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Fizza Zafar
University of Education
Zahra Noreen
University of Education
Anis Ali Shah
University of Education
Sheeraz Usman (miansheerazusman@gmail.com)
University of Education https://orcid.org/0000-0002-4218-0682

Research Article

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Co-application of humic acid, potassium dihydrogen phosphate and melatonin (osmo-regulators) ameliorate the effects of drought stress in Barley (*Hordeum vulgare* L.)

Fizza Zafar[1], Zahra Noreen[1], Anis Ali Shah[1], Sheeraz Usman[1*]

1. Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan.

[*]Corresponding Author: miansheerazusman@gmail.com

ABSTRACT

Purpose: Drought has an adverse impact on the production and growth of cereals globally. Due to drought stress, cereals' cultivation declined day by day, worldwide. Hence, ultimate yield does not fulfill the required demand. The current research investigated the consequences of drought stress on morpho-physiological, yield and biochemical parameters of barley plants and a comparison of different osmo-regulators and their ameliorating capacity towards drought stress.

Methods: A pot trial was held in a completely randomized (CR) design with three replicates manner to investigate the role of humic acid, potassium dihydrogen phosphate and melatonin (osmo-regulators) synergistic application in ameliorating drought stress. Three barely varieties (Haider-93, Sultan-17 and Jau-17) were selected for this experiment. The treatments applied were as follows; humic acid (400 ppm), potassium dihydrogen phosphate (20 ppm) and melatonin (0.5 mM) with two water levels i.e., Control (normal watering) and drought stress (stop watering).

Results: Results indicated that foliar application of all osmo-regulators improved propagation, antioxidants, proteins, chlorophyll, mineral contents and productivity parameters, while alleviate 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 given humic acid in control and drought stress in Jau-17. The order of effectiveness of osmo-regulators in this study was humic acid > melatonin > potassium dihydrogen phosphate.

Conclusion: Osmo-regulators examined in this study had potential role in combating against drought stress and could also be effective for various other abiotic stresses.

Keywords: Humic acid, Potassium dihydrogen phosphate, Melatonin, Drought stress, Antioxidant enzymes, foliar application, ROS species.

Statements and Declarations

Competing Interests: The authors have no relevant financial or non-financial interests to disclose. Besides this, all authors declare no conflict of interest.

Introduction

Plants are vulnerable to different ambient stresses during growth, and development by innate and agricultural circumstances. Drought is one of the most serious ambient stresses influencing plant fertility. (Brodersen *et al.,* 2019). World climate alteration typically result infrequent drought stress circumstances over wide regions at a scale globally (Adnan *et al.,* 2020). In future, most critical threat to global food safety is drought (Seleiman *et al.,* 2021). Growth phase, age, severity of drought, species of plant, and duration happen to the prime aspects that affect the plant responses to Drought conditions (Gray & Brady, 2016).

Hordeum vulgare L. (Barley) is the fourth most 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, 2016). Barley was one of the initial cultivated cereals, and old world agriculture crop (El-Hashash & El-Absy, 2019). Barley is a diploid (2n) self-pollinating plant, with each flower possess both male (anthers), and female (ovary) organs. It is a winter seasonal, rapid growing cereal that grow annually. It is mainly utilized as pasturage, and may also as a cover crop to sustain strength of soil, and yield via biological nitrogen fixation (Bishnoi *et al.*, 2022). Furthermore, barley is also a better model organism for inspecting the cereal botany. The reason is because of small life sequence i.e. 13 weeks, self-fertilized, and relatively diploid short genome (5.3 Gbp), specifically when contrasted to hexaploid wheat (18Gbp). So, it is easy to inspect the morphological, physiological, and genetic attributes (Giraldo *et al.,* 2019). Barley water is well-known to have numerous medicinal properties and facilitate in swift healing of multiple diseases or disorders (Chand *et al.,* 2008). It's associated predominantly to abundant healthy fibers, i.e., b-glucan constitution. Furthermore, barley considered as a magnificent cause of minerals, vitamins, starch, and protein. In short, considered as an ideal food supplement (Farag *et al.,* 2020).

The utilization of barley crop by the humans has now decreased substantially over the past years because the use of wheat crop has now become popular, and barley crop is promptly being used as poultry feed. But, barley has much richer in fiber, and cholesterol-lowering beta-glucan, and loses little nutrients during processing than wheat (Mandl, 2020). The crop may cultivated over broad span of agro-climatic conditions. However, drought is one of the serious threat which affect their production (Zargar *et al.*, 2017). The degree of Drought stress rely on the time duration, stress intensiveness, propagation stage, and genetic tolerance capability of plants (Nadeem *et al.*, 2019). As drought stress rises, lesser propagation, and yield were ascertained in barely (Behboudi *et al.*, 2018).

In Pakistan, bread wheat is the topmost food, even though its production cannot cope with requirement, resulting in a shortage (Mahmood *et al.*, 2020). Another grain crop i.e., barley is needed as a basic food to balance the gap to reduce the load on the bread wheat crop. But low production are partially due to the drought stress and inaccessibility of stress-tolerant high yielding varieties of barley (Elakhdar *et al.*, 2022). In Pakistan, unpredictable and more periodic rainfall exist in pre-spring, and winter. Although, more often drier, and intense period take place owing to reduced or no rainfall in initial fall, and summer seasons. (Karandish & Šimůnek, 2017).

Drought stress drought can stimulate 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 activate the membranes, organelles peroxidation, enzyme inactivation or activation, and disintegration of nucleic acids (Outoukarte *et al.,* 2019). CAT (Catalase), POD (Peroxidase), and SOD (superoxide dismutase) are antioxidant enzymes that play crucial roles in removing excessive ROS in cell, and sustain ROS homeostasis, and tolerance to Drought stress (Verma *et al.*, 2019).

Plants evolved several techniques, and schemes to reduce the negative upshots of Drought stress (Thanmalagan *et al.*, 2022). Agrologist are also utilizing different techniques for Drought stress tolerance, among which the practice of exogenous chemicals, regulators, artificial hormones, and compounds are of appreciable worth to elevate drought tolerance at various plant propagation phases. Practicing of plant growth modulators can inflate Drought tolerance in plants (Tariq *et al.*, 2022). One of the modern techniques is the utilization of phytohormones or plant bio-enhancers to increase the preservation, and adaptability of plants opposed to critical environmental circumstances. Several plant bio-enhancers are assessed to have constructive effects on different plant physiological systems (Cui *et al.,* 2017; Kamran *et al.,* 2018). Several chemicals like growth modulators, osmoprotectants, and stress prompting compounds are being used successfully opposed to various biological, and non-living stresses to trigger the tolerance (Abdelaal *et al.,* 2018). There is approach to enhance plants drought tolerance that is exogenously applied the plant development modulators e.g. Osmoprotectants, antioxidant compounds (Liang *et al.,* 2019).

Different chemicals used in this research were an ameliorating effects which sustain plant growth and expansion during drought stress. Outcomes indicated that drought revealed harmful effects on all attributes studied in barley plants (Hordeum vulgare L.). Three selected chemicals i.e., humic acid (HA), potassium dihydrogen phosphate and melatonin nominated as osmoregulators help in osmoregulation and maintaining plant growth under drought stress. Osmoregulators applied as foliar spray which entered through leaves and soil in plant and maintain turgor pressure. While using the same osmoregulators, under normal conditions also enhance all growth parameters and maximize the yield.

