L. (bread wheat), and *Oryza sativa* L. (rice) in both quantity production and cultivation acres (FAO 2022). Barley is a diploid (2n) self-pollinating, rapid-growing cereal that grows annually in winter (Bishnoi et al. 2022). However, drought is one of the serious threats which affect its production (Zargar et al. 2017). As drought stress rises, lesser propagation and yield were ascertained in barley (Behboudi et al. 2018). Low

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production is partially due to the drought stress and inaccessibility of stress-tolerant high-yielding varieties of barley (Elakhdar et al. 2022).

Barley is considered a magnificent cause of minerals, vitamins, starch, and protein (Farag et al. 2022). Utilization of barley crop by humans has now decreased substantially than other crops. But, barley is much richer in fiber, and cholesterol-lowering beta-glucan, and loses little nutrients during processing than wheat. Drought stress can cause senescence through malfunction of the chloroplast, reduced chlorophyll quantity, and lowered photosynthesis (Sabagh et al. 2019). ROS generation is linear with the severity of drought stress that activates the membranes and organelle peroxidation, enzyme inactivation or activation, and disintegration of nucleic acids (Outoukarte et al. 2019). The growth phase, age, the severity of drought, species of plant, and duration are the prime aspects that affect plant responses to drought conditions (Bakhoum Gehan et al. 2022). In Pakistan, unpredictable and more periodic rainfall exists in pre-spring and winter (Karandish and Šimůnek 2017).

Plants evolved several techniques and schemes to reduce the negative effects of drought stress (Thanmalagan et al. 2022). Antioxidant enzymes play crucial roles in removing excessive ROS in cells (Waris et al. 2023), and sustaining ROS homeostasis, and tolerance to drought stress (Abd Elhamid et al. 2021). Agrologists are also utilizing different techniques for drought stress tolerance, among which the practice of exogenous chemicals, regulators, artificial hormones, and compounds is of appreciable worth to elevate drought tolerance at various plant propagation phases (Tariq et al. 2022).

Humic substances are regarded as plant bio-fertilizers due to their impact in encouraging growth of plant through increasing root growth and mineral assimilation (Calvo et al. 2014). Humic acids (HA) disintegrate in water quite well, feasibly used through soil application and spraying. HA also upgrade chemical, physical, and biological features of soil (Roozbahani 2015). The HA foliar spray is promptly assimilated by leaves and improves cell growth and physiological processes as well as confronts the high nutrient requirement during some growth phases especially during grain-filling (Mahmoud and Youssif 2015). Utilization of HA appreciably increased the vegetative growth, photosynthetic pigments, and mineral value, aiding in the assimilation, and transport of minerals because of the complexes, and chelates synthesis, leading to a rise in yield of crops (El-Tahlawy and Ali 2022). Various researches have shown that HA can assist in mitigating abiotic stresses in plants, particularly drought stress (Moghadam et al. 2014; Vickers 2017).

Potassium (K^+) is one the most fundamental macronutrients which has a significant part in the enlargement and propagation of plants, and initiates more than 60 enzymes. K^+ also enhances water stress tolerance in plants by conserving water balance (Behairy et al. 2015). Furthermore, it plays effective roles in the photosynthesis process, protein, and carbohydrate development, nutrient and water transportation, and nitrogen (N) usage, thus provoking initial plant growth (Badr et al. 2022). Under drought stress, the plants consumed more K^+ for their inner regulation mechanism, and the application of K^+ mitigated the negative impact of the water shortage and maintained plant productivity (Hasanuzzaman et al. 2018). Foliar or soil application of K^+ is favorable for optimum plant physiological processes (Brestic et al. 2018). Therefore, the application of K^+ is of high significance for acquiring optimal crop yield grown under both rainfed areas or water-deficit conditions (Kumar et al. 2019).

Melatonin (MT, N-acetyl-5-methoxytryptamine), a pleiotropic hormone, is intricated in plant propagation and outgrowth regulation, such as vegetative progression stimulation, kernel germination, flowering, and rooting (Arnao & Hernández-Ruiz 2014; Li et al. 2012). MT hormone has several functions in plants and animals, and is established to be an abiotic anti-stress in plants (Manchester et al. 2015; Usman et al. 2023). Plants can not only assimilate MT through soil but also integrate from L-tryptophan (Nawaz et al. 2016). MT has been associated with plant propagation improvement and defense counter to different non-living stresses in various crops (Liang et al. 2019; Martinez et al. 2018).

Three chemicals, i.e., HA, KH_2PO_4, and MT, were selected that maintained plant growth under drought stress. These treatments applied as foliar spray which entered through leaves and soil in the plant that maintain turgor pressure. The aims of the study include the following: examine the morpho-physiological, biochemical, and yield attributes of barley plants under drought stress; compare the performance of two barley varieties (*Hordeum vulgare* L.) under drought stress. This study will also enable us to find ways for the potential role of different treatments (HA, KH_2PO_4, and MT) in mitigation of drought stress.

2 Material and Methods

2.1 Experimental Layout

The trial was arranged in complete randomized design (CRD) using triplet factors (variety, drought, and treatments) and three replicates in a total of 48 pots. Fifteen seeds were sown in each pot which was filled with an equal proportion of cleaned soil. The soil analysis was done to check different properties and types of soil as shown in Table 1. Plants were grown in the botanical garden of University of Education, Township Campus. The seeds of barley were acquired through Ayub Agricultural Research Institute (AARI), Jhang

Road, Faisalabad, i.e., barley varieties Sultan-17 and Jau-17. Thinning of seedlings was done after 3 weeks of sowing and only seven healthier and greenish seedlings were kept in each pot while the rest were discarded.

HA (400 mg L^{-1}), KH_2PO_4 (20 mg L^{-1}), and MT (116 mg L^{-1}) were applied foliarly on two varieties, i.e., Jau-17 and Sultan-17, as mentioned in Table 2. Moreover, the water level was maintained in two concentrations, i.e., control and drought stress. Plants were propagated in plastic containers and additionally shielded with transparent polyethylene to handle drought stress just in case of rainfall. The moisture in the soil that was placed in plastic pots was maintained at field capacity (FC) by regularly providing tap water to all plants for 27 days before drought application. Drought treatment was maintained until yield stage and was imposed by withholding watering for regular intervals of weeks after foliar application of treatments.

2.2 Growth Parameters Assessment

After 2 weeks of treatment, samples were collected and growth parameters were assessed. Roots were carefully taken out from the pot and excised from shoot. These were washed with adequate water to get rid of soil. Shoot and root fresh weight was taken on electronic weight balance in units of grams. For dry weight of shoot and root, samples were dried in an oven incubator for 24 h for 75 °C until constant weight was achieved and were estimated with the aid of electrical balance. Leaf area was computed manually by the formula: length × width in units of cm^2 (Carleton and Foote 1965).

2.3 Physiological and Biochemical Attributes

2.3.1 Determination of Carotenoid and Chlorophyll Content

Chlorophyll quantity was obtained at the tillering stage by using a renowned protocol (Arnon 1949). Fresh leaves were acquired from each pot and then weighed up to 0.05 g equally with the help of analytical balance. Leaves were mashed with the aid of a mortar and pestle of 80% acetone solvent in 10 mL. The sample was strained with Whatman Grade 42. The specimen was then kept in the refrigerator at 4 °C for 24 h. The filtrate was taken in a quartz cuvette and measurements were taken down at 480, 645, and 663 nm availing a double-beam UV–visible spectrophotometer (Metash-Model UV-9000).

The values were written down and chl *a* and chl *b* were calculated using formula in mg g^{-1} as follows:

$$Chl.a(mgg^{-1}\mathrm{f.wt}) = [12.7(OD663) - 2.69(OD645)] \times V/1000 \times Wf$$

$$Chl.b(\mathrm{mgg}^{-1}\mathrm{f.wt}) = [22.9(OD645) - 4.68(OD663)] \times V/1000 \times Wf$$

where V = volume of solvent (mL), Wf = weight of fresh leaf tissue (g), OD = optical density.

Carotenoid content was calculated using the below equation set by Lichtenthaler (1987).

$$Carotenoids(\mathrm{mgg}^{-1}\mathrm{f.wt}) = (1000 \times OD480) - (1.9 \times chla - 63.14 \times chlb)/214$$

2.3.2 Determination of RWC (%)

Leaf relative water content was analyzed by the Jones and Turner (1978) procedure. The same foliage size of each replicate was taken and recorded their respective fresh weight by electronic balance. Leaves were put down straightly in distilled water in Petri dishes, so they were soaked with water very well. Leave it for 3 h at room temperature in a dark place and leaf turgid weight was estimated. Then, leaves were placed in an incubator at temp 80 °C for 24 h for measuring dry leaf weight. Relative water content was measured by the formula given below:

$$RWC(\%) = [(f.wt - d.wt)/(t.wt - d.wt)] \times 100$$

where *t.wt*, *f.wt*, and *d.wt* represented the turgid weight, fresh weight, and oven-dried weight accordingly.

