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Melatonin as a Foliar Application and Adaptation in Lentil (*Lens culinaris* Medik.) Crops under Drought Stress

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Abstract: Here, we grow two different varieties of lentil (lentil-2009 and lentil-93) under different drought levels and with different applications of melatonin. Increasing the levels of soil water deficit significantly decreased numerous morphological and biochemical characteristics, including shoot length, total chlorophyll content, and transpiration rate, in both varieties of lentil. Contrastingly, drought stress increased the concentrations of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), and electrolyte leakage, an indicator of oxidative damage to membrane-bound organelles. The activities of enzymatic antioxidants and osmolytes were initially increased up to a drought level of 80% water field capacity (WFC) but gradually decreased with higher levels of drought stress (60% WFC) in the soil. At the same time, the results also showed that the lentil-2009 is more tolerant to drought stress than lentil-93. The negative impact of drought stress can be overcome by the application of melatonin. Melatonin increased plant growth and biomass, photosynthetic pigments, gas exchange characteristics, and enhanced the activities of various enzymatic and non-enzymatic antioxidants and proline content by decreasing oxidative stress. We conclude that foliar application of melatonin offers new possibilities for promoting lentil drought tolerance.

Keywords: melatonin; drought stress; legume family; oxidative stress; proline

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1. Introduction

Environmental variations due to abiotic stresses, such as drought, heat, cold, and salinity, adversely affect and limit agricultural productivity in developing countries, including Pakistan [1,2]. About 33% of the world's agricultural land is facing water imbalance and promoting drought vulnerability, which may drastically decrease the growth and yield of plants [3–5]. Abiotic stresses, such as drought, can lead to alterations in plant growth and composition and a decrease in growth-related attributes, affecting photosynthetic machinery, which ultimately causes a reduction in the dry biomass of the plant as it is unable to accumulate essential nutrients from the soil [6–8]. In addition, plants are typically exposed to a myriad of biotic and abiotic stresses, including feeding from wild animals and insects, weed infestation, mechanical injury, diseases, low soil fertility, drought, salinity, and others, that can diminish the plant photosynthetic area, and thus the attained total plant biomass or grain yield [9,10]. Water deficiency-induced impairment in photosynthesis is attributed to damage of thylakoid membranes in chloroplasts because the lipid contents of cell membranes are susceptible to the reactive oxygen species (ROS) produced as a

consequence of drought [11]. Stress conditions can disturb the dynamic equilibrium of ROS production, which promotes oxidative stress, membrane lipid peroxidation, and disrupts the structure and function of the cell membrane system [12–14]. The rate of photosynthesis decreases in many fodder grasses under drought stress, for example in *Festuca pratensis*, *Lolium perenne*, *Dactylis glomerata*, *Phleum pretense*, and *Arrhenatherum elatius* [15]. Activation of plant stress defensive mechanisms is important for survival.

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous bio-stimulating molecule, whose potential roles in plant growth, development, and stress responses have been progressively investigated in recent studies [16,17]. In plants, melatonin is involved in refining physiological processes such as photosynthesis, senescence, and reproduction [18,19]. Under stress conditions, melatonin mainly functions as a promoter of plant tolerance, and reduces oxidative damages by enhancing the antioxidant defense capacity of organelles, maintaining redox homeostasis [20,21]. It has been reported that exogenous applications of melatonin enhanced plant tolerance by providing protection against abiotic and biotic stresses such as drought stress [22–25]. Lentils (*Lens culinaris* Medik.) are a major cool seasonal food crop in India and the second most important winter-season legume after chickpea (*Cicer arietinum* L.) [26]. Lentils require low temperatures during vegetative growth, while at maturity, warm temperatures are required. The 'optimum' temperature for its best growth has been reported to be 18–30 °C [27]. Of the abiotic stresses experienced by lentils worldwide, drought and heat stress are considered the most important [28]. The susceptibility of lentils to hot and semiarid regions is supported by many studies [29–31]. The objective of the present study is to demonstrate the effect of drought stress, drought tolerance mechanisms, and management measures using melatonin application, for the alleviation of drought stress in lentil varieties. For this purpose, we designed a pot experiment using two varieties of lentil (lentil-2009 and lentil-93) to study: (i) the role of exogenous application of melatonin on growth and biomass, (ii) oxidative stress and antioxidant responses, and (iii) lentil sugar and osmolyte content in the drought-stressed environment. The results suggest that melatonin application may improve plant yield under drought-stressed conditions.

2. Materials and Methods

2.1. Seed Collection and Experimental Setup

Fresh and mature lentil seeds (*Lens culinaris* Medik.) named lentil-2009 and lentil-93 were collected from the Ayub Agriculture Research Institute (AARI) in Faisalabad, Pakistan. Both varieties were surface-sterilized with 0.1% bleaching powder for 10–20 min and washed gently with deionized water and sown in plastic pots (25 × 35 cm^2). The experiment was conducted at the Department of Botany, Government College University, Faisalabad, Pakistan (coordinates: 31.4162° N, 73.0699° E; elevation m a.s.l.: 186). The seedlings that emerged were thinned to maintain four almost uniform size seedlings per pot, and three pots were used for each treatment. After 21 days of seed germination, drought stress treatments, including control (100% water field capacity (WFC)) and drought-stressed (80% and 60% WFC), were initiated. Water levels were checked and maintained twice a week by weighing and adjusting the moisture level of the pots. After 30 days of drought stress, two levels of melatonin—control (no spray) and 3 mM—were applied as a foliar spray to stressed and non-stressed plants. Each pot was filled with 0.5 kg of sandy loam soil and five seeds were sown per pot. After one week of sowing, germination started. All plants in the glass house territory received natural light, with a day/night temperature of 35/40 °C and day/night humidity of 60/70%. The experiment was arranged in a completely randomized design (CRD) with three replications of each treatment.