Humic Acid (HA) with molecular weight of 30-300 kDa and < 30 kDa respectively, consequence in the development of soluble, and insoluble firm composite with micronutrients (Danyaei *et al.*, 2017). The nutrients foliar spray (Actosol and KH_2PO_4) is promptly assimilated by leaves, and improving the cell growth, and physiological processes as well to confront the high nutrients requirement during some growth phases especially at grain-filling period (Mahmoud & Youssif, 2015). Utilization of humic acid appreciably increased the vegetative growth, photosynthetic pigments, mineral value, aid in the assimilation, and transport of minerals because of the complexes, and chelates synthesis, leading to rise in yield in different plant crops (El-Tahlawy & Ali, 2022). HA disintegrate in water quite well, and also is dissolvable with other fluid fertilizers, feasibly used through soil application, spraying, and pressurized irrigation methods. Humic acid also upgrades chemical, physical, and biological features of soil (Roozbahani, 2015).

Potassium is one the most fundamental macronutrient which acts a significant part in enlargement and propagation of plants, and initiate above than 60 enzymes. Potassium is also enhances water stress tolerance in plants through conserving water balance (Behairy *et al.,* 2015). Furthermore, it play effective roles in the photosynthesis physiological process, protein, and carbohydrate development, nutrients and water transportation, nitrogen (N) usage, and provoke initial plant growth (Daniel *et al.,* 2016; Lakudzala, 2013). Under Drought stress, the plants consumed more K^+ for their inner regulation mechanism, and application of potassium mitigate the negative impact of the water shortage, and maintains the plant productivity (Hasanuzzaman *et al.*, 2018). Foliar or soil application of K^+ is favorable for the optimum plant physiological processes (Brestic *et al.,* 2018). Therefore, application of K^+ is of high significance for acquiring optimal crops yield grown under both rainfed areas or water deficit conditions (Kumar *et al.,* 2019).

Melatonin (MT, N-acetyl-5-methoxytryptamine), a pleiotropic hormone, intricate 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 have several functions in plants and animals, and established to be an abiotic antistresser in plants (Manchester *et al.,* 2015). Plants can assimilate MT, through soil but also organized from L-tryptophan (Nawaz *et al.,* 2016). Although 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).

By foliar application in this research, can mitigate the harmful effects of drought, and elevate the development and yield of plant. The outcome is both the quality and quantity of grains elevated. Comparison of different osmoregulators of separate composition and concentration on yield and growth related parameters observe in this study.

Research Methodology

Cumulation of Seeds

Seeds (barley) were sown in November 2020. The seeds of barley were acquired through Ayub Agricultural Research Institute (AARI), Jhang road, Faisalabad, Pakistan i.e. barley variety Sultan-17, Jau-17 and Haider-93. These were entirely dried and cleaned. It was exported in merely secure mode over parceling in a brown wrapper and in addition this wrapping were protected in polythene pouch for seed preventing from damp.

Seed Germination and Selection

Barley varieties were elected on the base of germination test. Total 15 seeds from each barley variety type placed in sterilized cell culture dishes having wet filter papers on them. Cell culture dishes were set within the research lab at ordinary temperature of room and were leftover there for five days period under 12 hour light.

Observed Proportion of effective germination was as followed:

i. Haider-93 (5 out of 15)
ii. Sultan-17 (10/15)
iii. Jau-17 (13/15)

Hence, on the base of result of varieties germination, two varieties i.e. sultan-17 and jau-17 were selected for further pot experiment.

Experimental Conditions

Experimental conditions for growth of plant were alike as ordinary environmental conditions of Township, Lahore besides the implementation of drought stress. Plants were propagate in plastic containers to handle stress of drought just in case of rainfall. Plastic containers were additionally shielded with transparent polyethylene film for further enhancement to handle drought stress.

Pots

The plastic pots of 23.5 cm × 20 cm were used. The pots were hole 1.5 inch from base of pot with pre-heated iron rode for drainage and aeration. The hole enclosed with white thin cotton, so soil did not leak out.

Collection of Soil and Soil Type

Fertile soil was brought for experimentation from local nursery near Ideal Park Township, Lahore. Soil taken in large sacs from local nursery to university fieldwork site in loaders. Soil was screen out for plant debris and picked the stone away prior to filling of pots. The soil analysis was done to check different properties and type of soil as shown in Table 1.

Soil Quantity

Every single plastic containers from 48 pots were filled up soil of 6.70 kg and overall 321 kg soil that were consumed in experiment.

Sowing

Seeds were sown in the end of 2nd week of November. Fifteen seed were seeded in every single pot with depth of 1.5 inches with the seed distance of 1 inch.

Thinning of Seedlings

The seeds germinated within a week but thinning of seedlings were done after 3 weeks of sowing. Only 7 healthier and greenish seedlings were kept in each pot while rest were discarded.

Experimental Layout

The trial was arranged in complete randomized (CR) design using triplet factors (variety, drought, treatments) and three replicates in total 48 pots. Two different varieties i.e. Jau-17 and Sultan-17 with three different selected osmoregulators applied as mentioned in table. Water level was maintained in 2 concentration i.e. Control (normal watering) and drought stress (stop watering).

Drought Stress Application

The moisture in soil that placed in plastic pots were sustained at field capacity (FC) by regular providing the tap water to all plants for 27 days prior to drought application. Drought treatment was imposed by withholding watering after foliar application of osmoregulators. After, round about 4 weeks of maturation, the pots divided into two equal sections (both varieties). One-half of the plastic containers from both of the varieties were normally watered, while the other section of plants treated with drought by holdback the supply of water. Plastic containers were additionally shielded with transparent polyethylene film to prevent it from precipitation for handling drought application.

Application of Osmoregulators

Chemicals used as osmoregulators were weighed by electrical balance accurately according to table mentioned above. They were dissolving in 1000 ml distilled water. Foliar application done with the help of water mister spray bottle. Foliar application done at evening time. It was ensure that all leaves were totally wet with chemicals applied with aid of hand sprayer.

Following growth parameters, anatomical, physiological and biochemical attributes were measured during the investigation.

Growth Parameters:

The accounts of morphological features were as following:

Fresh Weight of Shoots (g)

One plant from each individual pot was taken with the help of screwdriver. Plants, then was wash with tap water to eliminate debris and rinsed by using paper towel to remove the excessive water. The shoot was separated with root through knife. Shoot fresh weight was taken on electronic weight balance in units of grams.

Fresh Weight of Roots (g)

For root fresh weight, same procedure executes as done for shoot fresh weight. Roots was carefully taken out from pot and excised from shoot. These were washed by adequate water to get rid of soil. Weighing balance i.e. electronic utilized for took root fresh weight.

Total Leaf Area per Plant (cm^2)

Length and width from each leaf taken from plant was measured through a scale ruler. Leaf area was computed manually by formula; length ×width in units of cm^2 (Carleton & Foote, 1965).

Dry Weight of Shoot (g)

Fresh shoot was dried in an oven incubator for 24 hours for 75°C till constant weight was achieved. After 24 hours, dry weight of shoot was determined on digital analytical balance in units of grams.

Dry Weight of Root (g)

Initially, the washed roots dried with paper towel and then put in oven incubator at 75°C for 24 hours till constant weight was attained. Root dry weight were estimated with aid of electrical balance.