2.3.3 Determination of Relative Membrane Permeability (EC%)

The relative membrane permeability was determined by the procedure of Yang et al. (1996). Fully developed and young leaves excised from every single replicate have a uniform size. Leaves were chopped into small pieces and placed in test tubes possessing (20 mL) of distilled deionized H_2O. The test glass tubes were vortexed for 10 s and were assessed for electrical initial conductivity (EC_0) with the help of an electrical conductivity meter (Hanna HI-9811–5 EC portable meter). These glass tubes were covered by aluminum

Table 1 Different features of the experimental soil utilized in research

Parameter of soil	Value
pH	7.6
Electrical conductivity (µs/cm)	300
Water content (%)	13
Water holding capacity (%)	25
Silt (%)	49.4
Sand (%)	47
Clay (%)	3.5
Texture	Sandy loam

(Al) foil and kept for 24 h in a refrigerator at 4 °C and were tested for EC_1. These samples were covered and autoclaved at 121 °C for 20 min to find the EC_2. Relative membrane permeability was computed as:

$$RMP(\%) = (EC_1 - EC_0/EC_2 - EC_0) \times 100$$

2.3.4 Estimation of Ionic Content

The protocol was the same for both shoot and root ionic determination (Wolf 1982) with little modifications. For estimating the ion concentration, firstly plant pieces were kept in a lab oven for drying for 24 h at 75 °C. Weigh 0.1 g for shoot and for root separately and add slowly 2 mL conc. H_2SO_4 in each test tube containing samples. Samples were retained for 1 day and the digestion mixture (1 mL) which completely turns black was placed on a hot plate with gradually elevating temperature (50–150 °C). When heating started, add H_2O_2 in each test tube and wait until samples were completely turned colorless, remove them from the hot plate, and let them cool. Then, each solution was filtered with Whatman Grade 42 filter paper to remove any type of debris. The mixture was made up of a total volume, i.e., 50 mL, and was used for obtaining values of ions i.e., Ca^{2+}, K^+, bivalent and monovalent cations respectively with a (Sherwood-Model 360) flame photometer.

2.3.5 Assessment of Malondialdehyde (MDA) Content

Malondialdehyde was calculated in rates of absorption by thiobarbituric acid–reactive substances (TBARS) (Cakmak and Horst 1991). Take fresh leaves and weigh up to 0.2 g on an analytical balance. Each sample was shredded with the aid of pestle and mortar at 4 °C in 3 mL of 1.0% TCA solution, i.e., 0.5 g TCA and 50 mL H_2O (distilled deionized). The samples were centrifuged at 20,000 × *g* for 15 min (HERMLE Z 326 K). Extract half a milliliter from the supernatant and add 3 mL of 0.5% (v/v) TBA, i.e., thiobarbituric acid in 20% TCA in each sample. All samples were then incubated in a water-shaking bath at 95 °C for 45 min and a chemical reaction was discontinued by chilling the test tubes containing samples in an ice-water bath (Glas-Col model HSW-1/06), then were again centrifuged at 10,000 × *g* for 10 min. The clear supernatant in conical tubes was taken in a quartz cuvette and absorbance was observed at 532 nm with a UV–visible double-beam spectrophotometer (Metash-Model UV-9000). The assessed concentration for non-specific absorption at 600 nm was subtracted from all measurements taken at 532 nm. The absorption of TBARS was computed by applying the coefficient absorption, i.e., 155 $nmol^{-1}$ cm^{-1}.

$$MDAlevel(\text{nmol}) = \Delta(A532\text{nm} - A600\text{nm})/1.56 \times 105$$

Table 2 Treatments applied during experimentation on barley (*Hordeum vulgare* L.) with two different varieties and two water levels

Sr. no	Treatment	Abbreviation	Concentration used (mg L^{-1})
1	Humic acid	HA	400
2	Potassium dihydrogen phosphate	KH_2PO_4	20
3	Melatonin	MT	116

2.3.6 Total Soluble Protein Assay

The quantity of soluble proteins was investigated through the method given by Bradford (1976). Leaves were weighed up to 500 mg and finely ground in 10 mL of 50 mM buffer, i.e., orthophosphate having a 7.8 pH at 4 °C. The ground material was centrifuged (HERMLE Z 326 K) at 6000 × *g* for 20 min in the cold. Then, prepare Bradford reagent as dissolving chemicals, i.e., Coomassie brilliant blue (100 mg), 85% phosphoric acid (100 mL), 95% ethanol (50 mL), and then add distilled water to make a volume up to 1000 mL. Lastly, add 2 mL of Bradford reagent 100 µL of plant extract and take down the optical density at 595 nm in a double-beam UV–visible spectrophotometer (Metash-Model UV-9000).

2.3.7 Catalase (CAT) and Peroxidase (POD) Activity

Catalase and peroxidase activities were determined by the Chance and Maehly (1955) method with minor alterations. The CAT reaction solution (3000 µL) contained 1000 µL of 50 mM buffer (phosphate) having pH 7.0, 1900 µL of 5.9 mM H_2O_2, and enzyme extract (100 µL). Add enzyme extract at the end in the cuvette, so the chemical reaction was initiated. Using a double-beam UV–visible spectrophotometer (Metash-Model UV-9000), periodic changeovers in OD of the chemical reaction (solution) in the cuvette were recorded at 240 nm every 30 s (starting from 0 to 120 s).

For POD activity, the reaction solution contained 700 µL of 50 mM buffer orthophosphate (pH 7.0), 600 µL of 20 mM guaiacol, and 600 µL (40 mM) H_2O_2, enzyme essence (100 µL). The reaction was commenced by adding 0.6 mL H_2O_2 in a cuvette in reaction solution. Using a UV–visible spectrophotometer (Metash-Model UV-9000), the absorbance of the solution at 470 nm was assessed every 30 s (from 0 to 150 s).

2.3.8 Ascorbate Peroxidase (APX) Activity

Ascorbate peroxidase (APX) activity was analyzed by Nakano and Asada (1981) methodology with few amendments. The reaction chemical solution for APX was 3 mL containing 300 mM (0.1 mL) H_2O_2, 7.5 mM (0.1 mL) ascorbic acid, and 50 mM (2.7 mL) phosphate buffer. Adding 100 µL of enzyme extract, absorbance was noted at 290 nm for every 30 s (from 0 to 120 s) with a double-beam UV–visible spectrophotometer (Metash-Model UV-9000). The activity constant for APX is $E = 2.8$ mM/cm.

2.3.9 *Determination of H_2O_2*

For the determination of H_2O_2 levels in plant samples, the strategy of Velikova et al. (2000) was followed. Weigh 0.25 g of fresh leaves and grind with 5 mL of 0.1% TCA (w/v) trichloroacetic acid in refrigerated pestle and mortar. This solution was then centrifuged (HERMLE Z 326 K) at 12,000 × *g* for 15 min. Add 500 µL orthophosphate potassium buffer (pH 7.0) and 1000 µL potassium iodide (KI) to 500 µL of supernatant. This mixture was vortexed for 5 s and absorbance was calculated at 390 nm using a double-beam UV–visible spectrophotometer (Metash-Model UV-9000).

2.3.10 Evaluation of Free Proline Quantity

For the evaluation of proline quantity, the method of Bates et al. (1973) was followed. Fresh leaves (0.25 g) weighed from each sample were taken and ground in 10 mL of 3% of sulfo-salicylic acid. These samples were then filtered with a Whatman Grade 42 filter sheet and clear filtrate (2 mL) was mixed with 2 mL of acid ninhydrin, i.e., prepared by mixing 1.25 g of ninhydrin in 30 mL glacial acetic acid and 6 M (20 mL) orthophosphoric acid and 2 mL of CH_3COOH in a test glass tube. This composition was incubated at 100 °C for about 1 h and the sample was cooled in a chilled bath. Then, add toluene (4 mL) in each sample and vortex for 10–15 s by 1–2 min of continuous air passing stream. The superior layer having toluene was taken from the aqueous part by a glass pipette and warmed at room temperature. The OD was calculated at 520 nm on a double-beam (Metash-Model UV-9000) UV–visible spectrophotometer providing toluene as a blank and proline estimated by:

$$\mu moles proline \mathrm{g}^{-1} of fresh weight material = [(\mu g proline \mathrm{mL}^{-1} \times \mu\mathrm{L} toluol)/115.5](\mathrm{g}^{-1} specimen)$$

2.3.11 Total Phenolic Content Establishment

Phenolic proportions were figured by using the approach of Julkunen-Tiitto (1985). Fresh leaves from each replicate, weighing 50 mg, were crushed with 5 mL of 80% acetone solution with a mortar and pestle. The homogenized mixture was centrifuged at 10,000 × *g* for 600 s in cold (4 °C). Take out 0.1 mL of clear supernatant and dilute with 2 mL distilled purified water and 1 mL Folin–Ciocalteau's phenol reagent and shake strongly. Subsequently, add 5 mL of 20% Na_2CO_3 and the total volume was made up of 10 mL with distilled pure water. The mixture was swirled and the optical density (OD) was read at 750 nm utilizing a double-beam UV–visible spectrophotometer (Metash-Model UV-9000). The findings were indicated as milligrams per kilogram of fresh leaf.