2.2. Morphological Traits and Data Collection

After four weeks of foliar-applied melatonin, plant samples were collected from each replicate, and root and shoot fresh weight were measured separately using an electrical balance after harvest. Analyses of different biological parameters were performed at

Government College University, Pakistan. Shoot length was defined as the length of the plant from the surface growth medium line of the pot to the tip of the uppermost shoot, and root length was also measured. Shoot fresh weight was measured with the help of a digital weighing balance and root fresh weight was also measured. After that, plant samples were oven-dried for 1 h at 105 °C, then 65 °C for 72 h until the weight became uniform, and dry biomass was recorded. Roots were washed with distilled water and dipped in 20 mM of Na_2EDTA for 15–20 min, washed thrice with distilled water, and finally with deionized water, and then oven-dried for further analysis. The leaf in each treatment was picked at a rapid growth stage during 09:00–10:30 a.m. The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen, and stored in a freezer at a low temperature (−80 °C) for further analysis.

2.3. Determination of Photosynthetic Pigments

Leaves were collected for determination of their chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of Arnon [32].

Gas exchange parameters were also measured during the same days. Net photosynthesis (P*n*), leaf stomatal conductance (G*s*), transpiration rate (T*s*), and intercellular carbon dioxide concentration (C*i*) were measured from three different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf P*n*, G*s*, T*s*, and C*i* were measured with a LI-COR gas exchange system (LI6400; LI-COR Biosciences, Lincoln, NE, USA) with a red–blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 mmol mol^{-1} and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which is the average saturation intensity for photosynthesis in lentils [33].

2.4. Determination of Oxidative Stress Indicators

The degree of lipid peroxidation was evaluated by measuring malondialdehyde (MDA) content. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000× *g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, CA, USA) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532–A600)–0.56 A450. Lipid peroxidation was measured by using a method previously published by Heath and Packer [34].

To estimate the H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (*v*/*v*) H_2SO_4 and centrifuged at 6000× *g* for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by an extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured using the method presented by Jana and Choudhuri [35].

Stress-induced electrolyte leakage (EL) of the uppermost stretched leaves was determined by using the methodology of Dionisio-Sese and Tobita [36]. The leaves were cut into minor slices (5 mm length) and placed in test tubes containing 8 mL of distilled water. These tubes were incubated and transferred into a water bath for 2 h prior to measuring the initial electrical conductivity (EC_1). The samples were autoclaved at 121 °C for 20 min, and then cooled down to 25 °C before measuring the final electrical conductivity (EC_2). Electrolyte leakage was calculated by the following formula:

$$EL = (EC_1/EC_2) \times 100$$

2.5. Determination of Antioxidant Enzyme Activities

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol ethylenediaminetetraacetic acid (EDTA) and 0.15 mol NaCl. The homogenate was centrifuged at 12,000× *g* for 10 min at 4 °C, and the supernatant was used for the measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in a 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 µL enzyme extract. Finally, the sample was measured with a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, CA, USA). Enzyme activity was measured using the method of Chen and Pan [37] and expressed as U g^{-1} FW.

POD activity in the leaves was estimated using the method of Sakharov and Ardila [38] by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation were recorded for 2 min.

Catalase (CAT) activity was analyzed according to Aebi [39]. The assay mixture (3.0 mL) was comprised of 100 µL enzyme extract, 100 µL H_2O_2 (300 mM) and 2.8 mL 50 mM phosphate buffer, with 2 mM ETDA (pH 7.0). CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss (ε = 39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada [40]. The mixture containing 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H_2O_2 (300 mM), and 2.7 mL of 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm (ε = 2.8 mM^{-1} cm^{-1}).

2.6. Determination of Non-Enzymatic Antioxidant and Proline

Plant ethanol extracts were prepared for the determination of non-enzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of plant dry material was homogenized with 10 mL of ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol and the two extracts were pooled together to a final volume of 20 mL. The determination of phenolics [41], ascorbic acid [42], and total sugars [43] was measured in the extracts.

Fresh leaf material (0.1 g) was mixed thoroughly in 5 mL of aqueous sulphosalicylic acid (3%). The mixture was centrifuged at 10,000× *g* for 15 min and a 1 mL aliquot was poured into a test tube having 1 mL of acidic ninhydrin and 1 mL of glacial acetic acid. The reaction mixture was first heated at 100 °C for 10 min and then cooled in an ice bath. The reaction mixture was extracted with 4 mL of toluene and test tubes were vortexed for 20 s and cooled. Thereafter, the light absorbance at 520 nm was measured by using a UV-VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan). Free proline content was determined on the basis of a standard curve at 520 nm absorbance and expressed as µmol (g FW)$^{-1}$ [44].