Physiological and Biochemical attributes

Calculation of Carotenoid and Chlorophyll Content

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

The values were written down and chl a & chl b were calculated using formula in mg/g as follows:

$$Chl.a\ (mg\ g-1f.wt) = [12.7(OD663) - 2.69(OD645)] \times V/1000 \times Wf$$

$$Chl.b\ (mg\ g-1f.wt) = [22.9(OD645) - 4.68(OD663)] \times V/1000 \times Wf$$

V = volume of solvent (ml)

W_f = weight of fresh leaf tissue (g)

OD = optical density

Carotenoids content were calculated using the below correlation set by (Lichtenthaler, 1987).

$$Carotenoids = (1000 \times OD480) - (1.9 \times chl\ a - 63.14 \times chl\ b)/214$$

Analysis of RWC (%)

Leaf relative water content was analyzed by (Jones & Turner, 1978) procedure. Same foliage size of each replicate were taken and recorded their respective fresh weight by electronic balance. Leaves were put down straightly in twofold distilled water in petri dishes, so it soaked with water very well. Leave it up to 3 hours at room temperature in dark place. Leaves were dry with clean tissue for estimating leaf turgid weight. Then, leaves were place in an incubator at temp 80°C for 24 hours for measuring dried leaf weight. Relative water content was measured by formula given below:

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

Where $t.w_t$, $f.w_t$ and $d.w_t$, represented the turgid weight, fresh and oven-dried accordingly.

Determination of Relative Membrane Permeability (EC %)

The relative membrane permeability was ascertained by procedure of (Yang *et al.,* 1996). Wholly fresh developed leaves excised from plants from each single replicate having uniform size. The leaves were tearing into small pieces with scissor and place in test tubes possessing (20 ml) of distilled deionized H_2O. The test glass tubes were vortex for 10 s and infusion assessed for electrical initial conductivity (EC_0) with the help of electrical conductivity meter (Hanna HI-9811-5 EC portable meter). These glass tubes were covered by aluminum (Al) foil and kept in during 24 hours in refrigerator at 4°C. At that moment, infusion tested for EC_1. These samples that were covered, arranged in beaker containing chopped foliages were autoclaved on 121°C for 1200 sec to find out EC_2. Relative membrane permeability in percentage was computed as:

$$RMP\ (\%) = (EC1 - ECo/EC2 - EC0) \times 100$$

Estimation of Ionic Content

The underlying protocol were same for both shoot and root ionic determination (Wolf, 1982) with little modifications. Standard solutions were prepared by dilution of stock solutions. For estimating the ion concentration, firstly plant pieces were kept in a lab-oven for drying for 24 hours at 75°C. Weigh 0.1 g for shoot and 0.05 g for root and bring slowly 2 ml conc. H_2SO_4 in each test tube having samples very carefully. Samples were retained for one day. Digestion mixture (1 ml) which completely turns black were placed on hot plate having temperature (50-150 °C). When heating become started, add total 1000 µl H_20_2 in bits of 200 µl in each test tube. Wait for boiling it for 30 minutes. When samples were completely turns colorless, remove it from hot plate and let them cool. Then each solution was filter with Whatman Grade 42 filter paper to remove any type of debris. The mixture was made up total volume to (50 ml) in a graduated flask by distilled H_2O and preserve in plastic bottles. The filtered that kept in bottles were further manipulated for obtaining values of ions. Ca^{2+}, K^+ i.e. bivalent and monovalent cations respectively in digests were assessed with a (Sherwood-Model 360) flame photometer.

Assessment of Malondialdehyde (MDA) Content

Malondialdehyde was calculated in rates of absorption by (TBARS) thiobarbituric acid-reactive substances (Cakmak & Horst, 1991). Take fresh leaves and weight up to 0.2 g on analytical balance. Each sample was shredded with 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 900 sec (HERMLE Z 326 K). Extract half ml from supernatant and added trine ml of 0.5% (v/v) TBA i.e. (thiobarbituric acid) in 20% TCA in each sample. TBA of 0.5% was made by (liquefied 500 mg TBA in 0.1 liter 20% TCA). TCA (trichloroacetic acid) of 20% by (adding 0.02 kg TCA in 0.1 liter distilled refined water). 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 a frost water bath. Then were, again centrifuged at 10,000×g for 10 min. The clear supernatant in conical tubes were taken in quartz cuvette and absorbance were observe at 532 nm by UV-Visible double-beam spectrophotometer (Metash-Model UV-9000). The assessed concentration for non-specific engrossment at 600 nm was subtracted from all measurements taken at 532 nm. The absorption of TBARS were computed applying the coefficient absorption i.e. 155 $\text{nmol}^{-1}\,\text{cm}^{-1}$.

$$\text{MDA level (nmol)} = \Delta\,(\text{A 532 nm} - \text{A 600 nm})/1.56\times10^{5}$$

Total Soluble Protein Assay

Quantity of soluble proteins were investigated through tactics given by (Bradford, 1976). Leaves weight up to 500 mg and finely grinded in 10,000 µl of 50 mM buffer i.e., orthophosphate having a 7.8 pH on a frappe bathtub. The grounded material was centrifuged (HERMLE Z 326 K) at 6000 × g for 20 minutes at cold. Then prepare Bradford reagent as following chemicals i.e. Coomassie brilliant blue (100 mg), 85% Phosphoric acid (100 ml) 95% Ethanol (50 ml) and then addition of distilled pure water to above component to made a bulk to 1000 ml. Lastly, take 100 µl the plant extract and in addition 2 ml of Bradford reagent in each test tube and hold on for 300 sec. Then take down the optical density at 595 nm in double-beam UV-Visible spectrophotometer (Metash-Model UV-9000).

Activities of Antioxidant Enzymes

Fresh leaves (0.5 g) taken from each pot for determining activities of antioxidant enzymes. Leaves finely smashed in 10 ml of 50 mM buffer of phosphate having a pH 7.8 on an icing bath. This homogenate specimen was then transferred to labeled conical tubes and centrifuged 6000 × g for 20 min at 4°C. The resulting supernatant was expended and stored for further evaluating the activities of mentioned antioxidant enzymes:

Catalase (CAT) Activity:

Catalase and Peroxidase activity were determined by (Chance & Maehly, 1955) method with minor alterations. Their action intent on protein content. The CAT reaction infusion (3000 µl) contained:

- 1000 µl of 50 mM buffer (phosphate) having pH 7.0.
- 1900 µl of 5.9 mM H_2O_2
- Enzyme extract (100 µl)

Add enzyme infusion at the end in cuvette, so the chemical reaction was initiated. Using a double-beam UV-Visible spectrophotometer (Metash-Model UV-9000), periodic changeover in OD of the chemical reaction (solution) in cuvette were recorded at 240 nm in each 30 sec (starting from 0 sec to 120 sec). CAT one unit action was interpreted as an (OD) absorbance alterations of 0.01 units per min.

Ascorbate Peroxidase (APX) Activity:

Ascorbate peroxidase activity (APX) occurrence were analyzed (Nakano & Asada, 1981) methodology with little bit amendments.

The reaction chemical solution for APX was 3 ml. Total 3 reagents were needed for estimating APX activity:

i. 300 mM H_2O_2
ii. 7.5 mM ASA (ascorbic acid)
iii. 50 mM buffer (Phosphate)

Take 100 µl enzyme extract and add 0.1 ml ascorbic acid and 2.7 ml phosphate buffer in a labeled test tube. At the end, when all solution were in cuvette, add 0.1 ml H_2O_2 through micropipette and rapidly reading were noted at 290 nm by double-beam UV-Visible spectrophotometer (Metash-Model UV-9000). The absorbance were alleviates due to oxidation of ascorbate (from 0 sec to 120 sec). The activity constant for APX is E = 2.8 mM/cm.