2.4 Yield Parameters

For no. of grains per spike, some randomly chosen spikes of the same pot were detached from the barley plant and grains in each spike were counted. A total of 100 threshed grains were selected for grain yield per plant and the weight is measured on an electronic balance.

2.5 Statistical Analysis

CoStat statistical programs were utilized for ANOVA (analysis of variance) in results for all features studied (CoHort software's 1988, Monterey, California, USA). The version used was 6.303. LSD tests were applied to compare the mean values. R-software was used for Pearson's correlation and principal component analysis. The graphs in the results were analyzed in Microsoft Excel.

3 Results

Effect of HA, KH_2PO_4, and MT on Growth Attributes of *H. vulgare* Under Drought.

Drought decreased shoot fresh and shoot dry weight in Sultan-17 and Jau-17 from control. Under drought stress, all applied treatments increased shoot fresh and shoot dry weight compared to drought plants (Table 3, Fig. 1A and B). Jau-17 variety had high shoot fresh and dry weight as compared to Sultan-17 variety. Spraying of HA increases shoot fresh weight under drought and non-drought conditions as compared to their respective control. Dry and fresh shoot mass were also elevated under a water-deficit state with foliar application of KH_2PO_4. Exogenous application of MT lessened the harmful consequences of drought application in both varieties, i.e., Sultan-17 and Jau-17, and significantly increased growth attributes, i.e., root and shoot mass (Fig. 1C and D). Similarly, root fresh and dry weight reduced in both varieties, i.e., Sultan-17 and Jau-17, from control. Under drought and non-drought conditions, all three treatments elevated root (fresh and dry weight) in Sultan-17 and Jau-17. HA showed the highest root fresh and dry weight in Sultan-17, while MT showed the highest in Jau-17 compared to other treatments under non-drought conditions.

Drought application reduced leaf area (Fig. 2D) in Sultan-17 and Jau-17 from control. All three treatments increased leaf area under drought stress in Sultan-17 and Jau-17 from drought control. KH_2PO_4-treated plants showed the largest leaf area under drought stress in each variety, i.e., Sultan-17 and Jau-17.

Effect of HA, KH_2PO_4, and MT on Physiological Attributes of *H. vulgare* Under Drought.

Water content in leaves (i.e., RWC%) is the status mark for water in plants and used to evaluate drought tolerance. Drought application also declined relative water content in both varieties (Fig. 6A). Chlorophyll *a* and *b* and carotenoid contents dropped down in Sultan-17 as compared to Jau-17 under drought stress (Fig. 2A–C). Under drought conditions, HA, KH_2PO_4, and MT elevated chlorophyll *a* and *b* and carotenoids in Sultan-17 and Jau-17 as compared to control. HA showed the highest chlorophyll and carotenoid content in Jau-17 as compared to other treatments under drought conditions. Jau-17 had a relatively high chlorophyll value and RWC leading to better grain yield under drought stress as compared to Sultan-17.

3.1 Effect of HA, KH_2PO_4, and MT on Biochemical Attributes of *H. vulgare* Under Drought

3.1.1 *Relative Membrane Permeability (RMP), Hydrogen Peroxide (H_2O_2), and Malondialdehyde (MDA) Contents*

Relative membrane permeability (Fig. 6B), H_2O_2, and MDA content (Fig. 4C and D) were raised in Sultan-17 and Jau-17 under drought stress compared to control and all other treatments. In both varieties, Sultan-17 and Jau-17, HA, KH_2PO_4, and MT reduce RMP, MDA, and H_2O_2 content under drought stress equated to drought control. HA exhibited the least RMP in Jau-17 while it showed the least MDA and H_2O_2 production in both varieties under drought stress compared to other two treatments.

3.1.2 Total Soluble Proteins (TSP), Total Phenolics, and Proline Contents

Total soluble proteins (Fig. 3A) reduced while total phenolics and proline concentrations (Fig. 4A and B) were elevated in Sultan-17 and Jau-17 from control under drought stress. Under drought stress, HA, KH_2PO_4, and MT enhanced proline concentration, protein, and phenolics from drought control. Under drought stress, HA showed the highest total soluble proteins and phenolic content in Sultan-17, while MT application resulted in high total soluble proteins in Jau-17.

3.1.3 Antioxidant Enzymes (CAT, APX, and POD)

Antioxidant enzyme production demonstrated the alleviation in the harmful impacts of drought stress and an indication of well-adaptive mechanisms in barley. An increment in all antioxidant enzymes (CAT, APX, and POD) was observed under drought application stress in both barley varieties, compared with its control (Fig. 3B–D). Under drought stress, all treatments increased peroxidase, catalase, and APX levels respectively in Sultan-17 and Jau-17. HA and MT revealed the highest catalase value in Jau-17 and Sultan-17 respectively under drought stress.

3.1.4 *Inorganic Ions (K^+ and Ca^{2+}) in Shoot and Root*

Application of drought decreased ionic concentration (shoot and root), i.e., K^+ and Ca^{2+} content in Sultan-17 and Jau-17 from control (Fig. 5A–D). All treatments (HA, KH_2PO_4, and MT) increased K^+ and Ca^{2+} content in shoot and root from drought control in both varieties. Application of KH_2PO_4 showed maximum accumulation of K^+ ions in shoot and root under normal and drought conditions. Application of HA showed maximum accumulation of Ca^{2+} ions in roots under normal and drought conditions.

Effect of HA, KH_2PO_4, and MT on Yield Attributes of *H. vulgare* Under Drought.

Evaluation of the present data displayed that the grain yield of barley under drought stress was affected severely (Fig. 6C and D). The high grain yield was linked with a high no. of grains per spike, and water and chlorophyll content. Under drought stress, barley productivity reduced greatly. Yield attributes (no. of grain per spike and 100-grain weight) were reduced under drought stress in Sultan-17 and Jau-17 from control. Under drought stress, all treatments elevated the no. of grain per spike and 100-grain weight from drought control in both varieties. Under drought stress, MT and HA displayed the greatest no. of grains per spike in Sultan-17 and Jau-17 respectively. MT and HA indicated maximum 100-grain weight under drought and non-drought states in Sultan-17 and Jau-17 respectively.

3.2 Pearson's Correlation and Principal Component Analysis

The results of Pearson's correlation and principal component analysis (PCA) for both *H. vulgare* varieties were nearly the same and comparable to one another (Fig. 7). Pearson's correlation clearly showed that all studied parameters can be divided into two groups whose members (studied parameters) are positively correlated with each other, which depicts increase in one resulted in incrementation of others and vice versa. But they are negatively correlated to parameters of second group, illustrating that decrement in one can result

Table 3 The mean square values from analysis of variance of data for different physiological and biochemical parameters of two varieties of barley (*Hordeum vulgare* L.) with the foliar application of three different chemicals