2.7. Statistical Analysis

Statistical analysis was performed with analysis of variance (ANOVA) by using the statistical program Co-Stat version 6.2 (Cohorts Software, 2003, Monterey, CA, USA). All the data obtained were tested by one-way ANOVA. Thus, the differences between treatments were determined by using ANOVA, and the least significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means. Logarithmic or inverse transformations were performed for data normalization, where necessary, prior to analysis. Pearson's correlation analysis was performed to quantify relationships between various analyzed variables. Graphs were drawn in Origin-Pro 2017 (Systat Software Inc., San Jose, CA, USA). RStudio was used to calculate Pearson's correlation.

3. Results

3.1. Impact of Melatonin Application on Plant Growth and Photosynthesis in Lentil Varieties Grown under Drought Conditions

We measured various growth and photosynthetic parameters in both varieties of lentils grown under the varying levels of drought (100%, 80%, and 60% WFC), both with and without the application of melatonin. Morphological traits are presented in Figure 1 and data regarding photosynthetic pigments and gas exchange attributes are presented in Figure 2. Decreasing soil water levels significantly decreased shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, net photosynthesis, stomatal conductance, and transpiration rate in lentil varieties, compared to 100% WFC. Under the same levels of drought in the soil, lentil-2009 showed better growth and development compared to the lentil-93. Melatonin also increased plant growth and biomass and photosynthetic pigments even in the plants grown in the drought-stressed environment. The application of melatonin increased shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, net photosynthesis, stomatal conductance, and transpiration rate. However, drought stress did not affect the intercellular CO_2 in both varieties of lentil and the application of melatonin also did not have any significant effect on the levels of intercellular CO_2.

3.2. Impact of Melatonin Application on Oxidative Stress and Antioxidant Enzymes in Lentil Varieties Grown under Drought Conditions

We measured various markers of oxidative stress in both lentil varieties grown in drought conditions, including malondialdehyde, hydrogen peroxide, and electrolyte leakage (EL) (Figure 3A–C). We also measured antioxidant capacity in the form of super oxidase dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) (Figure 3D–G, respectively). Increasing levels of drought stress in the soil increased the concentrations of MDA, EL, and H_2O_2 in the tissues of both lentil varieties. The maximum increase was observed by the lentil-sensitive variety, i.e., lentil-2009, compared to the control. The activities of various antioxidant enzymes (SOD, POD, CAT, and APX) initially increased up to a water-deficit level of 80% WFC, but then decreased significantly at the highest level of drought in the soil. In addition, the activities of enzymatic antioxidants were higher in the drought-tolerant varieties, i.e., lentil-2009, compared to the drought-sensitive variety, i.e., lentil-93. However, we also noticed that the application of melatonin decreased the concentrations of MDA, EL, and H_2O_2 in both varieties of lentil, compared to those which did not have melatonin applied. Similarly, increasing levels of melatonin significantly increased the activities of SOD, POD, CAT, and APX in the leaves of both lentil varieties, compared to those which were not treated with melatonin (Figure 3).

3.3. Impact of Melatonin Application on Osmolytes and Proline of Enzymatic Antioxidants in Lentil Varieties Grown under Drought Conditions

We also measured the contents of phenolics, ascorbic acid, soluble sugar, and proline from both varieties of lentil grown under varying levels of drought stress, i.e., 100%, 80%, and 60% WFC, with or without the application of melatonin. Decreasing levels of water (80% and 60% WFC) in the soil significantly induced ($p < 0.05$) the phenolic acid, ascorbic acid, soluble sugar, and proline content of both varieties of lentil, compared to control plants (Figure 4). Phenolics and ascorbic acid contents first increased up to a drought level of 80% WFC but gradually decreased with more drought in the soil (60% WFC). The application of melatonin also increased the content of phenolics, ascorbic acid, soluble sugar, and proline in both varieties of lentil compared to plants grown without the application melatonin.

