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Melatonin Decreases Negative Effects of Combined Drought and High Temperature Stresses through Enhanced Antioxidant Defense System in Tomato Leaves

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Citation: Annadurai, M.K.K.; Alagarsamy, S.; Karuppasami, K.M.; Ramakrishnan, S.; Subramanian, M.; Venugopal, P.R.B.; Muthurajan, R.; Vellingiri, G.; Dhashnamurthi, V.; Veerasamy, R.; et al. Melatonin Decreases Negative Effects of Combined Drought and High Temperature Stresses through Enhanced Antioxidant Defense System in Tomato Leaves. *Horticulturae* **2023**, *9*, 673. https://doi.org/10.3390/horticulturae9060673

Academic Editors: Małgorzata Majewska, Barbara Hawrylak-Nowak and Agnieszka Hanaka

Received: 3 May 2023
Revised: 24 May 2023
Accepted: 30 May 2023
Published: 6 June 2023

Abstract: In tomato (*Lycopersicon esculentum* L.), the effects of combined drought (D) and high temperature (HT) stress during the flowering stage had not been studied in detail. Therefore, this study was conducted with an objective of quantifying the effects of foliar spray of melatonin under individual and combined drought and HT stress. At flowering stage, D stress was imposed through withholding irrigation, while HT stress was imposed through exposing the plants to ambient temperature (AT) along with an increase of +5 °C. Under D + HT, plants were first subjected to drought followed by a + 5 °C increase in AT. The duration of individual or combined stress was ten days. At 80% available soil moisture, 100 µM melatonin was sprayed on D, HT, or D + HT treated plants. Among the stresses, D + HT stress increased the thylakoid membrane damage and decreased the photosynthetic rate and fruit yield more than D or HT stress. Foliar spray of 100 µM melatonin produced decreased thylakoid membrane damage [D: 31%, HT: 26%, and D + HT: 18%] and increased antioxidant enzyme, viz., superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase, activity over stress-control plants. The photosynthetic rate [D: 24%, HT: 22%, and D + HT: 19%] and fruit yield [D: 32%, HT: 23%, and D + HT: 16%] were increased over stress-control plants. Hence, it is evident that the increased photosynthetic rate and fruit yield in D + HT and 100 µM melatonin-sprayed plants may be associated with an increased antioxidant defense system. Melatonin as a novel biostimulator has a great potential in scavenging free radicals through increased antioxidant activity, which shields the photosynthetic membrane from damage and therefore helps in stress mitigation.

Keywords: melatonin; drought; high temperature; antioxidants; free radicals; photosynthesis; lipid peroxidation; mitigation

1. Introduction

Climate variability is associated with releasing greenhouse gas emissions [1,2]. The Intergovernmental Panel on Climate Change (IPCC) indicates that the increase in air temperature from baseline should be less than 1.5 °C, and if it exceeds the threshold, it will affect crop productivity [3]. Similar to high temperature (HT), drought (D) is also an abiotic stress which is more frequent due to reduced precipitation and water vapor

fluxes in the atmosphere, which affects crop productivity [4]. From 1970 to 2000, the percentage of drought-affected area was doubled [5]. The global population is projected to increase significantly by 2050, demanding increased crop production or productivity to meet food security [6]. Hence, to meet the global food demand and sustain the crop yield under a changing climate, developing a crop management solution to mitigate drought or high-temperature stress is mandatory [7].

Drought inhibits photosynthesis [8], thus decreasing the assimilate partitioning and lowering fruit yield [9]. Drought causes decreased stomatal conductance due to which diffusion of CO_2 also decreases, which in turn results in stomatal closure [10]. Additionally, high-temperature stress denatures the photosynthetic pigments involved in the light reaction and damages the thylakoid membrane responsible for producing $NADPH_2$ and ATP [11]. Under abiotic stress, increased malondialdehyde levels indicate oxidative damage in plants. Therefore, plants rely on the enzymatic antioxidants, viz., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR), to scavenge reactive oxygen species (ROS) produced under stress, thereby protecting the membrane from damage [12].

Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine compound, was discovered in the pineal gland of animals [13] and has similarities with other tryptophan derivatives [14]. Studies suggest that melatonin has various roles in the plant developmental process, namely, improved seed germination and seedling growth [15,16], photosystem activity [17], antioxidant defense system [18], osmoregulation [19], rooting depth [20], and fruit yield and quality [21,22]. In contrast, melatonin decreases the leaf senescence process [23]. It is predicted that in the current and future climate, crop yield will be affected by two or more abiotic stresses during their reproductive phase [24,25]. The effect of melatonin on drought or high-temperature stress in tomato has been studied in detail [26,27]. However, the impacts of combined drought and high-temperature stress on plants have not been quantified.

Tomato is one of the most popular and commercially grown vegetable crops and is susceptible to drought or HT stress which could cause a yield loss of 70% [28]. In tomato, the reproductive stage is more sensitive to drought or high-temperature stress because it affects the pre- and post-fertilization processes, and carbohydrate translocation from source to sink, thus, reducing fruit yield [29]. Previous research on tomato confirmed that melatonin could increase antioxidant enzymes [30]. The antioxidant molecules are used to mitigate the detrimental effects of abiotic stress through (i) decreasing thylakoid membrane damage (F_0/F_m ratio), (ii) increasing the photosynthetic activity due to less damage in photosystem II (PSII), where the initial reaction of photosynthesis take place in the thylakoid membrane, and (iii) decreasing chlorophyll degradation via protecting the chlorophyll biosynthetic enzyme [31]. In contrast, antioxidants will reduce levels of (i) malondialdehyde, (ii) free radicals, and (iii) electrolyte leakage [32,33]. The effect of melatonin on crops is presented in Supplementary Table S1 [34–47]. Based on this, we hypothesize that melatonin could increase the antioxidant defense system, resulting in increased photosynthetic rate and yield. The main aim of this study is to exploit the antioxidant potential of melatonin against drought, high-temperature, or combined drought and high-temperature induced oxidative stress; its protective role in the photosynthetic system; and its impact on membrane integrity.

2. Materials and Methods

2.1. Experimental Details

An experiment was conducted in a completely randomized block design with two factors and four replications. The first factor was the type of stress with three levels (drought, high temperature, and combined drought and high temperature), and the second factor was the foliar spray of melatonin with four levels: (i) absolute control (plants were grown in ambient temperature, maintained under 100% field capacity, and received no spray), (ii) stress control (for drought stress, plants were maintained under

drought stress and received no spray; for high-temperature stress, plants were maintained under high-temperature stress and received no spray; and for combined drought and high-temperature stress, plants were drought and high-temperature stressed and received no spray), (iii) 80 µM melatonin, and (iv) 100 µM melatonin (Figure 1).