Source of variation	DF	Shoot FW	Shoot DW	Root FW	Root DW	Chlorophyll *a*	Chlorophyll *b*
Variety	1	8.365698***	0.233662***	0.4517872***	0.02146***	0.4060766***	0.175678***
Drought	1	7.656657***	0.241826***	4.7648162***	0.17100***	2.5786993***	1.854577***
Treatments	3	0.981110***	0.043279***	0.2620255***	0.01157***	0.3546299***	0.222687***
Variety × drought	1	0.032854^{ns}	0.014456**	0.0298402 ^{ns}	0.00212*	0.0714379**	0.063516*
Variety × treatments	3	0.221388**	0.003237 ^{ns}	0.2307161***	0.00483***	0.0116956 ^{ns}	0.038232*
Drought × treatments	3	0.310194***	0.011849***	0.1687596***	0.00191**	0.0725108***	0.053266**
Variety × drought × treatments	3	0.559761***	0.012950***	0.1983431***	0.00689***	0.0240456*	0.012762 ^{ns}
Error		0.039775	0.00128	0.0097609	4.0102e-4	0.0075429	0.008692
		Carotenoids	RMP	RWC	H_2O_2	MDA	Phenolics
Variety	1	1.4534941***	1.97487 ^{ns}	971.3641 ^{ns}	2.21673 ^{ns}	4.577742***	1240.841***
Drought	1	9.7103295***	130.82795***	4987.3447***	542.06881***	36.044753***	11,220.108***
Treatments	3	1.0353042***	10.85533***	522.1443***	25.87283***	4.766992***	1031.330***
Variety × drought	1	0.4853211***	6.71027***	204.3451*	57.30468***	2.420439***	546.007***
Variety × treatments	3	0.0849039 ^{ns}	1.62174*	10.4145 ^{ns}	2.99846 ^{ns}	0.677752*	460.663***
Drought × treatments	3	0.3878528***	0.88411**	130.7999***	0.45149 ^{ns}	0.291744 ^{ns}	192.339**
Variety × drought × treatments	3	0.0348082 ^{ns}	0.25260***	0.9498 ^{ns}	1.0999171 ^{ns}	0.819808**	234.544***
Error		0.0363851	0.271502	10.94594	1.166865	0.1795907	29.768521
		TSP	Leaf area	POD	APX	CAT	Proline
Variety	1	0.0487539***	82.22352***	14.72735***	0.592131***	0.460419**	0.002508***
Drought	1	0.2517286***	293.05566***	58.12212***	11.800792***	67.554075***	0.028998***
Treatments	3	0.0141931***	16.81281**	6.28730***	0.567341***	2.774592***	0.003554***
Variety × drought	1	0.0245617***	7.87563 ^{ns}	0.72065 ^{ns}	0.024869 ^{ns}	0.030810 ^{ns}	3.699e-4 ^{ns}
Variety × treatments	3	8.7021e-4 ^{ns}	4.11592 ^{ns}	2.26577**	0.056695 ^{ns}	0.176038*	2.289e-4 ^{ns}
Drought × treatments	3	0.0010811 ^{ns}	3.02395 ^{ns}	1.48688*	0.048455 ^{ns}	1.419262***	1.243e-4 ^{ns}
Variety × drought × treatments	3	0.0013323 ^{ns}	12.10802**	0.37603 ^{ns}	0.087875 ^{ns}	0.567187***	2.059e-4 ^{ns}
Error		9.7681e − 4	2.3170414	0.4589076	0.0322755	0.0406263	1.5141e − 4
		K^+ (shoot)	Ca^{2+} (shoot)	K^+ (root)	Ca^{2+} (root)	No. of grains per spike	100-grain weight
Variety	1	46.20706*	1.3772898**	12.469912***	5.7274228***	31.3471***	9.973906***
Drought	1	816.79125***	6.4762336***	66.141752***	5.9445763***	214.4188***	16.381552***
Treatments	3	112.59914***	0.7725599**	14.336105***	0.5240082**	18.8587***	1.628451***
Variety × drought	1	1.61883 ^{ns}	0.0222224 ^{ns}	1.689975 ^{ns}	0.0012628 ^{ns}	7.7940**	0.075026 ^{ns}
Variety × treatments	3	4.58395 ^{ns}	0.0592582 ^{ns}	0.249454 ^{ns}	0.0604855 ^{ns}	10.5346***	0.372429***
Drought × treatments	3	10.11602 ^{ns}	0.1305172 ^{ns}	1.285837 ^{ns}	0.0331525 ^{ns}	3.8697*	0.024471 ^{ns}
Variety × drought × treatments	3	4.86230 ^{ns}	0.0801406 ^{ns}	0.527654 ^{ns}	0.0252429 ^{ns}	3.2437*	0.210121***
Error		6.8036059	0.159511	0.5492617	0.1010624	0.9571857	0.028104

Where treatments included humic acid (HA 400 mg L^{-1}), potassium dihydrogen phosphate (KH_2PO_4 20 mg L^{-1}), and melatonin (MT 116 mg L^{-1}) applied under drought and non-drought conditions. Various abbreviations used are as follows: *FW*, fresh weight; *DW*, dry weight; *RMP*, relative membrane permeability; *RWC*, relative water content; *H_2O_2*, hydrogen peroxide; *MDA*, malondialdehyde; *TSP*, total soluble proteins; *POD*, peroxidase; *APX*, ascorbate peroxidase; *CAT*, catalase; *K^+*, potassium ion; *Ca^{2+}*, calcium ion

in an opposite change in the parameters of the second group. Figure 7 shows that growth parameters (like SFW, SDW, RFW, RDW, and LA), physiological attributes (chl *a*, chl *b*, carotenoids, and RWC), inorganic minerals (shoot K^+, root K^+, shoot Ca^{+2}, and root Ca^{+2}), and yield parameters (like total soluble proteins, grains spike^{-1}, and 100-grains weight) lie in same group and are positively correlated to each other. PCA graph of parameters (Fig. 8) also depicts the same findings. All these aforementioned parameters lie in PC1 (first principal component) with contributions of 78.21% in variety Sultan-17 and 77.65% in variety Jau-17. Similarly, parameters included in the second group or PC2 (second principal component) were stress markers (i.e., RMP, H_2O_2, and MDA) and antioxidants (enzymatic: APX, CAT, POD; nonenzymatic: proline, total phenolics). The contributions of PC2 parameters were 11.13 and 8.87% in varieties Sultan-17 and Jau-17, respectively. All the parameters in PC1 had a negative correlation with those of PC2 and vice versa, as

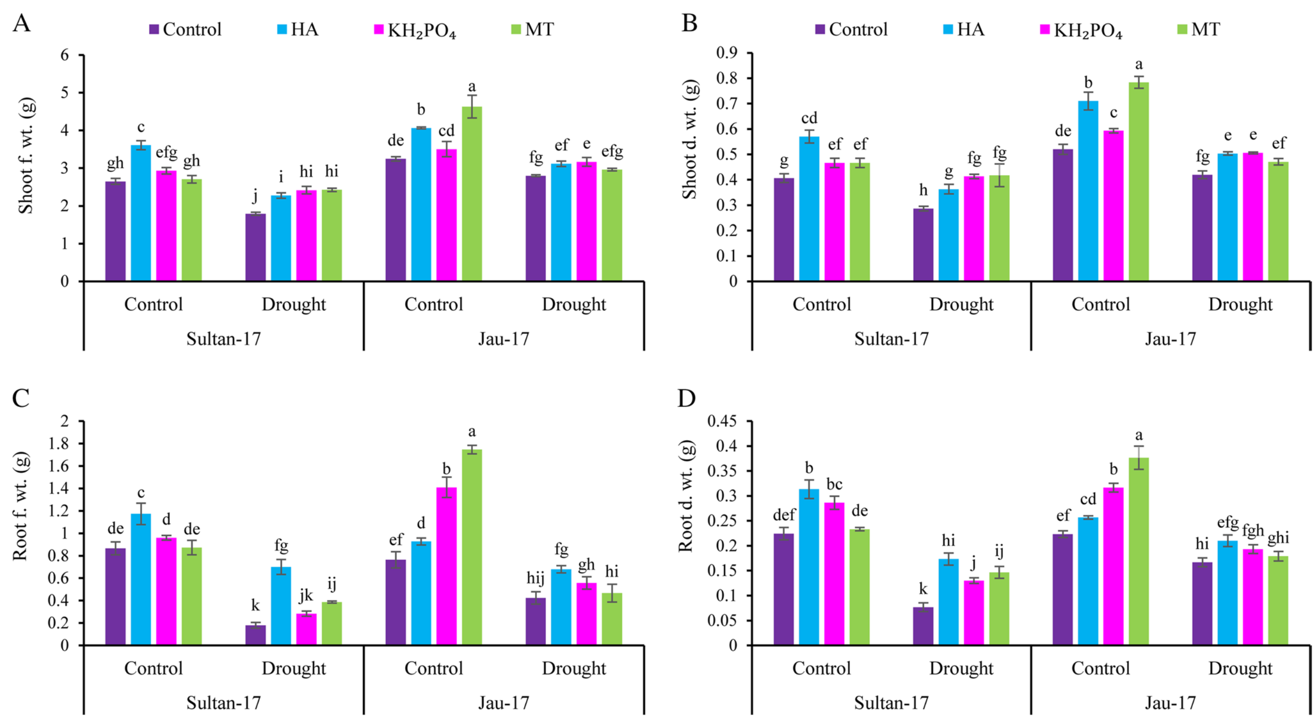

Fig. 1 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) shoot fresh weight, (B) shoot dry weight, (C) root fresh weight, and (D) root dry weight of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

shown in Fig. 8. Hence, this might be perceived that drought may have certainly resulted in stomatal closure, or disturbed or inhibited photosynthesis process that mean low energy production. Plants facing drought adjusted their metabolism accordingly and may have reduced biomass, leaf expansion, and growth. In secondary effects of drought stress, it also includes generation of ROS (H_2O_2) that alters membrane permeability and caused lipid peroxidation. Correlation and PCA showed that these stress markers were negatively correlated with growth, physiological, and yield parameters, thus indicating that generation of ROS, altered RMP, and increased lipid peroxidation resulted in hampered growth and physiological processes, and low yield. Positive correlation of RMP, H_2O_2, and MDA with antioxidants (mentioned previously) depicted that plants, by themselves or with aid of different treatments applied in this study, can induce antioxidant accumulation under drought. Plants used it as an adaptive strategy to cope with drought.