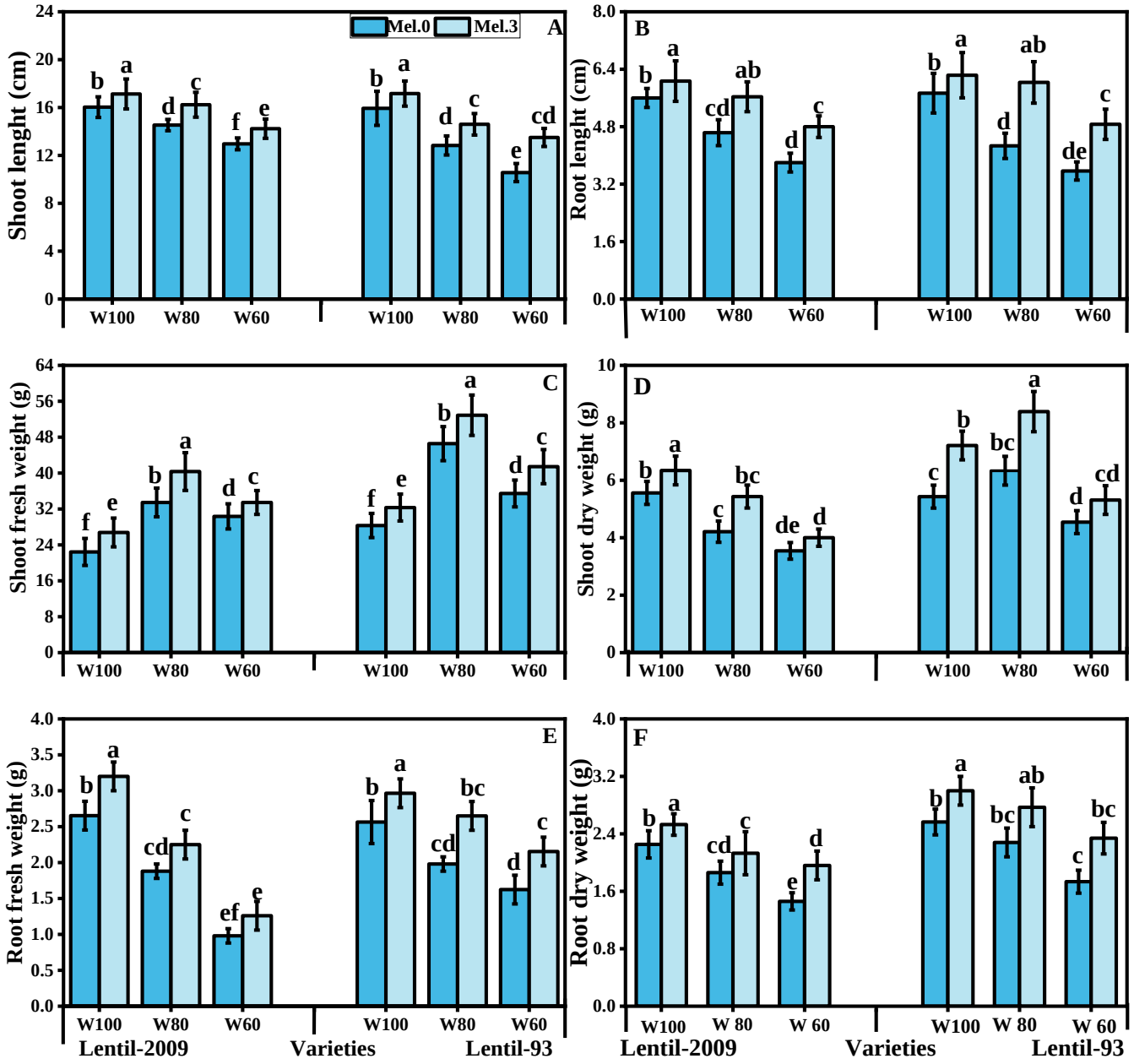

Figure 1. The impact of melatonin application on different morphological traits. The graphs show shoot length (**A**), root length (**B**), shoot fresh weight (**C**), shoot dry weight (**D**), root fresh weight (**E**), and root dry weight (**F**) under various water-deficit conditions (W100 (100% WFC), W80 (80% WFC), and W60 (60% WFC)) in both varieties of lentil (lentil-2009 and lentil-93), either with or without melatonin (0 or 3 mM). Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of four repeats (n = 4) of just one harvest of lentil varieties ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments.