Peroxidase (POD) Activity

For POD activity, reaction solution of 2 ml was used in cuvette, and it contained:

- 700 µl of 50 mM buffer orthophosphate (pH 7.0),
- 600 µl of both 20 mM methylcatechol and 40 mM H_2O_2
- Enzyme essence (100 µl)

Reaction was commenced by placing 0.6 mL of H_2O_2 in a cuvette ahead all other components described above. Using a double-beam UV-Visible spectrophotometer (Metash-Model UV-9000), swap in OD of the solution at 470 nm were assessed each and every 30 seconds (from 0 sec to 150 sec). POD one unit action was interpreted as an absorbance alterations of 0.01 constituent per min. For blank of CAT, POD, all reaction combination were taken except enzyme extract.

Determination of H_2O_2

For the specification of H_2O_2 level in plant sample, strategy of (Velikova *et al.,* 2000) was followed. Weigh 0.25 g of fresh leaves and grounded with 5000 µl of 0.1% TCA (w/v) trichloroacetic acid in refrigerated pestle and mortar. TCA of 0.1% contained following components:

- 1000 ml distilled water
- 10 g TCA

This solution was then centrifuged (HERMLE Z 326 K) at 12000 × 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 intermixture was vortexed for 5 seconds and its (OD) absorbance was calculated at 390 nm using double-beam UV-Visible spectrophotometer (Metash-Model UV-9000).

Evaluation of free Proline Quantity

For the evaluation of proline quantity the method of (Bates *et al.,* 1973) was followed. Newly picked leaves 0.25 g weight from each sample taken and grinded in 10 ml of three per centum of sulfide-salicylic acid. These samples, then filter with Whatman Grade 42 filter sheet. The clear filtrate (2000 µl) was assorted with 2ml of acid ninhydrin i.e., prepared by mixing 1250 mg of ninhydrin in 30 ml glacial acetic acid and 6 M (20 ml) orthophosphoric acid and 2000 µl of CH_3COOH in a test glass tube. This composition was oven incubated for 100 °C for about one hour. The sample was moderated in a chilled bath. Then, addition of toluene (4000 µl) in each sample and vortex for 10-15 sec by 1-2 min of continuous air passing stream. The superior stratum having toluol was taken from aqueous part by glass pipette and warm up at ambient temperature. The OD was calculated at 520 nm on a double-beam (Metash-Model UV-9000) UV-Visible spectrophotometer providing toluol as a blank.

The proline fraction was worked out from a well-established curve as mentioned below:

µmoles proline/g of fresh weight material: [(µg proline/ml) x µl toluol) /115.5] / (g specimen).

Total Phenolic Content Establishment

Overall phenolic proportion were figured by using approach of (Julkunen-Tiitto, 1985). Fresh leaves from each 2 replicates, weighing 50 mg were macerated with 5 ml of 80% dimethyl ketone solution with mortar & pestle. Homogenized was centrifuged at 10,000 × g for 600 sec in cold (4°C). Take out 0.1 ml of clear supernatant and was diluted with 2000 µl distilled purified water and 1000 µl of Folin-Ciocalteau's phenol reagent and shaken strongly. Subsequently, added 5 ml of 20% Na_2CO_3 and the total magnitude were made up to 10,000 µl with distilled pure water. The mixture were swirled and the optical density (OD) read at 750 nm utilizing a double-beam UV-Visible spectrophotometer (Metash-Model UV-9000). The findings were indicated as mg /kg of fresh leaf.

Yield Parameters

Number of Grains per Spike

Randomly, choose three spikes from different plants of same pots for counted the seeds per ear. Every single spike was detached from the barley plant and thrashed its seeds manually and were counted. Their mean value was taken for each plant.

Yield of Grain per Plant (100-Seed Weight)

Total 100 threshed kernels, were selected for grain yield per plant. The weight measured on electronic balance. Hundred seeds of individual replicate considered as grain yield per plant. The seeds were stored in concealed plastic bags for later use.

Statistical Analysis

CoStat statistical program were utilized for ANOVA (analysis of variance) in results for all features studied (CoHort software's 1988, Monterey, California, USA). Version used was 6.303. LSD test were applied to compare the mean values. The graphs in results were analyzed in Microsoft excel (2013).

Results and Discussion

Application of drought decreases shoot fresh and shoot dry weight in Sultan-17 and Jau-17 from control. Under drought stress, all treatments increased shoot fresh and shoot dry weight compared to drought plants. There is reduction in dry and fresh weight of shoot under drought situation as compared to drought control plants and this likely due to the lowering of osmosis through the soil.

As an outcome, mitosis and elongation decreased, hence plant growth of barley reduced. This result related with findings of (Abdelaal *et al.*, 2017; Esmail *et al.*, 2019) in corn and wheat crop respectively. Jau-17 variety had high shoot fresh and dry weight as compared to Sultan-17 variety. 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.*, 2016b) and in sunflower (Razzaq *et al.*, 2017). Spray of humic acid increases shoot fresh weight under drought application and non-drought stress as compared to their respective control and increases tolerance to droughts stress, noted by (Bijanzadeh *et al.*, 2019; Moghadam *et al.*, 2014).

Foliar application of KH_2PO_4 elevated dried and fresh shoot mass under moisten deficit state because of K^+ ions presence. Comparable outcomes was accounted by (Abdelaal *et al.*, 2018). Melatonin exogenously applied 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 dried mass and fresh mass root and shoot lengthiness under normal or drought conditions. These consequences are in agreement with findings of (Li *et al.,* 2018) in rapeseed; (Sadak & Bakry, 2020) and in flax plant (Ahmad *et al.,* 2019).

Root fresh and dry weight reduced in both varieties i.e., Sultan-17 and Jau-17 from control. Under drought and non-drought conditions, humic acid, potassium dihydrogen phosphate and melatonin elevated root fresh and dry weight in Sultan-17 and Jau-17. Humic acid showed highest root fresh and dry weight in Sultan-17. While melatonin showed highest in Jau-17 compared to other treatments under non-drought conditions. Melatonin application stimulated lateral root growth under moisture stress as determined by (Dai *et al.,* 2020).

Drought application reduced leaf area in Sultan-17 and Jau-17 from control. Humic acid, potassium dihydrogen phosphate and melatonin increase leaves area under drought stress in Sultan-17 and Jau-17 from drought control. Potassium dihydrogen phosphate showed largest leaf area under drought stress in each variety i.e., Sultan-17 and Jau-17. Drought stress were caused disorders in all growth parameters of *Hordeum vulgare* L. (barley) that accordance with previous report of (Abdelaal *et al.*, 2020; Abdelaal *et al.*, 2018) in barley and (El-Sabagh *et al.*, 2017; Elewa *et al.*, 2017; Sadak, 2016) in various other plants. Humic acid application increases all growth characters including dried and fresh weight of shoot in both varieties, similar findings obtained by (Roozbahani, 2015) in barley and (Al-Fraihat *et al.,* 2018) in onion. Potassium upraised all morphological characters under drought stress as it maintain water cellular balance, reported in sugar corn (Bijanzadeh *et al.*, 2019; Rao *et al.*, 2012). Excessive K^+ application has been demonstrated to enhance growth parameters, photosynthesis and maximizes yield under drought and non-drought stress, previously reported in tobacco plants (Bahrami-Rad & Hajiboland, 2017).