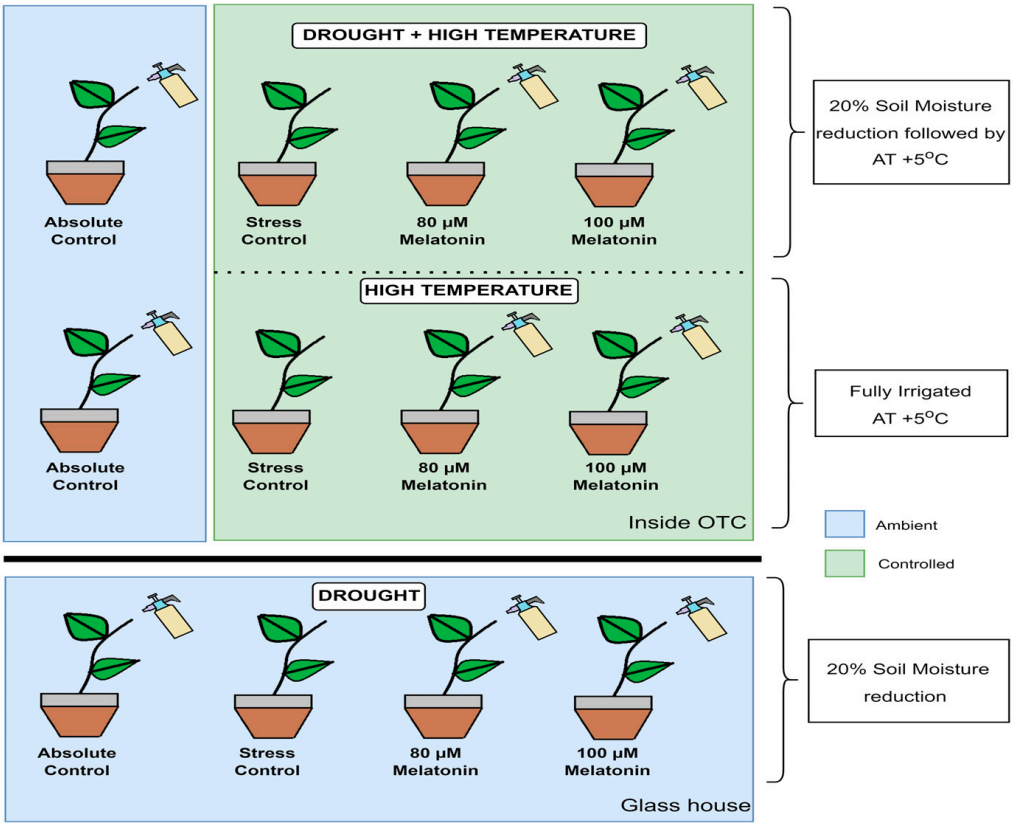


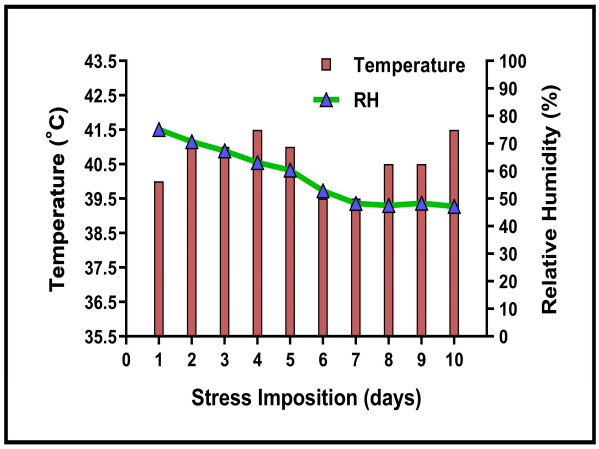
Figure 1. The image represents the stress imposition methodology and foliar treatment details used in this experiment conducted in OTC and glass house for 10 days.

The seedlings of tomato hybrid 'Shivam' were grown in portrays containing a vermicompost and coir pith. Based on uniform growth and good health, twenty-one-day-old tomato seedlings were used for transplanting. This experiment was conducted in the Glasshouse and Open Top Chamber (OTC) at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India, from March to June 2022. The twenty-one-day-old seedlings were moved to large-sized plastic pots (46 cm in length and 60 cm in diameter) containing a mixture of red soil, sand, and vermicompost in a ratio of 3:1:1. In a pot, two plants were maintained, and the plants were watered on alternate days. All the pots were maintained under open sunlit condition. Ten days after transplanting, plants were supplied with a recommended dose of nutrients. During crop growth and development, the required crop management practices were followed as per the horticulture crop production guide [48].