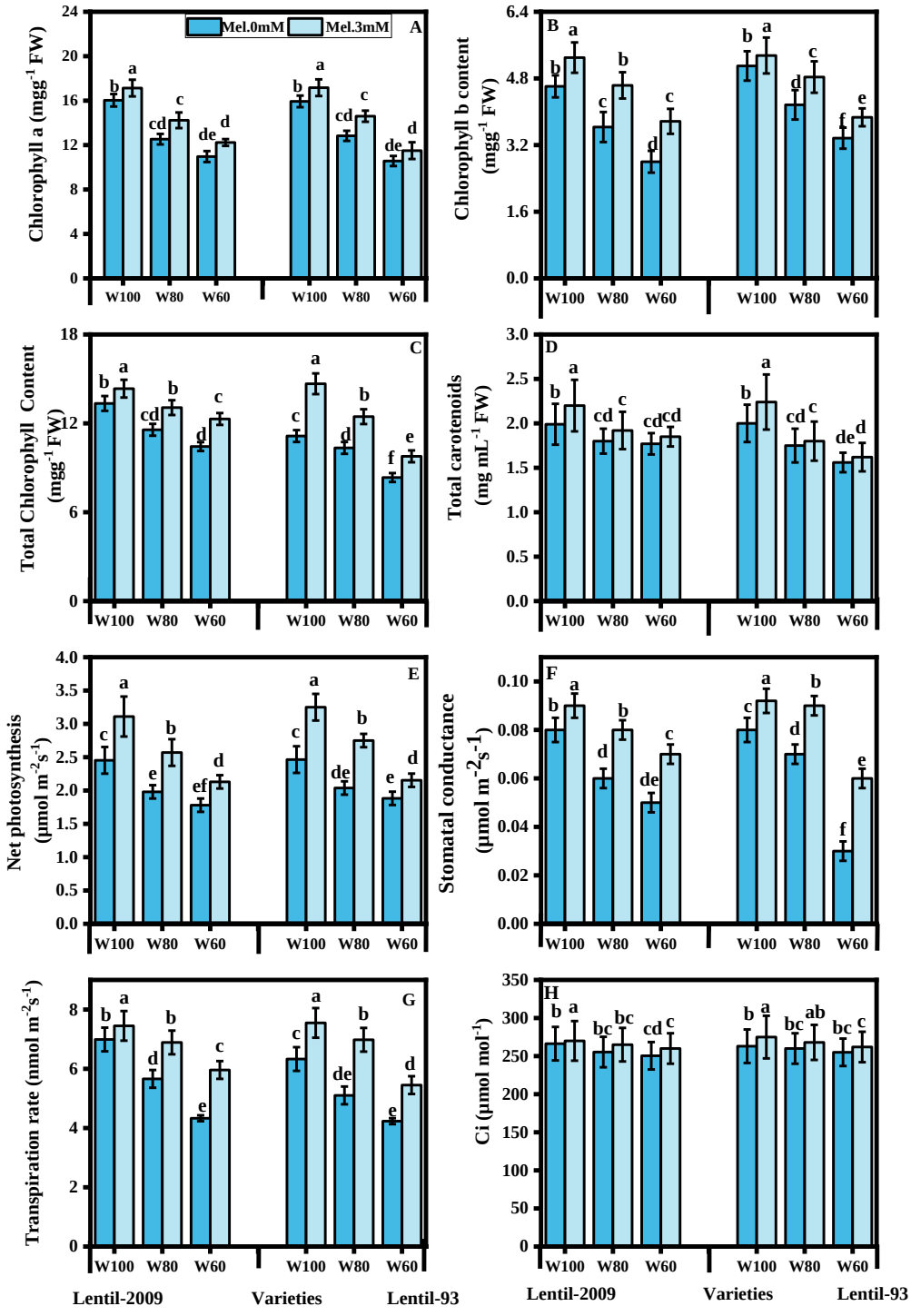

Figure 2. Impact of melatonin application on different photosynthetic pigments and gas exchange attributes. The graphs show chlorophyll "a" (**A**), chlorophyll "b" (**B**), total chlorophyll content (**C**), total carotenoid content (**D**), net photosynthesis (**E**), stomatal conductance (**F**), transpiration rate (**G**), and intercellular CO_2 (**H**) under various water-deficit conditions (W100 (100% WFC), W80 (80% WFC), and W60 (60% WFC)) in both varieties of lentil (lentil-2009 and lentil-93). Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of four repeats (n = 4) of just one harvest of lentil varieties ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments.