Chlorophyll a, b, total chl and carotenoids reduced under drought in Sultan-17 as compared in Jau-17 where it reduced from control. Drought application also declined water content and in both varieties. Under drought conditions, humic acid, potassium dihydrogen phosphate and melatonin were elevated Chlorophyll a, b, total chl and carotenoids in Sultan-17 and Jau-17 from drought plants. Humic acid showed highest Chlorophyll a, b, total chl and carotenoids content in Jau-17 as compared to other treatments under drought conditions. Water deficit resulted in stress reflected in statistically notable decreases in chlorophyll a & b, beta carotenoids and RWC in plant of barley, these results supported by (Abdelaal *et al.*, 2020; Goodarzian *et al.*, 2015; Jaleel *et al.*, 2009).

Water content in leaves is status mark for water in plants 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 cereals photosynthetic capability as its quantity decreased under drought, plant normal growth disturbs. Jau-17 relatively huge chlorophyll value, relative H_2O proportion leading to better grain yield under drought stress as compared to Sultan-17. Different genotypes i.e.Sultan-17 and Jau-17 were difference in their relative chlorophyll and water content proved by findings of (Dai *et al.*, 2020; El-Shawy *et al.*, 2017). One variety have high-level of relative water content i.e. Jau-17 was higher relative water content than Sultan-17, similar results supported by (Rampino *et al.*, 2006; Tounekti *et al.*, 2018). There is relatively less decrease and stability in chlorophyll under drought stress in Jau-17 and possibly an indication of drought tolerance and proved by the past report (Sakya *et al.*, 2018).

HA notably elevate chl a & b, carotenoids, and total chl in both two barley varieties under application of drought stress with respect to drought control, previous researched by (Abdelaal *et al.*, 2018; El-Bassiouny *et al.*, 2014; Shen *et al.*, 2020). HA and potassium significantly increase RWC under drought stress application as determined by (Shen *et al.,* 2020) and (Zahoor *et al.*, 2017) respectively. KH_2PO_4 application lifted photosynthesis process and as a result carbohydrate content improved in both varieties under water deficit stress conditions (Mahmoud & Youssif, 2015; Marschner, 2012). Melatonin raise the values of both chl b and chl a in barley under drought state and were higher than control drought and previously reported by (Ahmad *et al.*, 2019; Cao *et al.*, 2019; Liang *et al.*, 2019). Melatonin improved chlorophyll in both varieties under normal and stressed conditions, noted by (Sadak & Bakry, 2020).

Relative membrane permeability, MDA and H_2O_2 content raises in Sultan-17 and Jau-17 under drought stress from control. In both varieties Sultan-17 and Jau-17, humic acid, potassium dihydrogen phosphate and melatonin reduce MDA and H_2O_2 content, RMP under drought stress from drought control. Humic acid exhibited least RMP in Jau-17 while it showed least MDA and H_2O_2 production in both varieties under drought stress.

Results demonstrated that under drought stress, MDA, relative membrane permeability and H_2O_2 magnified in both varieties and were superior than barley control plants and other applied osmo-regulators, supported by findings of (Abdelaal *et al.*, 2018; Bijanzadeh *et al.*, 2019; Mihaljević *et al.*, 2021). 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 variety. Low MDA accompanied with low membrane leakage were associated to induce drought stress tolerance in barley. The lower H_2O_2 production with low MDA content in drought stress perhaps due to the triggering of antioxidant enzyme activities especially CAT which minimizes H_2O_2 accumulation. Similar outcomes reported by (Outoukarte *et al.*, 2019; Umar & Siddiqui, 2018).

Treatment of Humic acid alleviates the MDA content and relative membrane permeability in both varieties under drought and non-drought conditions, observed by (Shen *et al.,* 2020). Potassium dihydrogen phosphate and humic acid improving plasmalemma stability and reduction in MDA concentration and membrane permeability, founded by (Abdelaal *et al.*, 2018; Aydin *et al.*, 2012). Melatonin spray remarkably declined the MDA and H_2O_2 content, proved by the experiments of (Kabiri *et al.*, 2018; Liang *et al.*, 2019). This declined might be due to action of antioxidant enzymes.

Proline elevates while protein and phenolic concentration alleviate in Sultan-17 and Jau-17 from control under drought stress. Under drought stress, humic acid, potassium dihydrogen phosphate and melatonin enhance proline concentration, protein and phenolics from drought control. Under drought stress, humic shows highest total soluble proteins and phenolic content in Sultan-17 while melatonin in Jau-17. Proline particularly marked indication for drought tolerance as its level increases in plants and were reported by (Du *et al.*, 2023). Under drought, proline accumulation found by (Sallam *et al.,* 2019) in cereals. An increase in proline concentration were analyzed during research under application of drought in both varieties compared to control and previously reported by (Fayez & Bazaid, 2014; Habib, 2020).

Drought stress caused remarkably decreased in protein content in barley, were seen by (Liang *et al.*, 2019; Pazirandeh *et al.*, 2013). Protein and proline concentration was higher in one variety than other and they are more in the Jau-17 were supported by (Anjum *et al.*, 2016a; Maghsoudi *et al.*, 2019). One variety (Jau-17) was higher phenolic contents as compared to other variety (Sultan-17) and were previously reviewed the variety difference of phenolic content (Outoukarte *et al.*, 2019; Sallam *et al.*, 2019). Barley plants with better photosynthetic capacity had an extent level of total phenolics and were supported by (Vicas *et al.*, 2019).

Protein and proline concentration were enhanced up under the exogenous osmo-regulators and drought application. The results concluded that humic application under drought stress promoted proline concentration and may oppose the negative effect of drought stress were aggress with results of (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. Potassium application triggered accumulation of proline in both varieties and to conserve tissue water were interpreted by (Ahanger *et al.,* 2017). K^+ ions increased phenolics in drought state as contrast to control were disclosed by (Fayez & Bazaid, 2014) that used KNO_3 in experiment. Melatonin treatment further boosted proline and soluble protein content under moisture stress. It suggested that melatonin could repress the breakdown of protein and enhance production of new proteins were earlier informed by (Ahmad *et al.*, 2019; Liang *et al.*, 2019). MT application increases phenolic proportion under drought state as contrast to control were determined by (Sadak & Bakry, 2020; Tan *et al.*, 2012).

An increment in all antioxidant enzymes (CAT, APX, POD) were observed under drought application stress in both barley varieties with respect to control in this study. Under drought stress, humic acid, potassium dihydrogen phosphate and melatonin incrementing peroxidase, catalase and APX levels respectively in Sultan-17 and Jau-17. Humic acid revealed highest catalase value in Jau-17 and melatonin in Sultan-17 under drought stress. Comparable results founds that antioxidants elevated (Cao *et al.*, 2019; Maghsoudi *et al.*, 2019; Yasmeen *et al.*, 2013) in different plants under drought conditions.

Antioxidant enzymes production demonstrate to have lessen the harmful impacts of drought application stress and a probably a well-adaptive mechanism in barley. Drought stress amplifies the ROS content in barley especially H_2O_2 activity which counteract by the CAT production and previously reported by (Sadak & Bakry, 2020). Tolerant barley plants were high activity of POD, assessed by (Outoukarte *et al.*, 2019; Sallam *et al.*, 2019; Shen *et al.*, 2020). CAT and APX is much stronger in Jau-17 barley plant as compared to Sultan-17 and that is similar to results of (Goodarzian *et al.*, 2015; Laxa *et al.*, 2019).