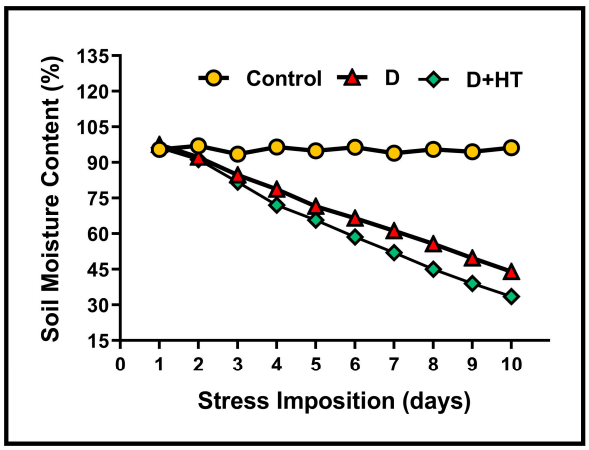
2.2. Stress Imposition and Treatment Details

The plants were maintained under well-watered and ambient temperature conditions until the flower initiation stage, which coincides with the last week of April. At 50% flowering stage, plants were moved to the controlled environment facility for imposing Drought (D), high-temperature (HT), or combined drought or high-temperature stress (D + HT) for 10 days. The duration of stress imposition for ten days depends on the reduction in soil moisture content up to 60 to 70 percent under D and D + HT stress, while in case of HT stress, it depends on reduction in relative humidity up to 40 percent. Well-watered and D-stressed

plants were maintained under ambient conditions, whereas the high-temperature-stressed plants were placed inside an Open Top Chamber (OTC) maintained at AT + 5 °C. In case of D + HT stress, plants were first exposed to drought (20% soil moisture reduction) and then subjected to AT + 5 °C. During the experimental period, the relative humidity ranged between 47 and 75 percent. Meteorological data of OTC are shown in Figure 2A. In D and D + HT stress experiments, soil moisture was regularly measured using a theta probe, the moisture content was calculated based on a reduction from 100% field capacity, and the soil moisture data are recorded and presented in Figure 2B. Melatonin (80 µM or 100 µM) was sprayed at 80% field capacity, and observations were recorded at the end of D, or HT, or D + HT stress. Plants exposed to HT stress were maintained at fully irrigated conditions and on the fourth day of stress, the plants under D, HT, and D + HT stress were sprayed with either 80 µM or 100 µM of melatonin.



(**A**) OTC



(**B**) Soil moisture

Figure 2. Temperature and soil moisture data recorded during the experiment. (**A**) Daily temperature and relative humidity under OTC during ten days of stress imposition; (**B**) soil moisture content under drought and combined stress for a period of ten days.

2.3. Preparation of Melatonin Solution

Melatonin chemical was purchased from Sigma-Aldrich Pvt. Ltd. India and stored at −20 °C. Irrespective of varieties, a previous study reported the significant results of 0.1 mM melatonin among different concentrations [49]. However, a preliminary lab study was performed on germination parameters using various concentrations of melatonin, viz., 20 µM, 40 µM, 60 µM, 80 µM, 100 µM, and 120 µM, under the PEG-induced drought and temperature-inductive response methodology. Based on the results obtained from initial screening, 80 µM and 100 µM melatonin showed significant difference among other treatments. Therefore, stock solution was prepared using the required quantity of melatonin, dissolving in 99.9% ethanol, and made to final volume using distilled water. The two final concentrations of melatonin (80 µM or 100 µM) were prepared via diluting the stock solution, and 0.25 mL of surfactant (Tween 20) was added to the melatonin solution to increase its absorption efficiency in leaves.

2.4. Sampling

The leaf samples were collected at the end of the stresses, and the collected leaf was used for physiological and biochemical analysis in one of the two plants. The yield and yield components were recorded in both plants, and the average was presented. Fresh leaves were collected at the end of the stress and immediately dipped in liquid nitrogen, grounded using liquid nitrogen, to assess biochemical parameters and enzyme activity.