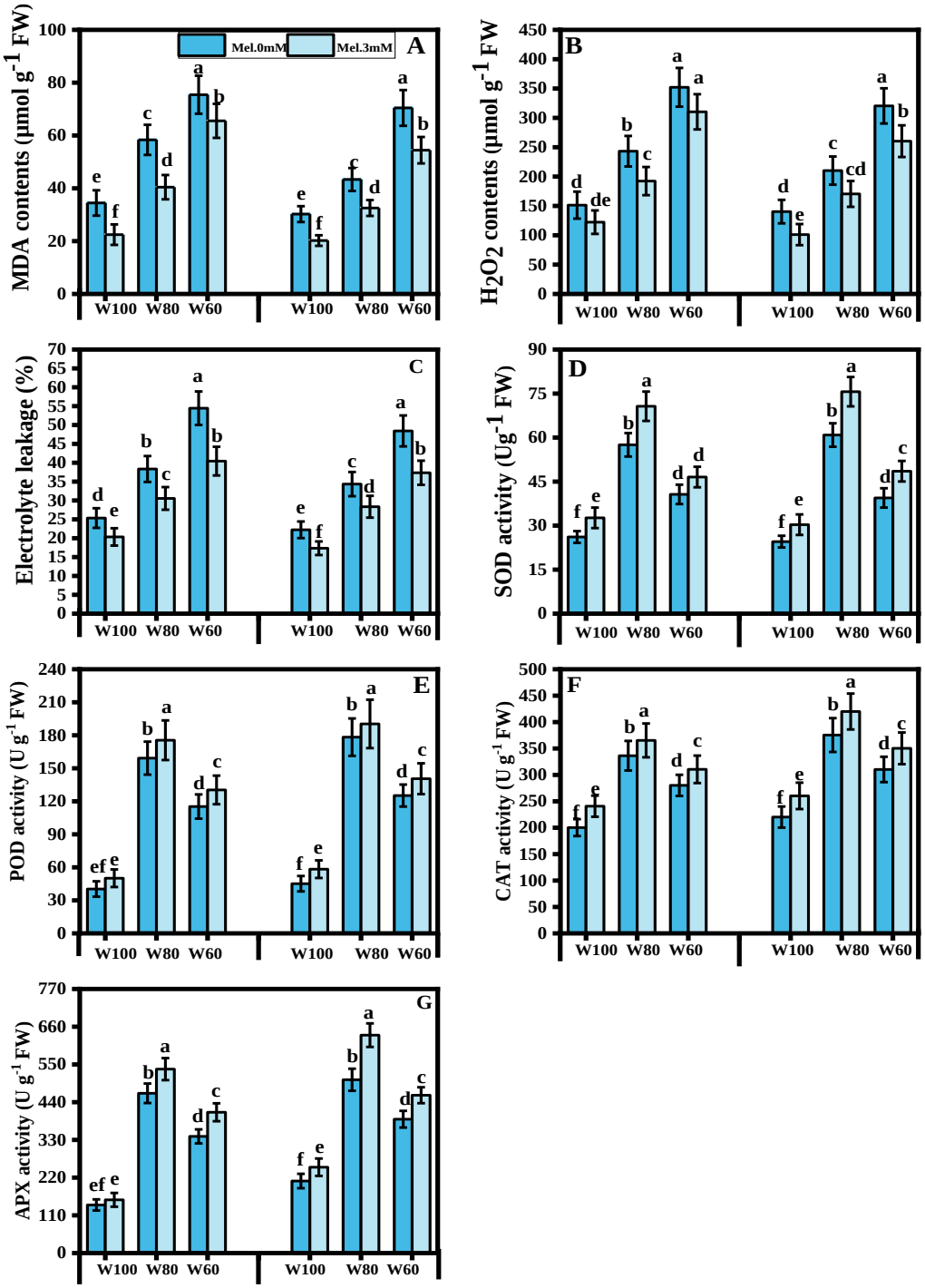

Figure 3. Impact of melatonin application on different markers of oxidative stress. The graphs show malondialdehyde content (**A**), hydrogen peroxide content (**B**), electrolyte leakage (**C**), and enzymatic antioxidants superoxidase dismutase (SOD) (**D**), peroxidase (POD) (**E**), catalase (CAT) (**F**), and ascorbate peroxidase (APX) (**G**) under various water conditions (W100 (100% WFC), W80 (80% WFC), and W60 (60% WFC)) in both varieties of lentil. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of four repeats (n = 4) of just one harvest of lentil varieties ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments.

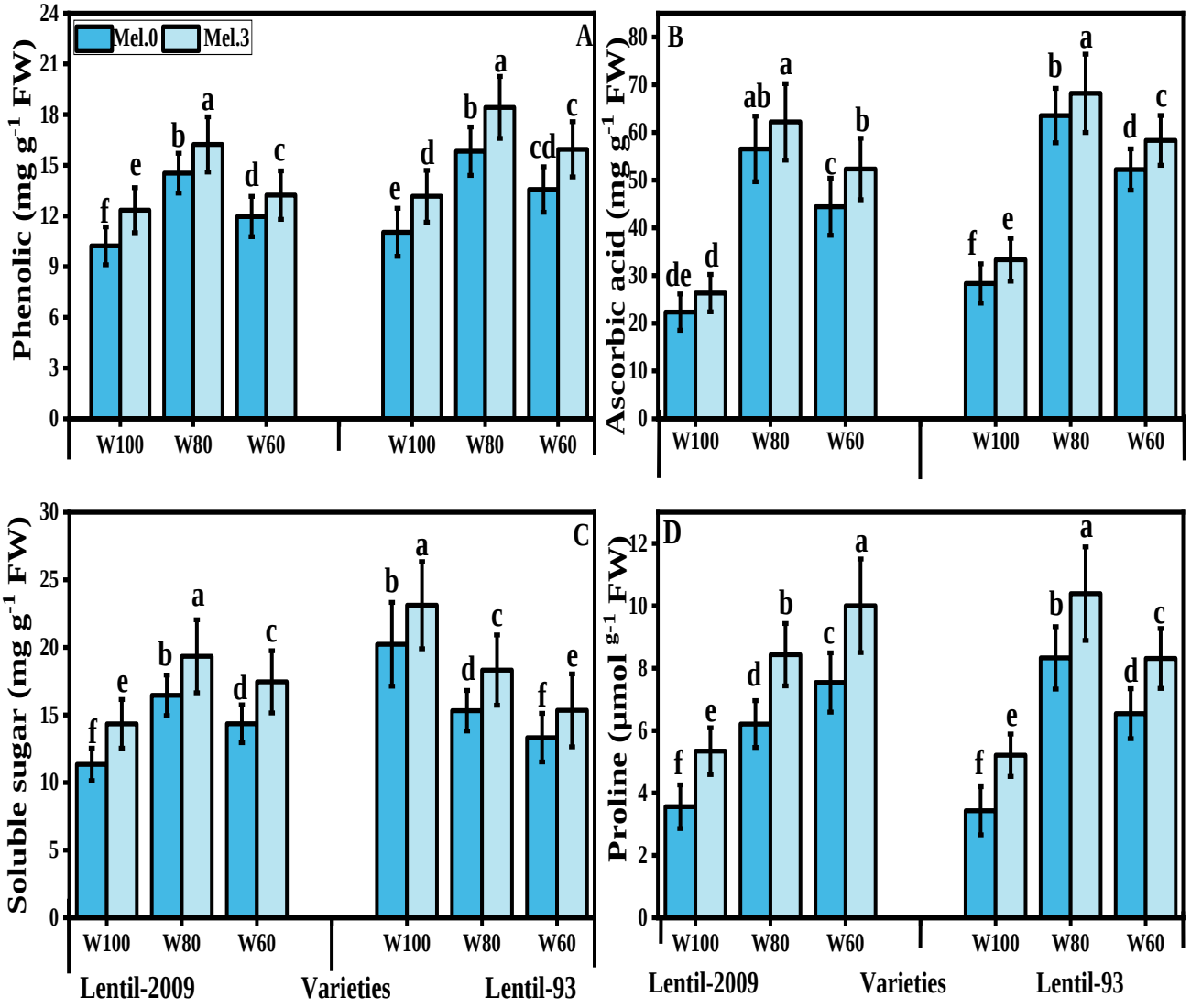

Figure 4. Impact of melatonin application on phenolics content (**A**), ascorbic acid (**B**), soluble sugar (**C**), and proline (**D**) under various water conditions (W100 (100% WFC), W80 (80% WFC), and W60 (60% WFC)) in both varieties of lentil. Means sharing similar letter(s) within a column for each parameter do not differ significantly at $p < 0.05$. Data in the figures are means of four repeats (n = 4) of just one harvest of lentil varieties ± standard deviation (SD). Different lowercase letters on the error bars indicate significant difference between the treatments.