Present studied revealed the foliar misting of humic acid could amplifies antioxidant enzymes production in both varieties under drought conditions, observed by (Shen *et al.,* 2020). Exogenous potassium dihydrogen phosphate induced upregulation of these ROS- scavenging enzymes proved by finding of (Bharti & Barnawal, 2019). An increment in an enzymes i.e. (antioxidant) like POD (peroxidase) and (catalase) CAT, by exogenously applied of potassium dihydrogen phosphate and humic acid under drought stress conditions as supported by, earlier reported by (Abdelaal *et al.,* 2018). Melatonin enhanced catalase (CAT) activity of drought plants (Dai *et al.,* 2020). Exogenous applied melatonin in a drought stress, the antioxidant enzymes were elevated, founded by (Ahmad *et al.*, 2019; Cao *et al.*, 2019; Li *et al.*, 2018).

Application of drought decreases ionic concentration (shoot and root) i.e., potassium and calcium content in Sultan-17 and Jau-17 from control. All treatments (humic acid, potassium dihydrogen phosphate and melatonin) increase potassium content from drought control in Jau-17. Under drought conditions, humic acid, potassium dihydrogen phosphate and melatonin increase potassium and calcium ions in Sultan-17 and in Jau-17 from control. Application of potassium dihydrogen phosphate showed maximum accumulation of potassium ions in root and shoot under normal and drought conditions. Application of humic acid showed maximum accumulation of calcium ions in roots under normal and drought conditions.

Drought stressed barley plants were decreased ions contents especially K^+ contents were earlier monitored by (Abdelaal *et al.*, 2018; Fayez & Bazaid, 2014). The levels of N-P-K were lesser in drought-stressed plants in both varieties. Foliar spray of all osmo-regulators enhances the ions content (potassium and calcium) in plant under drought and non-drought conditions in both varieties. Humic acid and potassium dihydrogen phosphate increased K^+ contents in barley plants compared to drought state plants (Abdelaal et al., 2018). Application of potassium dihydrogen phosphate markedly increase in K^+ contents that were subjected to drought stress were related to the results given by (Fayez & Bazaid, 2014; Zahoor *et al.*, 2017). Under drought and non-drought conditions, it is notes that ionic contents elevate after the application of humic acid as contrasted to control plants in both varieties (El-Bassiouny *et al.,* 2014).

All yield attributes (spike length, no. of spikes, no. of grain per spike and one hundred grain weight) reduced under drought stress in Sultan-17 and Jau-17 from control. Under drought stress, Sultan-17 and Jau-17, all treatments i.e., humic acid, potassium dihydrogen phosphate and melatonin elevate the no. of grain per spike and 100 grain weight from drought control. Humic acid and potassium dihydrogen phosphate showed same results under drought as well as non-drought in Sultan-17. Under drought stress, melatonin and humic acid displayed greatest no. of grains per spike in Sultan-17 and Jau-17 respectively. Melatonin indicated maximum 100-grain weight under drought and non-drought state in Sultan-17 whereas humic acid showed maximum 100-grain weight under drought and non-drought state in Jau-17.

Evaluation of present data display that grain yield of barley under drought stress affected severely. The high grain yield is linked with high no. of grains per spike, water and chlorophyll content, earlier monitored by (Sallam *et al.,* 2019) in wheat. Under drought stress, barley productively greatly reduced due to no. of grains decreased were supported by (Abdelaal et al., 2020). The yield elements like spike length, spikes quantity per plant, grains amount per spike and one hundred seed weight were deceased under drought condition in both varieties. Comparable outcomes were accounted by (Abdelaal *et al.*, 2018; Habib, 2020; Sadak & Bakry, 2020). No. of grains and 100 grain weight were higher in non-drought conditions in both varieties. Recent studies revealed that the yield elements (100-grain weight, spike length in both varieties remarkably enhanced with exogenously applied humic acid. Same results determined by (El-Bassiouny *et al.*, 2014; Roozbahani, 2015). Elevated 100 seed weight and no. of seeds per spike were observed with foliar application of potassium dihydrogen phosphate were agreed by (El-Abady *et al.*, 2009; Zareian *et al.*, 2014). Practicing of humic acid and potassium fertilizer increases the yield of barley plant, were seen by (El-Sheshtawy *et al.*, 2019).

Conclusion

It has been revealed from current work that drought reduces the fresh and dry weight of shoot & root, leaf area, relative water content, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Drought raised the concentration of H_2O_2, MDA, and relative permeability while the application of osmo-regulators alleviated their concentrations. Both varieties i.e. Sultan-17 and Jau-17 affected by drought stress of barley plant (*Hordeum* *vulgare* L.). Although the exogenous application of different osmo-regulators i.e. humic acid, potassium dihydrogen phosphate and melatonin significantly alleviated the negative effects of drought stress in barley, however, the notable effects were observed when plants were sprayed by humic acid. Overall performance of Jau-17 variety interpreted that it may be the better choice of variety under drought stress and further foliar application enhances the features under drought and normal conditions.

Recommendations for future work:

Potassium dihydrogen phosphate increases 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 effectiveness of these interpretations. The trials at different concentrations of various osmo-regulators may be conducted to be commonly use on commercial scale.

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Table 1: Different features of the experimental soil that 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

Table 2: Details of the osmo-regulators applied during experimentation in barley (*Hordeum vulgare* L.) with two different varieties and two water levels.

Sr. no	Name of Osmo-regulators	Abbreviation	Concentration used
1	Humic acid	HA	400 ppm or mgL^{-1}
2	Potassium dihydrogen phosphate	KH_2PO_4	20 ppm or mgL^{-1}
3	Melatonin	MT	0.5 mM

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

Source of variation	df	Shoot fr. Wt.	Shoot dry wt.	Root fr. Wt.	Root dry wt.	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**	**RCW**	**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***	11220.108***
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
		Total soluble proteins	**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

Osmoregulators (HA, KH_2PO_4, MT) under drought and non-drought conditions.