2.5. Physiological Attributes

The chlorophyll index was determined in the second distal leaflet of the second and fourth leaf from the top using a chlorophyll meter (SPAD) (Minolta, Japan). The photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (Ci) were measured in third leaf using a portable photosynthesis system (LI-6400 XT; LI-COR Inc., Lincoln, NE, USA). The leaf chlorophyll fluorescence was measured in the third leaf using a chlorophyll fluorometer [50]. Upon dark adaptation of the leaf using clips for 30 min, minimal fluorescence (F_0), maximum fluorescence (F_m), and variable fluorescence ($F_v = F_m - F_0$) were measured. The ratio of minimum fluorescence to maximum fluorescence (F_0/F_m ratio) was calculated using the data taken. The F_0/F_m ratio is referred to as thylakoid membrane damage. These observations were taken from 10:00 am to 12:30 pm simultaneously from the fully expanded leaf below the apex.

2.6. Histochemical Detection of ROS

Hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) generation rate was detected histochemically, as mentioned in Lei et al. [51] using the 3,3-diaminobenzidine (DAB) and nitro blue tetrazolium (NBT) staining method. Fresh leaves were dipped in 1 mg mL^{-1} DAB solution containing 50 mM sodium phosphate buffer (pH 3.8) and incubated for 5 h in the dark, during which brown precipitates were formed, indicating H_2O_2 accumulation. To detect superoxide anions, the leaves were immersed in 50 mM sodium phosphate buffer (pH 7.5) containing 0.2% NBT. The formation of dark blue insoluble formazan detects O_2^- accumulation. The destaining was followed with ethanol, glacial acetic acid, and glycerol in the ratio of 3:1:1, respectively, and the excess stain was removed via two to three washes using distilled water. Samples were placed in 80% glycerol, and photographs were taken.

2.7. Analysis of Hydrogen Peroxide and Superoxide Anion Content

Hydrogen peroxide content (H_2O_2) was measured as per Velikova and Loreto's method [52] through measuring the absorbance at 390 nm and expressed in µmol per gram of fresh weight. The superoxide anion (O_2^-) was estimated as per the method of Doke [53]. 0.5 g leaves was placed in the test tube containing 7 mL of 50 mM sodium azide and incubated for 5 min in the dark. From this solution, 2 mL was taken and subjected to heating at 85 °C for 15 min, then cooling on ice for 5 min. The data is expressed as an increase in absorbance at 580 nm per gram of fresh weight.

2.8. Membrane Integrity

Malondialdehyde content was estimated using the thiobarbituric acid method, according to Heath and Packer [54]. 500 mg of the leaf samples was taken and macerated with 0.1% TCA and centrifuged at 5000 rpm for 10 min, and the supernatant was collected, to which 4 mL of 20% TCA containing 0.5% TBA was added and subjected to heating at 95 °C for 30 min in a water bath followed by cooling and centrifugation. Finally, MDA content was calculated via subtracting the absorbance at 532 nm and 600 nm and expressed as µmol per gram. Leaf discs were made from the fresh leaf of drought or high-temperature stress or the combined drought-and-high-temperature-stressed plant. The leaf was immersed in distilled water and incubated for 24 h; then, the leakage was determined initially with a conductivity meter (EC_1). Then, these samples were heated at 100 °C for one hour, and the electrical conductivity of the solution was recorded (EC_2). The electrolyte leakage of the sample was expressed as a percentage [55].

2.9. Antioxidant Enzyme Activity

One gram of leaf sample was macerated with 50 mM phosphate buffer containing (pH 7.0), 0.1 mM EDTA, 0.1 mM phenyl methane sulfonyl fluoride, 1% PVP (*w*/*v*), and 0.2% (*v*/*v*) Triton X-100 using pre-chilled pestle and mortar and centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was used to estimate the antioxidant enzyme activity as described in Camejo et al. [56].