3.4. Relationship between Various Growth Parameters of Lentil

A Pearson's correlation graph depicts the relationship between various growth and physiological parameters in lentils (lentil-2009) (Figure 5). Malondialdehyde was positively correlated with electrolyte leakage, hydrogen peroxide, catalase, ascorbate peroxide, superoxide dismutase, peroxidase, phenolics, ascorbic acid, sugar, and proline. Malondialdehyde was negatively correlated with shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, net photosynthesis, stomatal conductance, and transpiration rate. Similarly, electrolyte leakage was positively correlated with malondialdehyde, hydrogen peroxide, catalase, ascorbate peroxide, superoxide dismutase, peroxidase, phenolics, ascorbic acid, sugar, and proline, while negatively correlated with shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll a, chlorophyll b,

total chlorophyll, carotenoid, net photosynthesis, stomatal conductance, and transpiration rate. This relationship showed a close connection between various attributes of lentil.

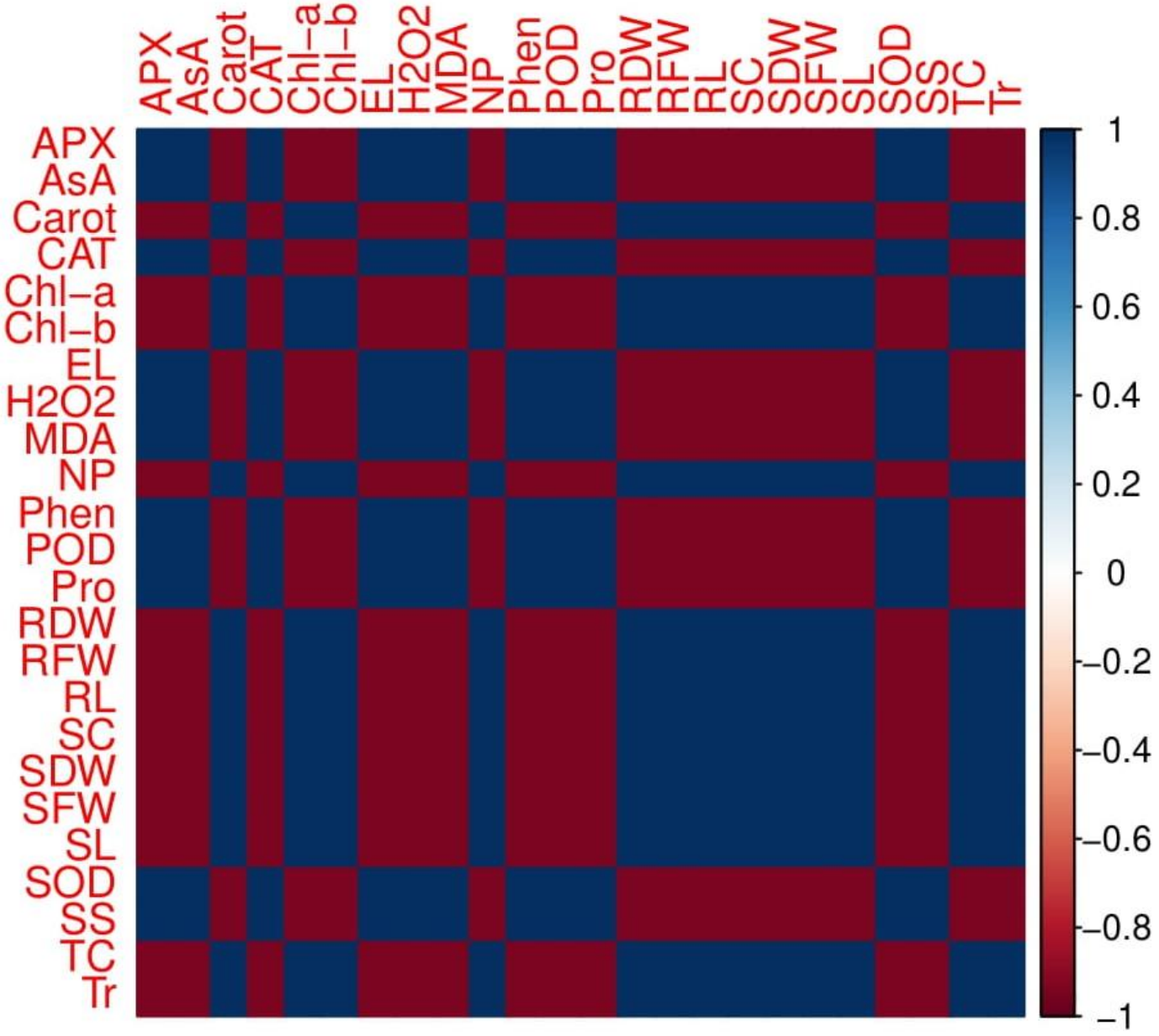

Figure 5. Correlation between various lentil growth parameters under the different water-deficit conditions with or without the application of melatonin. Different abbreviations used are as follows: (MDA) malondialdehyde contents, (EL) electrolyte leakage percentage, (H_2O_2) hydrogen peroxide content, (CAT) catalase activity, (APX) ascorbate peroxide activity, (SOD) superoxide dismutase activity, (POD) peroxidase activity, (Phe) phenolic content, (AsA) ascorbic acid content, (SS) sugar content, (Pro) proline contents, (SL) shoot length, (RL) root length, (SFW) shoot fresh weight, (SDW) shoot dry weight, (RFW) root fresh weight, (RDW) root dry weight, (Chl-a) chlorophyll a content, (Chl-b) chlorophyll b content, (TC) total chlorophyll content, (Carot) carotenoid content, (NP) net photosynthesis, (SC) stomatal conductance, and (Tr) transpiration rate.