4 Discussion

Drought revealed harmful effects on all growth attributes studied in barley plants (*Hordeum vulgare* L.), whereas foliar application of all treatments mitigated the harmful effects of drought. There was a reduction in the dry and fresh weight of barley shoots under drought situations as this is likely due to the lowering of osmosis through the soil and reducing elongation of cell. Hence, overall barley growth decreased and these results were related to the findings of Abdelaal et al. (2017) and El-Bassiouny et al. (2023) in corn and wheat crops respectively. Jau-17 variety had high shoot fresh and dry weight as compared to Sultan-17 variety and seemed to be drought tolerant. It was examined that drought-tolerant varieties had more shoot dry and fresh weight as compared to drought-sensitive varieties in maize (Anjum et al. 2016 b) and in sunflowers (Razzaq et al. 2017).

Spraying of HA increases shoot fresh weight under drought application and non-drought stress as compared to their respective control as noted previously by Bijanzadeh et al. (2019) and Moghadam et al. (2014). Dry and fresh shoot mass were elevated under a water-deficit state with foliar application of KH_2PO_4 and comparable outcomes were accounted by Abdelaal et al. (2018). The improved growth as result of MT introduction is in agreement with the findings of Li et al. (2018) in rapeseed and Sadak and Bakry (2020) in flax plants (Ahmad et al. 2019). MT application stimulated lateral root growth under moisture stress (Dai et al. 2020).

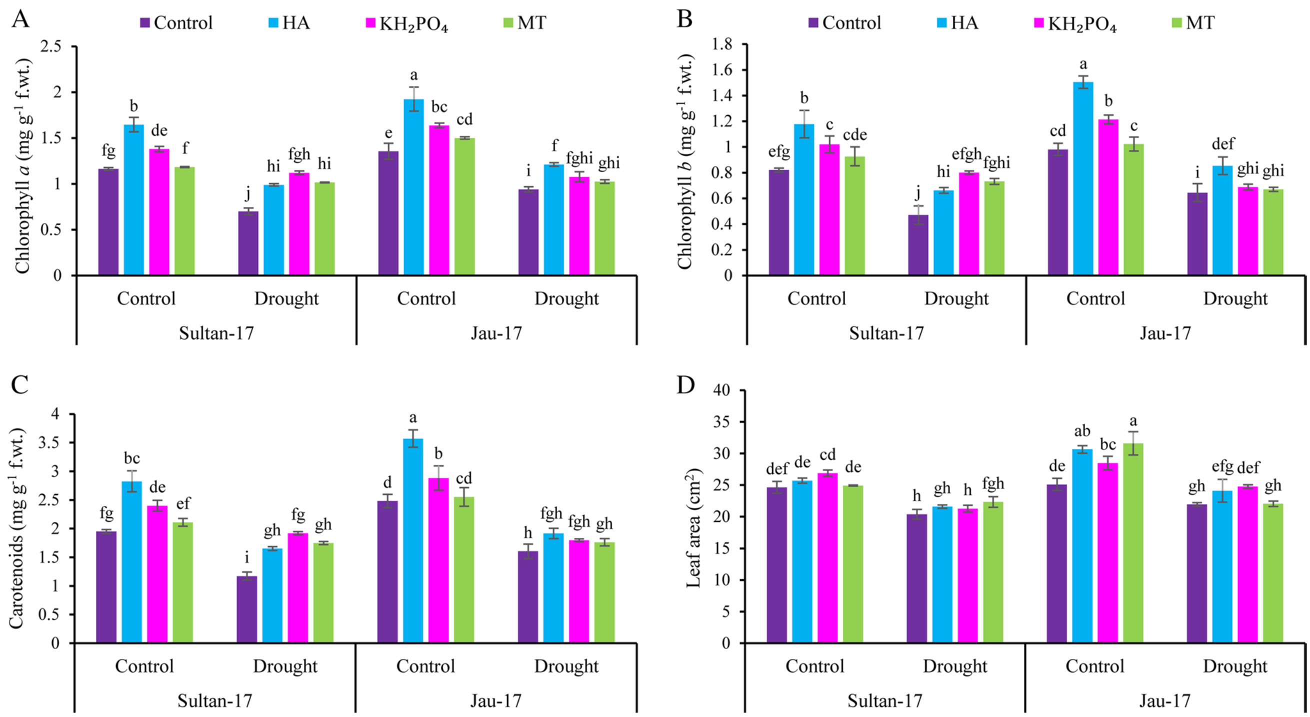

Fig. 2 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) chlorophyll *a*, (B) chlorophyll *b*, (C) carotenoid contents, and (D) leaf area of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

In this study, drought stress caused impairment in all growth parameters of *H. vulgare*, in accordance with a previous report in barley (Abdelaal et al. 2020, 2018) and various other plants (El-Sabagh et al. 2017; Elewa et al. 2017; Sadak 2016). The HA application increases all growth characteristics including dry and fresh weight of shoot in both varieties, with similar findings obtained by Roozbahani (2015) in barley and Al-Fraihat et al. (2018) in onion. K^+ upraised all morphological characters under drought stress as it maintains water cellular balance as reported in sugar corn (Bijanzadeh et al. 2019; Rao et al. 2012). Excessive K^+ application as KH_2PO_4 has been demonstrated to enhance growth parameters which helps plants attain better yield under drought and non-drought stress, previously reported in tobacco plants (Bahrami-Rad and Hajiboland 2017).

Water content in leaves is the status mark for water in plants and used to evaluate drought tolerance. Chlorophyll and carotenoids play a vital role in plant energy generation, and it is well known that drought minimizes the cereal's photosynthetic capability and this disturbed the normal plant growth. Water-deficit conditions result in notable decreases in chlorophyll *a* and *b* and carotenoids and relative water content in the barley plant and these results were supported by previous literature (Abdelaal et al. 2020; Goodarzian et al. 2015; Jaleel et al. 2009). Both varieties, i.e., Sultan-17 and Jau-17, had varying chlorophyll and water content. Different barley varieties respond differently in respect to RWC and chlorophyll contents when exposed to drought (Dai et al. 2020; El-Shawy et al. 2017). There was a relatively slight decrease in chlorophyll under drought stress in Jau-17 and possibly an indication of drought tolerance as proved by the past report (Sakya et al. 2018).

HA notably elevated chl a and b and carotenoids in both barley varieties under the application of drought stress concerning drought control (Abdelaal et al. 2018; El-Bassiouny et al. 2014; Shen et al. 2020). HA and K^+ significantly increase RWC under drought stress as determined by Shen et al. (2020) and Zahoor et al. (2017) respectively. KH_2PO_4 application lifted the photosynthesis process and as a result chl content improved in both varieties under water-deficit stress conditions (Mahmoud and Youssif 2015; Marschner 2012). MT raised the values of both chl *a* and *b* content in barley under drought state compared to drought control (Sadak and Bakry 2020; Ahmad et al. 2019; Cao et al. 2019; Liang et al. 2019).

Results demonstrated that under drought stress, MDA, relative membrane permeability, and H_2O_2 content were raised in both varieties and were superior to control and other applied treatments, as supported by findings (Abdelaal et al. 2018; Bijanzadeh et al. 2019; Mihaljević et al.