The enzyme superoxide dismutase (SOD) was determined using the nitroblue tetrazolium (NBT) method described in Beauchamp and Fridovich [57]. The reaction mixture (3 mL) contained 0.1 mL of enzyme extract, 1.5 mL of 50 mM phosphate buffer (pH 7.8), 0.1 mL of 2 mM EDTA, 0.2 mL of 9.9 mM L-methionine, 0.1 mL of 0.02% Triton X-100, 0.1 mL of 55 µM NBT, and 0.1 mL of 1 mM riboflavin. The absorbance of control and blank was measured at 560 nm, and SOD activity was expressed as units per mg of protein. One unit of SOD is the quantity of enzymes necessary to inhibit NBT by 50% at 25 °C. According to Lowry et al. [58], the total protein was estimated using bovine serum albumin as a standard. The reaction mixture (3 mL) contained 0.1 mL enzyme extract and 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0). 0.1 mL of 15 mM H_2O_2 was added, and the absorbance was recorded at 240 nm for 2 min. Catalase (CAT) activity was assessed based on the disappearance of H_2O_2 during the reaction initiation and calculated using an extinction coefficient of 43.6 mM^{-1} cm^{-1} and expressed as enzyme units per mg of proteins [59]. The peroxidase (POD) activity was measured according to the procedure of Kumar and Khan [60]. A 0.1 mL enzyme extract was added to the reaction mixture (3 mL) containing 1 mL of 100 mM phosphate buffer (pH 7.0), 0.5 mL of 10 mM pyrogallol, and 0.5 mL of 5 mM H_2O_2. Later, the solution was incubated for 5 min at 25 °C, and the reaction was terminated through adding 0.5 mL of 2.5 N H_2SO_4. The absorbance was recorded at 420 nm for 3 min at 30 s intervals, and the activity was calculated using the extinction coefficient of 12 mM^{-1} cm^{-1} and expressed in µmol of purpurogallin min^{-1} mg of $protein^{-1}$. According to Chen and Asada [61], ascorbate peroxidase (APX) activity was determined using 1 mL of the reaction mixture comprised of 0.05 mL enzyme extract, 0.85 mL of 50 mM phosphate buffer (pH 7.0), 0.05 mL of 0.1 mM ascorbate, and 0.05 mL of 0.3 mM H_2O_2, and the measure of absorbance was recorded at 290 nm for 1 min. APX activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed in units per mg of protein. Glutathione reductase (GR) was quantified as per the procedure of Smith et al. [62]. The enzyme activity was measured with 1 mL of reaction mixture containing enzyme extract, 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 150 µM NADPH, and 500 µM oxidized glutathione. The enzyme activity was measured at an absorbance of 340 nm and expressed as enzyme units per mg of protein.

2.10. Relative Tolerance Index (RTI)

The tolerance level of plants exposed to stress and foliar spray was indirectly calculated using stomatal conductance [63]. The RTI was calculated using the formula:

$$\text{RTI } (\%) = \frac{\text{Stomatal conductance of stressed plant}}{\text{Stomatal conductance of unstressed plant}} \times 100$$

2.11. Yield

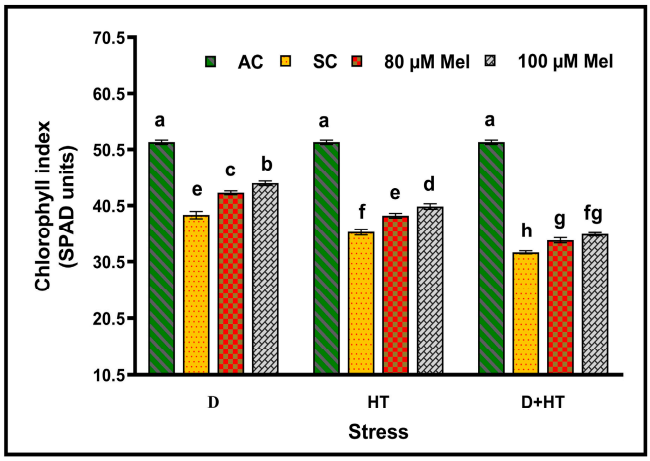
Fruit was harvested for seven pickings; the number of fruits harvested per picking was counted, and the total was represented as the total fruits per plant. The weight of tomato fruit at each harvest was recorded and expressed as fruit yield per plant.