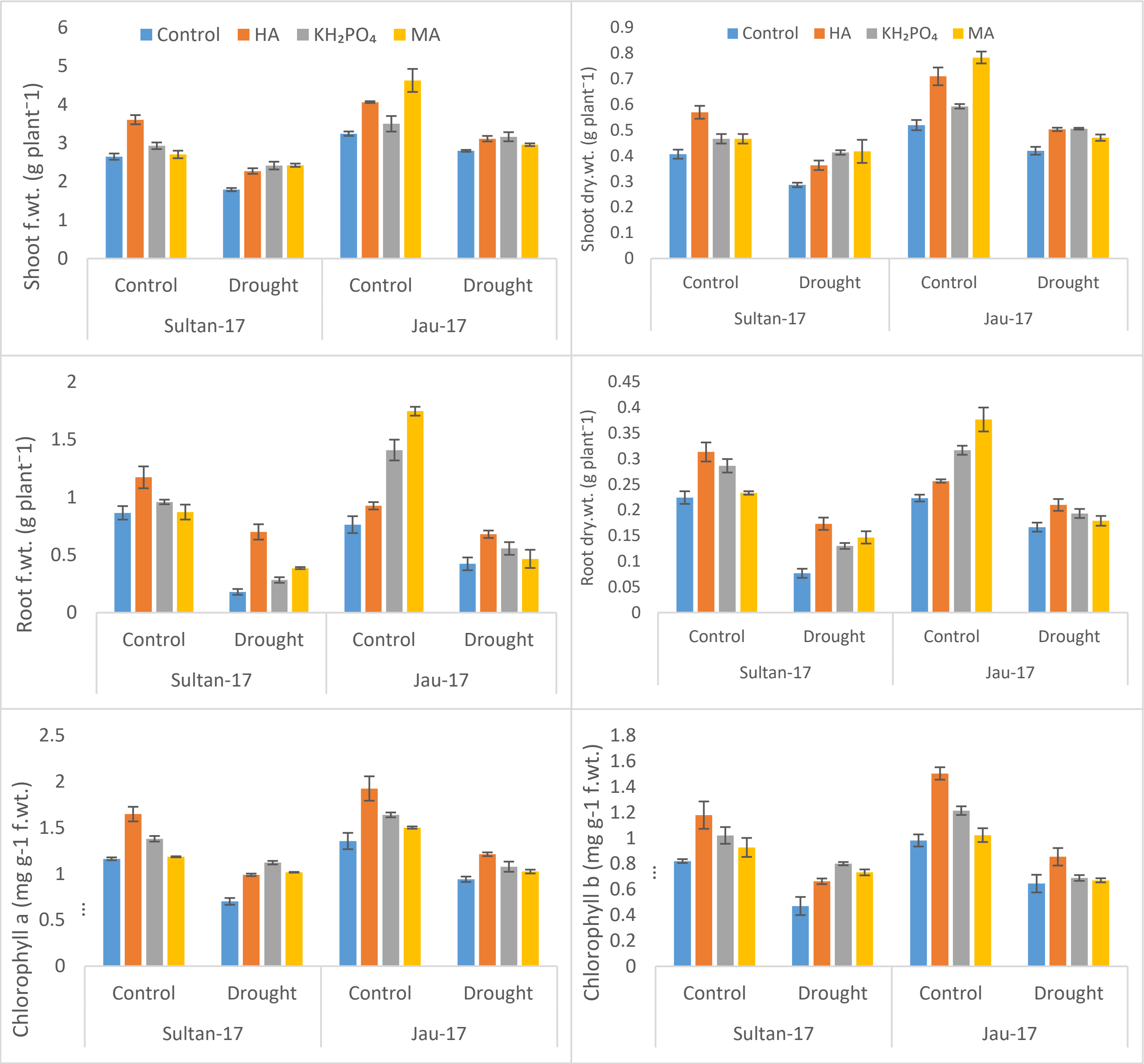

Fig. 1 Shoot and root fresh and dry weights and chlorophyll ‘a’ and ‘b’ of fifty-one days old barley (*Hordeum vulgare* L.) with the foliar application of three different osmoregulators (HA, KH_2PO_4, MT) under drought and non-drought conditions.

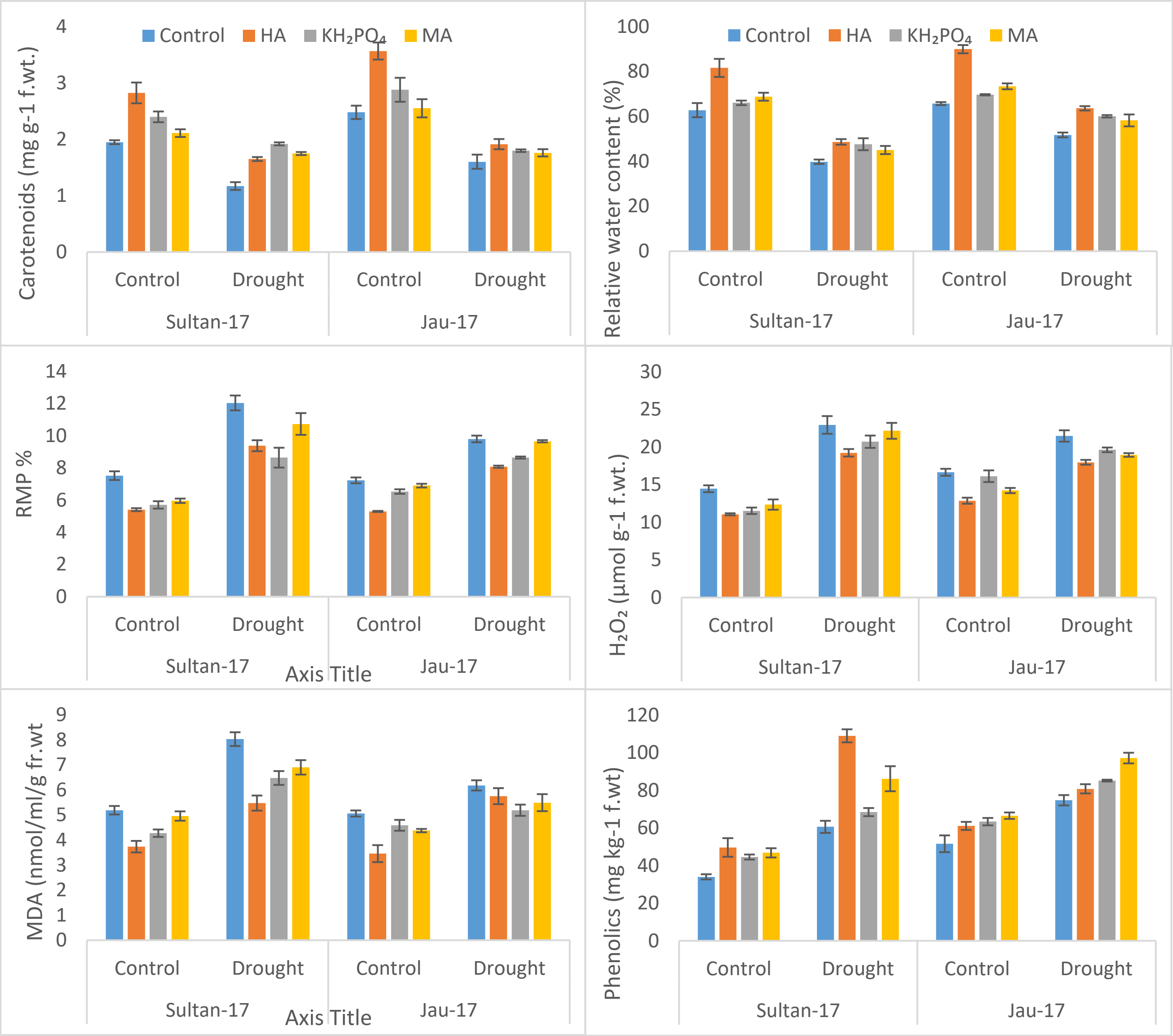

Fig. 2 Carotenoid contents, relative membrane permeability, relative water content, H2O2, malondialdehyde contents and total phenolics of fifty-one days old barley (*Hordeum vulgare* L.) with the foliar application of three different osmoregulators (HA, KH_2PO_4, MT) under drought and non-drought conditions.