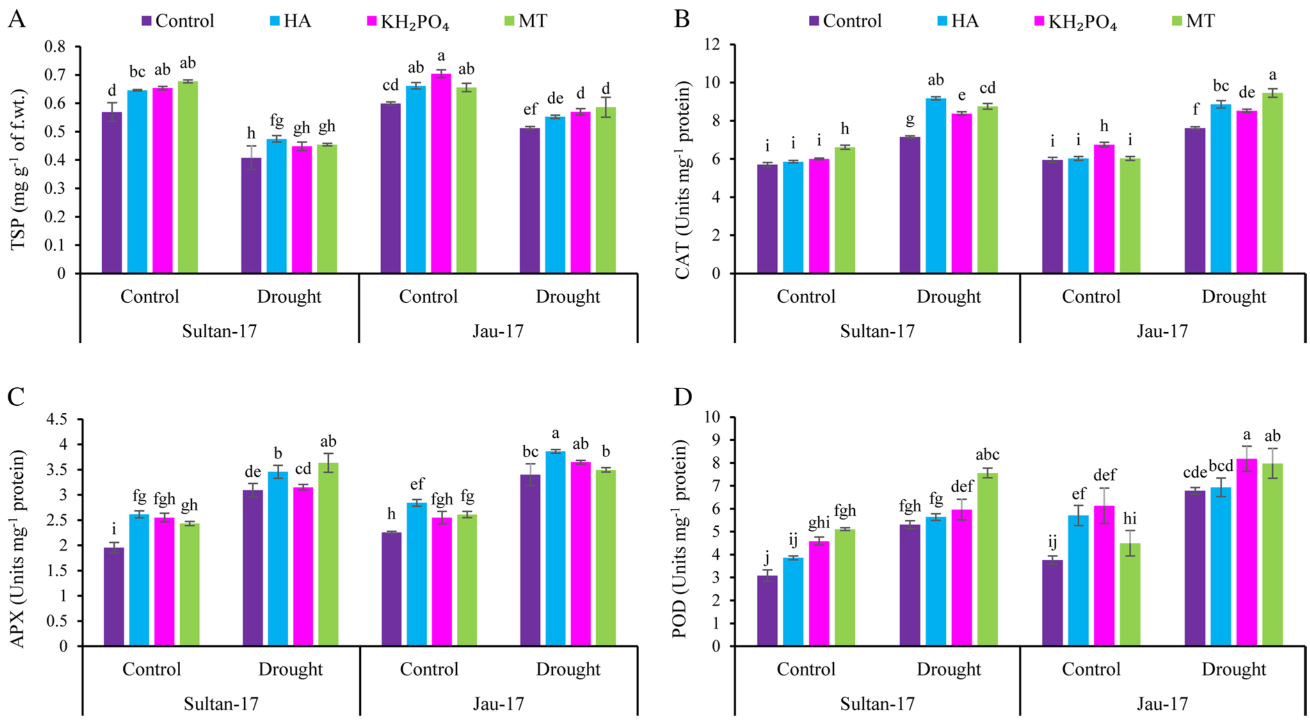

Fig. 3 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) TSP (total soluble proteins), (B) CAT (catalase), (C) APX (ascorbate peroxidase), and (D) POD (peroxidase) of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

2021). The present study indicated that higher MDA content in drought-stress plants was linked with excessive H_2O_2 production. It increases more in Sultan-17 as compared to other varieties used in this research. Low MDA was accompanied by low membrane leakage and this was associated to induce drought stress tolerance in barley. The lower H_2O_2 production with low MDA content under drought stress is perhaps due to the triggering of antioxidant enzyme activities especially CAT which minimizes H_2O_2 accumulation. Similar outcomes were reported by Outoukarte et al. (2019) and Umar and Siddiqui (2018). Treatment of HA alleviates the MDA content and relative membrane permeability in barley under drought and non-drought conditions (Shen et al. 2020). KH_2PO_4 and HA improve plasmalemma stability which in turn reduce MDA concentration and membrane permeability, as found by Abdelaal et al. (2018) and Aydin et al. (2012). MT spray remarkably declined the MDA and H_2O_2 content (Kabiri et al. 2018; Liang et al. 2019).

Proline particularly marked an indication of drought tolerance as its level increases in plants under drought stress (Du et al. 2023). Under drought, proline accumulation was found by Sallam et al. (2019) in cereals. An increase in proline concentration was analyzed under the application of drought in both varieties compared to control and previously reported by Fayez and Bazaid (2014) and Habib et al. (2020).

Drought stress caused a remarkable decrease in protein content in barley (Liang et al. 2019; Pazirandeh et al. 2013). Barley plants with better photosynthetic capacity had an extended level of total phenolics and were supported by Vicas et al. (2019). Protein and proline concentrations were enhanced under the three exogenous different treatments and drought application. The results concluded that HA application under drought stress promoted proline concentration and may oppose the negative effect of drought stress (Shen et al. 2020). HA enhanced phenol and proline content in barley under drought application as found by El-Bassiouny et al. (2014) in wheat. K^+ application triggered the accumulation of proline in both varieties and to conserve tissue water was interpreted by Ahanger et al. (2017). That K^+ ions increased phenolics in drought state in contrast to control was disclosed by Fayez and Bazaid (2014). It is suggested that MT could repress the breakdown of protein and enhance the production of new proteins as earlier informed by Ahmad et al. (2019) and Liang et al. (2019). MT application increased phenolic proportion under a drought state in contrast to control as determined by Sadak and Bakry (2020) and Tan et al. (2012).

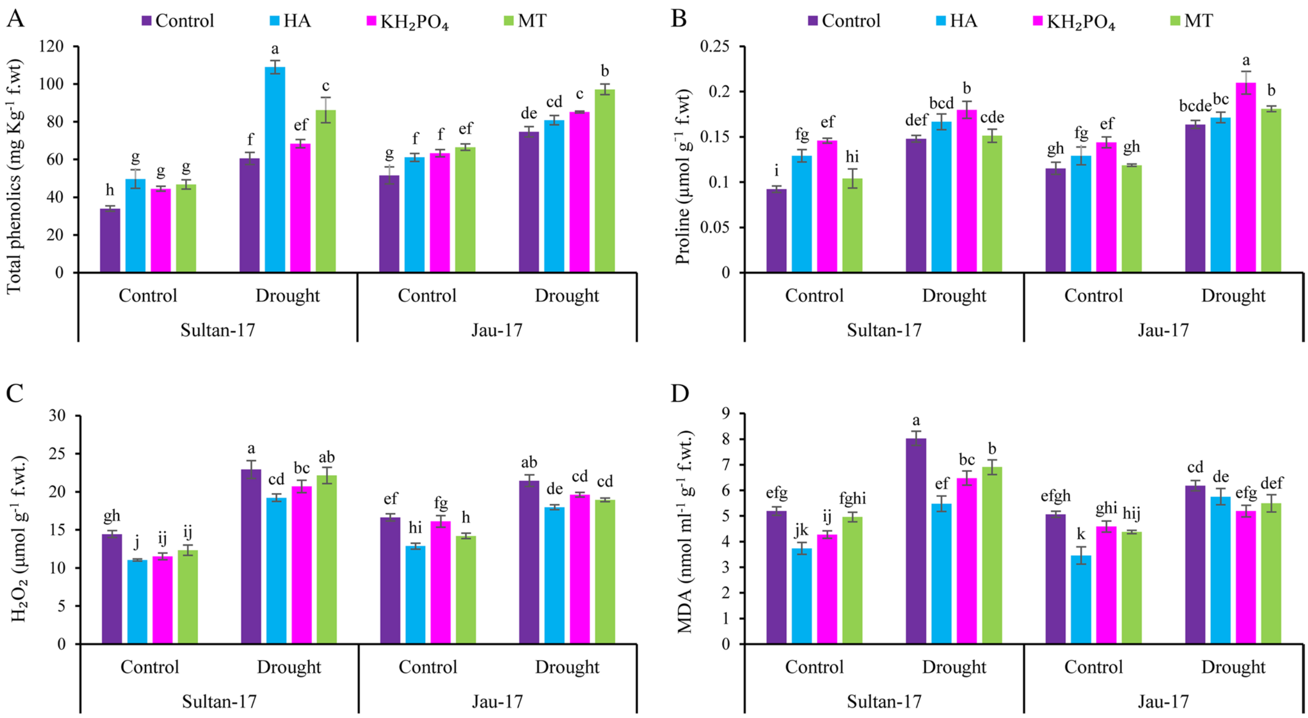

Fig. 4 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) total phenolics, (B) leaf proline content, (C) H_2O_2 (hydrogen peroxide) content, and (D) MDA (malondialdehyde) content of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