4. Discussion

Drought stress adversely affects morphological aspects of plants, such as early germination, plant height, relative root length, root diameter, the total biomass of leaves and roots,

the number of leaves, and branch number [11]. We assessed the influence of melatonin on growth, osmolyte accumulation, and enzymatic and non-enzymatic antioxidants in lentil varieties under drought stress conditions. Drought-induced reductions might be due to photosynthesis, respiration, cell extension, and enzymatic activities [6,8] because drought-stressed plants had a diminished number of leaves, and the development of new leaves, stems, and leaflets, and leaf area were reduced compared to those in the control plants. This might be attributed to the impact of water stress on the physiological cycles in plants, such as photosynthesis, leaf zone extension, nucleic acid metabolism, protein synthesis, and the partitioning of assimilates [30,45,46]. In addition to this water deficiency, decreased photosynthesis restricts the mechanism of cell development and cell enlargement closed stomata [47], eventually reducing the yield [27,48].

Water-deficient environments are generally known to initiate oxidative stress in plants by the production of extra reactive oxygen species (ROS) [49–51] and antioxidative enzymes that play a protective role in reducing metal toxicity by scavenging ROS [52,53]. Excessive reactive oxygen species (ROS) production causes oxidative stress, as reported for many crops under drought stress treatment, and is likely to be initiated by molecular oxygen excitation (O_2) to generate singlet oxygen or by electron transfer to O_2 and genesis of free radicals, i.e., O^{2-} and OH^- [11,27]. Plant responses to oxidative stress also depend upon plant species and cultivars, and ROS are removed in plants by a variety of antioxidant enzymes, such as SOD, POD, CAT, and APX [54,55]. The increase in the activities of antioxidant enzymes is concomitant with the generation of extra ROS. It has also been reported that an increase in the activities of various antioxidant enzymes under environmental stress conditions is due to a reduction in the glutathione level [56,57]. Plants produce a variety of antioxidants (ascorbic acid, glutathione, proline, carotenoids, phenolic acids, and flavonoids) that improve tolerance against drought stress [58,59]. Phenolics are potent antioxidants against drought-induced oxidative damage and efficiently scavenge ROS [60]. Proline accumulation in plant tissue/organs is a response to drought stress, which might be associated with signal transduction and prevents membrane distortion [61]. Studies related to our results found that drought increased oxidative stress by increasing MDA, EL, and H_2O_2 in wheat [46], cucumber [62], and canola [63].

Many efforts have been made to mitigate the hazardous impacts of drought stress by using various plant growth regulators, such as salicylic acid [64], polyamine [65], abscisic acid [66], glycine betaine [67], and melatonin [18,23,24]. Melatonin is an amphiphilic biological (indolamine) hormone found throughout the animal and plant kingdoms. Melanin is produced by the shikimate pathway in chloroplasts from tryptophan [68,69]. Melatonin plays a vital part in plant growth and development by regulating plant physiology and root regeneration [17], antioxidant activity [16], photosynthesis [22], senescence of leaves [70], and immunological enhancement [71]. Melatonin might also boost the antioxidative capacity to fortify a variety of plant species from various abiotic stresses, especially drought stress [23,70], by altering the expression of salt tolerance genes, upregulating antioxidant enzymes, and directly scavenging ROS. Previously, melatonin has been reported to enhance resistance against drought stress in various crops, including barley [72], soybean [73], and tomato [74]. Many possibilities could be suggested to explain how melatonin can help plants to alleviate the adverse effects of various environmental conditions. One of the most important defensive mechanisms in this respect is the protection of the photosynthetic apparatus via improving the scavenging efficiency of reactive oxygen species (ROS) and reducing the stress-induced oxidative damages [71,75]. Under drought stress, melatonin joins in the readjustment of the cell osmotic potential and accumulation of osmolytes such as proline and soluble sugars [76]. Moreover, melatonin can maintain the water status of water-stressed plants through regulating stomatal movement [20] and modulating a broad spectrum of anatomical aspects, i.e., preserving the integrity of cell membranes [74] and increasing the cuticle and/or wax accumulation [77]. In addition, it has been confirmed that during the exposure to stress, melatonin has a close linkage in plant signal transduction

and can trigger cascades of reprogramming primary metabolites, transcriptomes, and proteomes [16].

5. Conclusions

In this study, we investigated the influence of melatonin on the growth of lentil varieties in well-watered (100% WFC) and water-depleted conditions (80% and 60% WFC). Drought conditions had a harsh impact on plant growth, and as well as influencing photosynthetic measurements, they induced oxidative stress, antioxidant enzymes, and osmoprotectants. The application of melatonin is useful in alleviating oxidative stress by accelerating the activities of antioxidants and increasing the content of soluble sugars and other enzymatic and non-enzymatic antioxidants, even in drought conditions. Hence, we suggest that the application of melatonin offers new opportunities by promoting greater drought tolerance and enhancing the capacity to adapt to future environmental challenges.

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