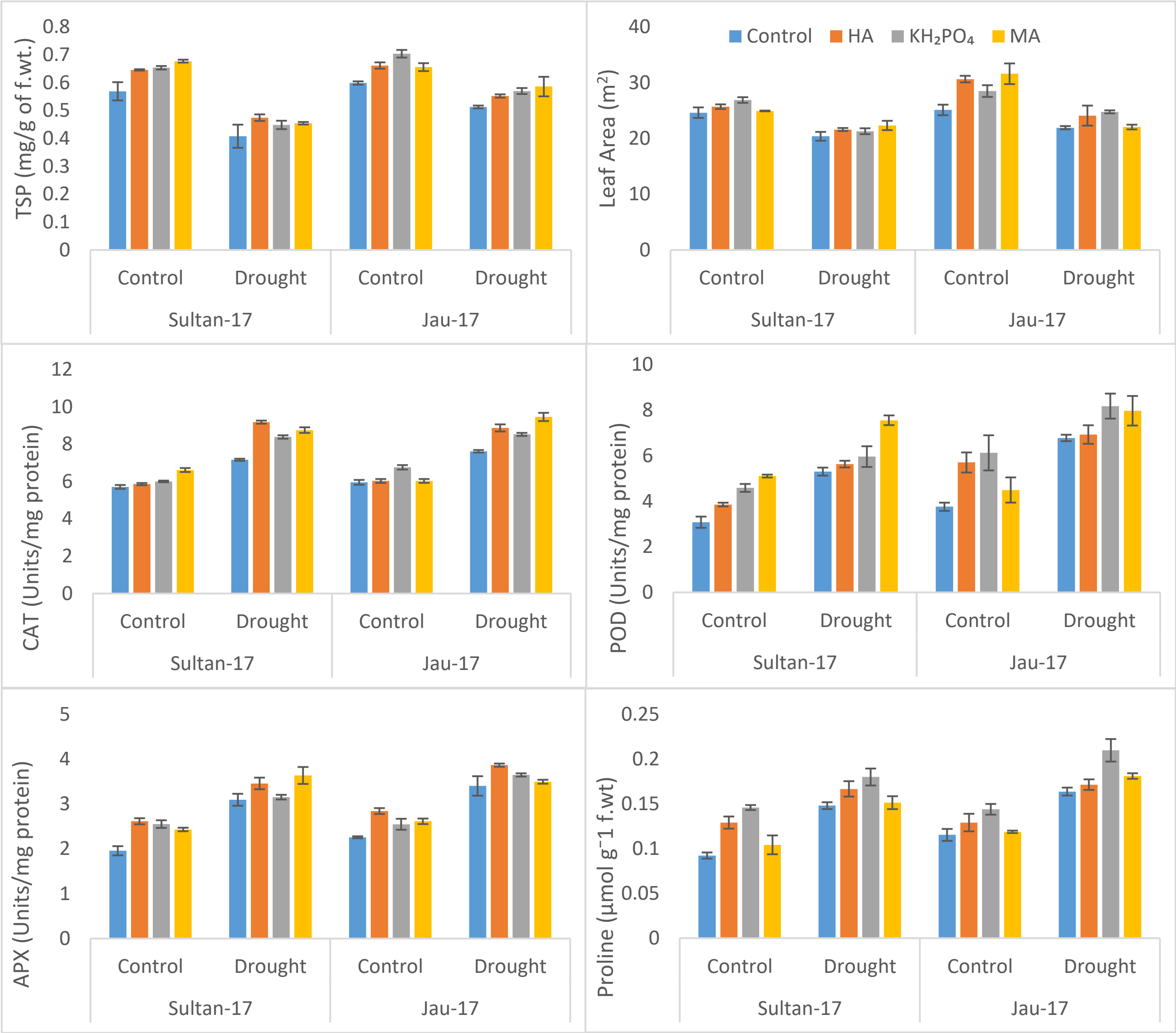

Fig. 3 Total soluble proteins, total leaf area, catalase, peroxidase, ascorbate peroxidase activities and free proline of fifty-one days old barley (*Hordeum vulgare* L.) with the foliar application of three different osmoregulators (HA, KH_2PO_4, MT) under drought and non-drought conditions.

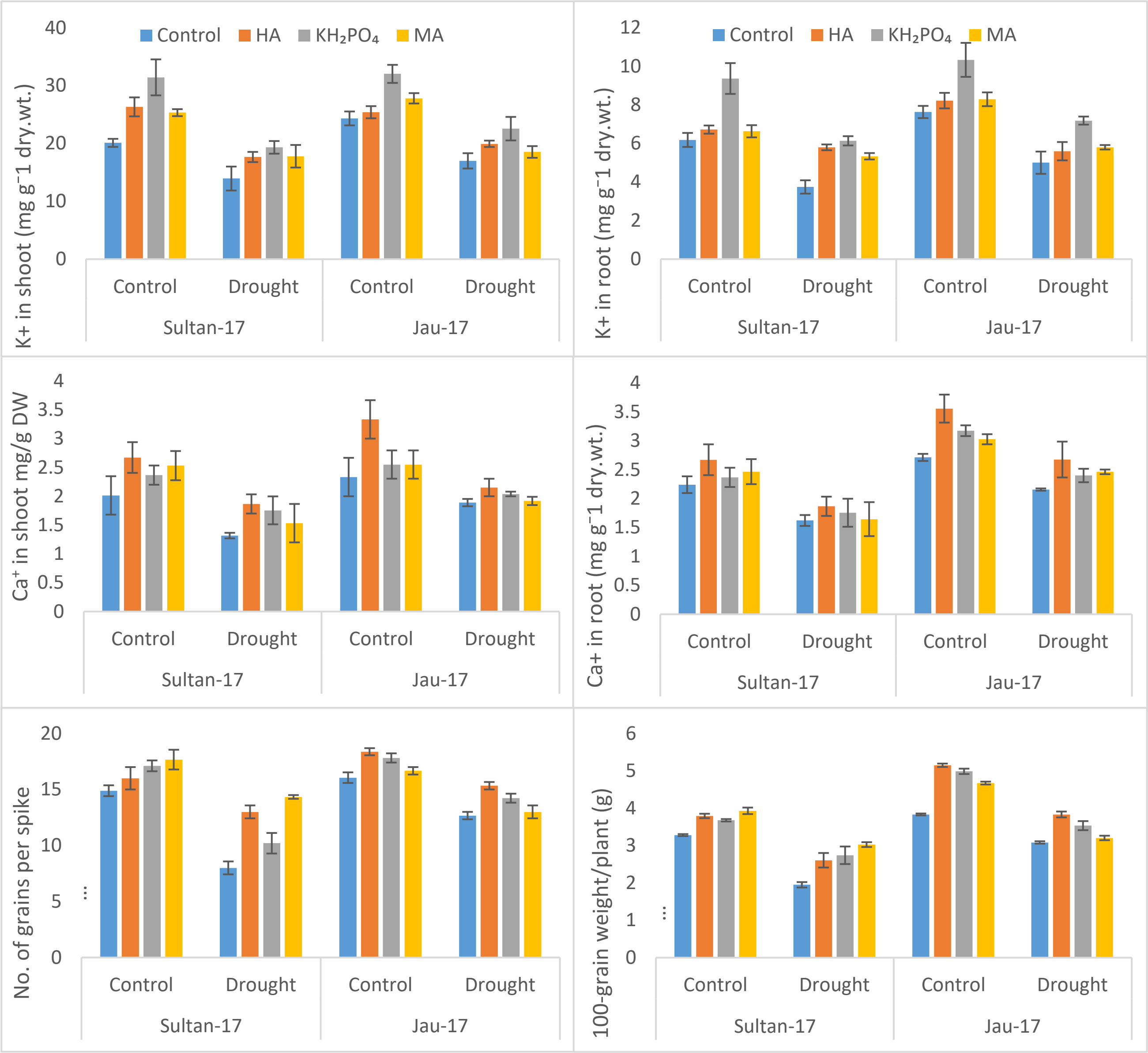

Fig. 4 Shoot and root concentrations of K^+, Ca^{2+} mineral contents, no. of grains per spike and 100-grain weight of fifty-one days old barley (*Hordeum vulgare* L.) with the foliar application of three different osmoregulators (HA, KH_2PO_4, MT) under drought and non-drought conditions.

Supplementary Files

- GraphicalAbstract.docx