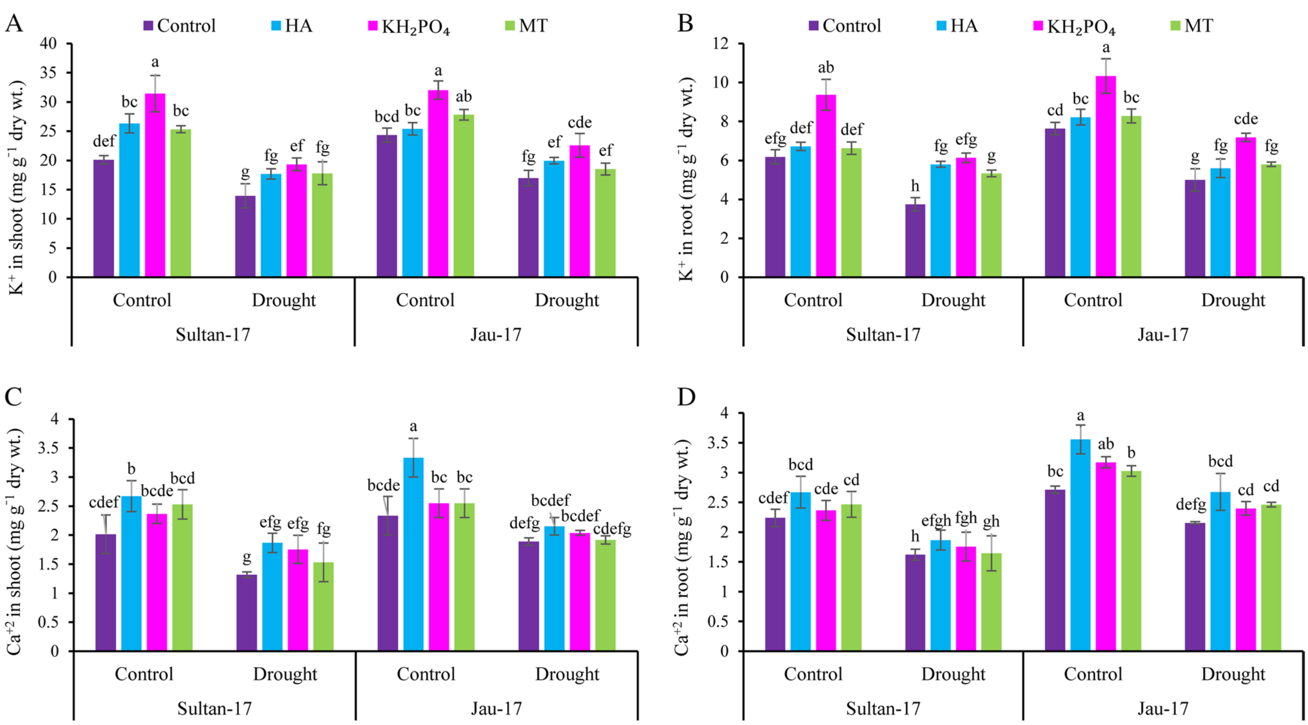

Fig. 5 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) K^+ (potassium) in shoot, (B) K^+ (potassium) in root, (C) Ca^{2+} (calcium) in shoot, and (D) $Ca.^{2+}$ (calcium) in root of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

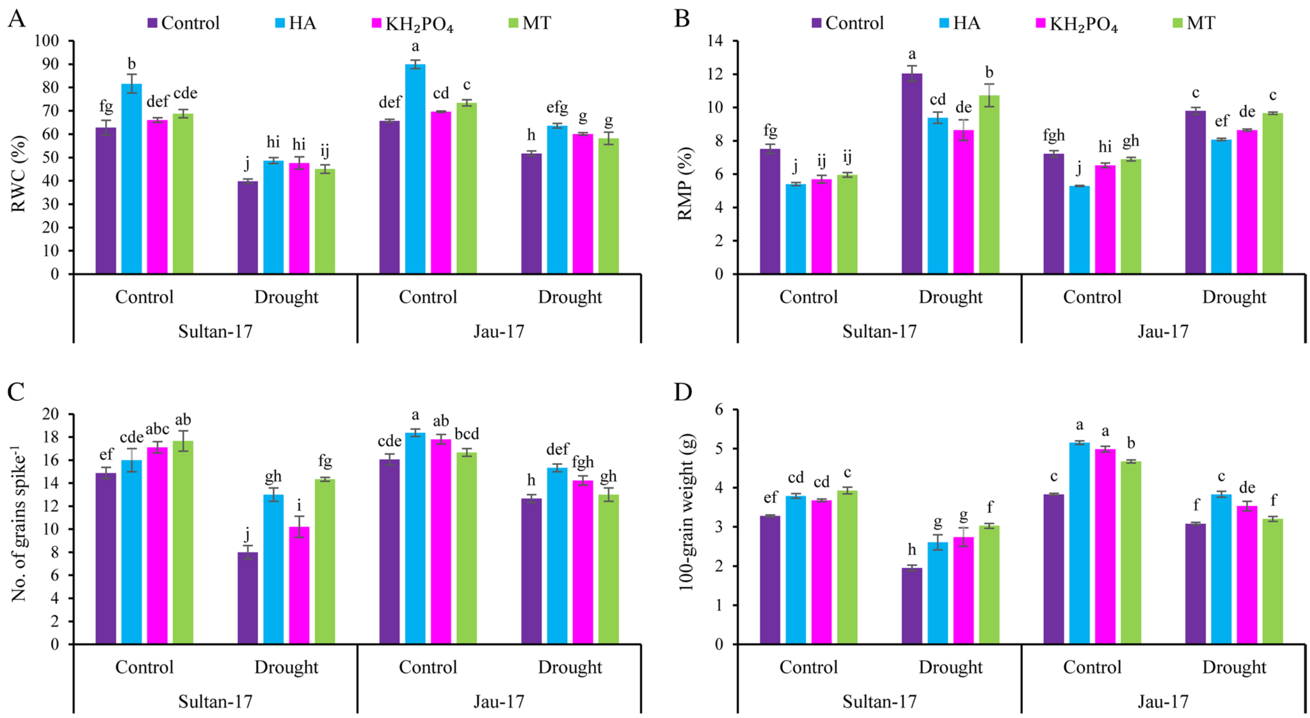

Fig. 6 Effect of foliar application of HA (humic acid), KH_2PO_4 (potassium dihydrogen phosphate), and MT (melatonin) on (A) RWC (relative water content), (B) RMP (relative membrane permeability), (C) number of grains per spike, and (D) 100-grain weight of two *Hordeum vulgare* L. varieties (Sultan-17 and Jau-17) under drought and non-drought conditions. Data presented in (A)–(D) is average of three replicates for each treatment ± standard error (SE). Different letters (a, b, c, and so on) above graph bars show statistically significant differences among treatment groups as determined by three-way ANOVA, followed by LSD test ($p < 0.05$)

Antioxidant enzyme production demonstrated the alleviation in the harmful impacts of drought stress and an indication of well-adaptive mechanism in barley. Drought stress amplifies the ROS content in barley especially H_2O_2 activity which counteracts by the CAT production, as previously reported by Sadak and Bakry (2020). Tolerant barley plants had high activity of POD, found by Outoukarte et al. (2019), Sallam et al. (2019), and Shen et al. (2020). CAT and APX were much greater in Jau-17 barley plant as compared to Sultan-17, similar to results of Goodarzian et al. (2015) and Laxa et al. (2019). An increment in all antioxidant enzymes (CAT, APX, and POD) was observed under drought application stress in both barley varieties compared to its control in this study (Fig. 3). Under drought stress, all treatments increased peroxidase, catalase, and APX levels respectively in Sultan-17 and Jau-17. Comparable results found that antioxidants elevated in different plants under drought conditions (Cao et al. 2019; Maghsoudi et al. 2019; Yasmeen et al. 2013).

The present study revealed the foliar misting of HA could amplify antioxidant enzyme production in both varieties under drought conditions. Exogenous KH_2PO_4 induced upregulation of these ROS-scavenging enzymes proved by finding (Bharti & Barnawal 2019). An increment in enzymes, i.e., (antioxidant) like POD and CAT, was showed by exogenously applied KH_2PO_4 and HA under drought stress conditions (Abdelaal et al. 2018). MT enhanced catalase (CAT) activity of drought plants (Dai et al. 2020; Tabassum et al. 2024). Exogenously applied MT elevated all the antioxidant enzymes under a drought stress (Ahmad et al. 2019; Cao et al. 2019; Li et al. 2018).

Drought-stressed barley plants had low ion contents especially K^+ (Abdelaal et al. 2018; Fayez and Bazaid 2014). HA and KH_2PO_4 increased K^+ contents in barley plants as compared to drought-stress plants (Abdelaal et al. 2018). Application of KH_2PO_4 markedly increased K^+ contents in plants that were subjected to drought stress, related to the results given by Fayez and Bazaid (2014) and Zahoor et al. (2017). Under drought and non-drought conditions, it is noted that ionic contents elevated under the application of HA as contrasted to control plants (El-Bassiouny et al. 2014).