2.12. Statistical Analysis

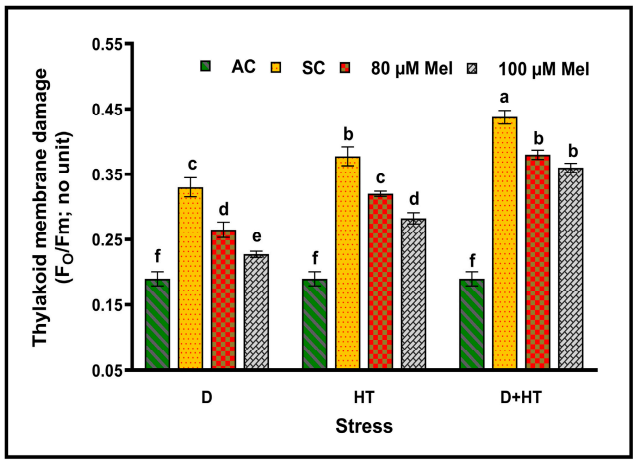
The experiment was laid out in a Factorial Completely Randomized Design (FCRD) with four replications. The data were statistically analyzed using SPSS for windows, version 16.0. Chicago, SPSS Inc., USA, and the graphs of observed variables were obtained using Graphpad prism software for windows, version 9.0.0. The results were presented as the mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted using small letters, given that the means with same letters are not statistically significant at $p = 0.05$. The mean value of each trait is presented in Supplementary Tables S2–S16.

3. Results

The effect of stress, foliar spray, and the interaction of stress and foliar spray was significant ($p < 0.05$) for the chlorophyll index (Figure 3A) and thylakoid membrane damage (Figure 3B). Among the stresses, D + HT stress decreased the chlorophyll index by a greater magnitude than D or HT stresses alone. Among the foliar sprays, a higher level of chlorophyll index was observed in 100 µM melatonin-treated plants than in other treatments. Application of 100 µM melatonin to D (15%), HT (13%), and D + HT (10%) stressed plants increased the chlorophyll index more than other treatment combinations. In contrast, the thylakoid membrane damage was more remarkable in D + HT-stressed plants than D or HT-stressed plants (Figure 3B). Foliar spray of 100 µM melatonin to D + HT-stressed plants decreased the thylakoid membrane damage by 18%, which was lower than D + 100 µM melatonin (31%), and HT + 100 µM melatonin (26%) sprayed plants (Figure 3B).