The high grain yield is linked with a high no. of grains per spike, and water and chlorophyll content, earlier

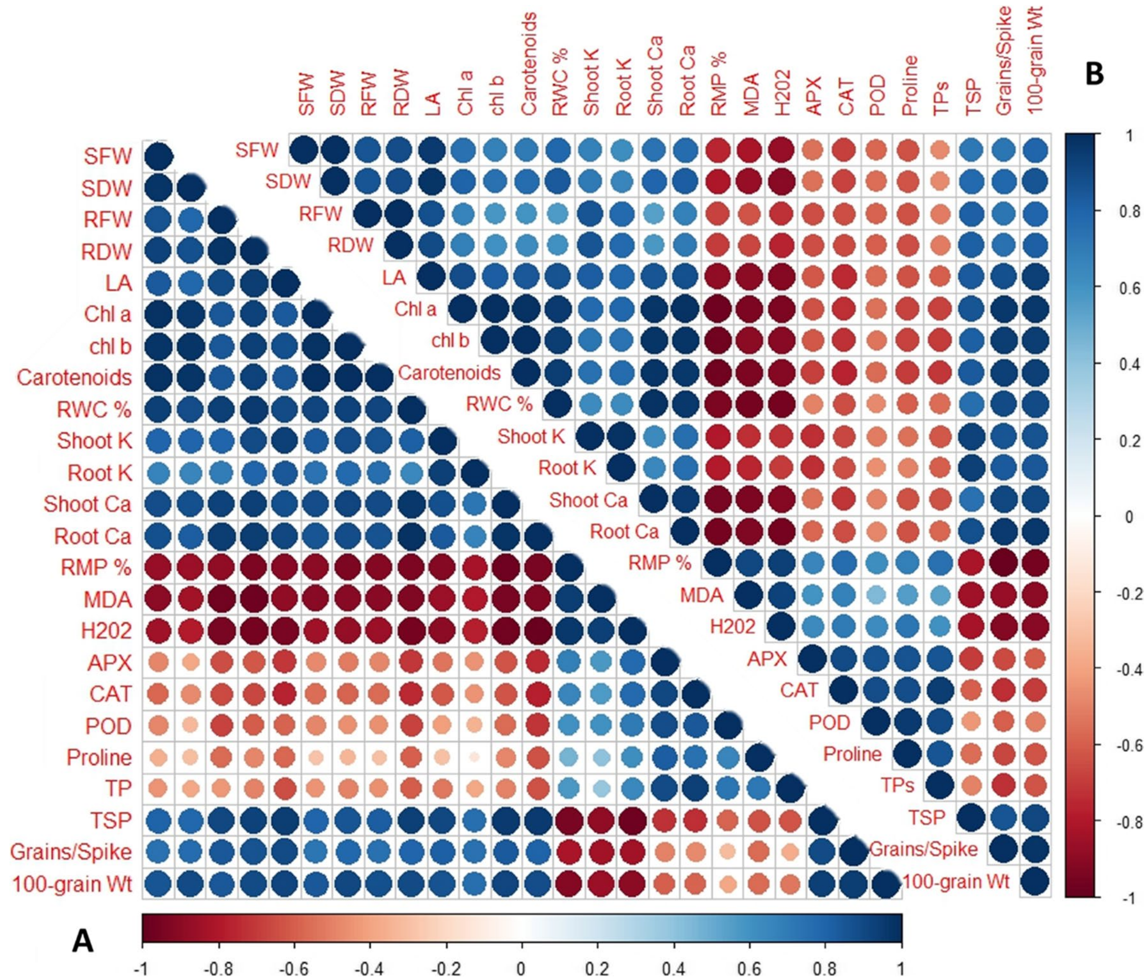

Fig. 7 Pearson's correlation for all studied parameters of two *Hordeum vulgare* varieties (A = Sultan-17 and B = Jau-17) subjected to three treatments (HA, KH_2PO_4, and MT) under drought and non-drought conditions (various abbreviations used are as follows: SFW, shoot fresh weight; SDW, shoot dry weight; RFW, root fresh weight; RDW, root dry weight; LA, leaf area; Chl, chlorophyll; RCW, relative water content; RMP, relative membrane permeability; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; TP, total phenolics; TSP, total soluble proteins; Wt, weight)

monitored in wheat (Sallam et al. 2019). Under drought stress, barley productivity was greatly reduced due to the decreased no. of grains, as supported by Abdelaal et al. (2020). Comparable outcomes were accounted by Abdelaal et al. (2018), Habib et al. (2020), and Sadak and Bakry (2020). This study revealed that 100-grain weight in both varieties was remarkably enhanced with exogenously applied HA. The same results were determined by El-Bassiouny et al. (2014) and Roozbahani (2015). Elevations in 100-seed weight and no. of seeds per spike were observed with foliar application of KH_2PO_4, in agreement with El-Abady et al. (2009) and Zareian et al. (2014). Practicing of HA and K^+ fertilizer increased the yield of barley plants, as seen by El-Sheshtawy et al. (2019). Different chemicals used in this research ameliorated the effects that sustain plant growth and development during drought stress and hence quantity of grains elevated.

5 Conclusion

It has been revealed from the current work that drought reduces the fresh and dry weight of shoot and root, leaf area, relative water content, chlorophyll *a*, chlorophyll *b*, and carotenoids. Drought raised the concentration of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and relative permeability while the application of all substances alleviated their concentrations. Both varieties, i.e., Sultan-17 and Jau-17 of the barley plant (*Hordeum vulgare* L.), were affected by drought stress. Although the exogenous application of different substances, i.e., humic acid (HA), potassium dihydrogen phosphate (KH_2PO_4), and melatonin (MT), significantly alleviated the negative effects of drought stress in barley; however, the notable effects were observed when plants were sprayed with HA. The overall performance of the Jau-17 variety interpreted that it may be the better choice

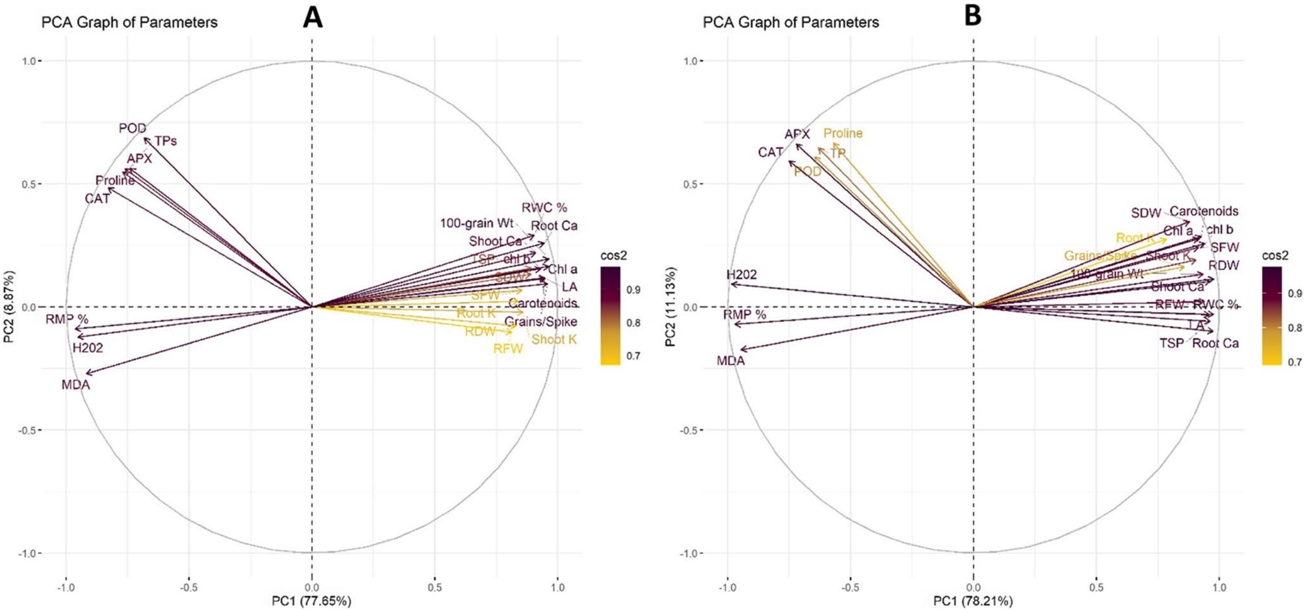

Fig. 8 Principal component analysis for all studied parameters of two *Hordeum vulgare* varieties (A = Sultan-17 and B = Jau-17) subjected to three treatments (HA, KH_2PO_4, and MT) under drought and non-drought conditions (various abbreviations used are the same as in Fig. 7)

of variety under drought stress and further foliar application enhances the growth and yield under drought and normal conditions.

6 Recommendations for Future Work

Potassium dihydrogen phosphate (KH_2PO_4) treatment increases the majority of parameters studied but if its concentration will increase, it will be more effective. More work is needed to identify more drought-tolerant varieties. The changing of timing in drought application can give better results. Further molecular studies are important for the effectiveness of these interpretations. The trials at different concentrations of various treatments may be conducted to be commonly used on a commercial scale.

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Author Contribution Conceptualization: Z.N.; methodology: F.Z.; formal analysis and investigation: F.Z. and A.A.S.; writing—original draft preparation: S.U.; writing—review and editing: A.A.S. and S.U.; supervision: Z.N. All authors read and approved the final manuscript.

Data Availability The data will be provided whenever required.

Declarations

Competing Interests The authors declare no competing interests.

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