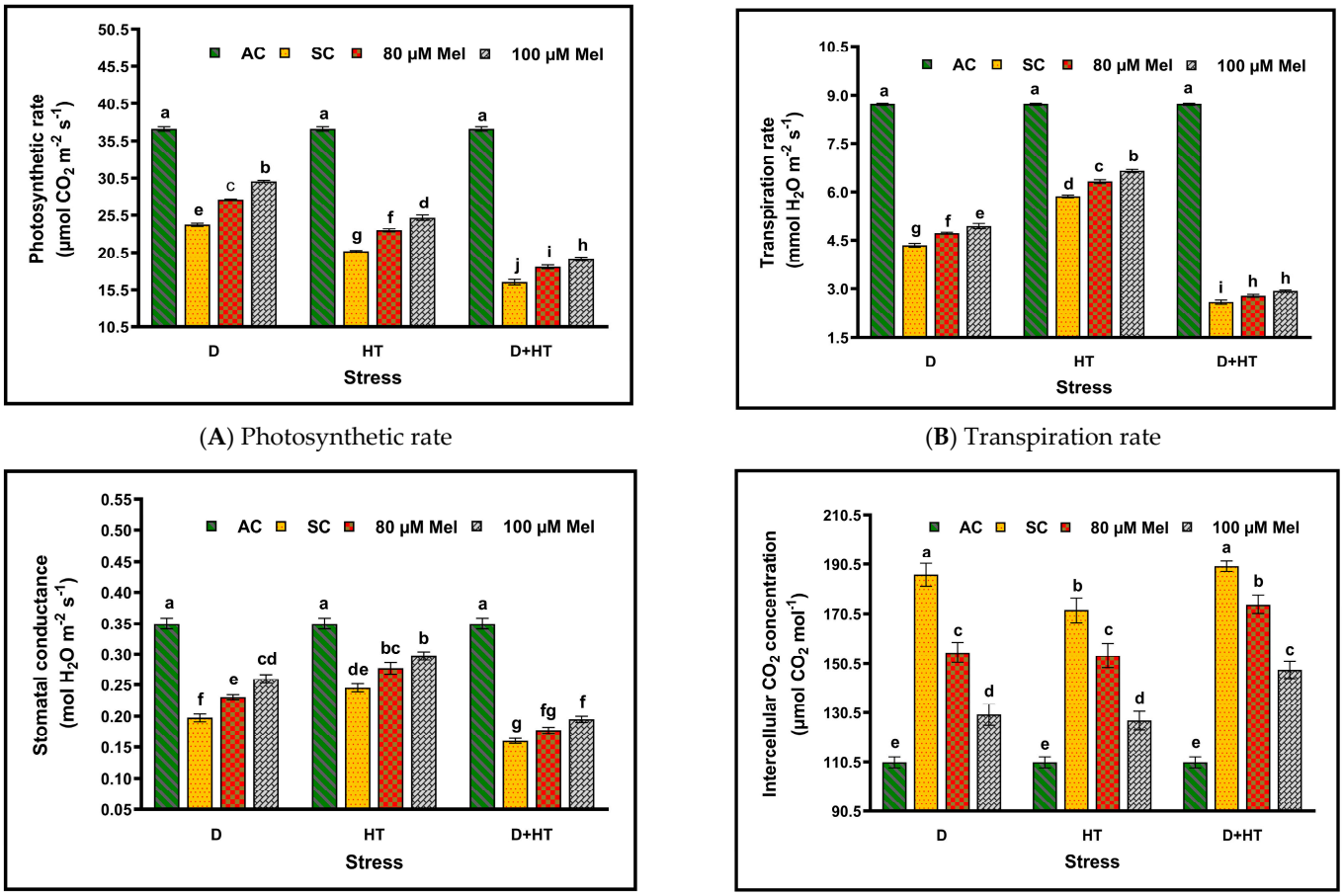
(**A**) Chlorophyll index



(**B**) Thylakoid membrane damage

Figure 3. Effect of stress (drought—D, high temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM melatonin—80 µM Mel, and 100 µM melatonin—100 µM Mel) on (**A**) chlorophyll index (SPAD units) and (**B**) thylakoid membrane damage (F_0/F_m) in tomato on 10th day of stress. The results were presented as mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted by small letters, given that the means with same letters are not statistically significant at $p = 0.05$.

The gas exchange parameters, viz., P_n (Figure 4A), E (Figure 4B), g_s (Figure 4C), and Ci (Figure 4D), were significantly ($p < 0.05$) influenced by stress, foliar spray, and their interactions (Figure 4A–D). Among the stresses, a higher decrease in P_n, E, and g_s was recorded in D + HT-stressed plants than D or HT stress (Figure 4A–D). Among the foliar sprays, 100 µM melatonin-treated plants showed an increased P_n, E, and g_s and decreased Ci compared to other foliar spray treatments (Figure 4A–D). A foliar spray of 100 µM melatonin on D-stressed plants yielded a higher increase in P_n (24%), E (14%), and g_s (32%) than HT + 100 µM melatonin and D + HT + 100 µM melatonin-sprayed plants (Figure 4A–D).



(**A**) Photosynthetic rate

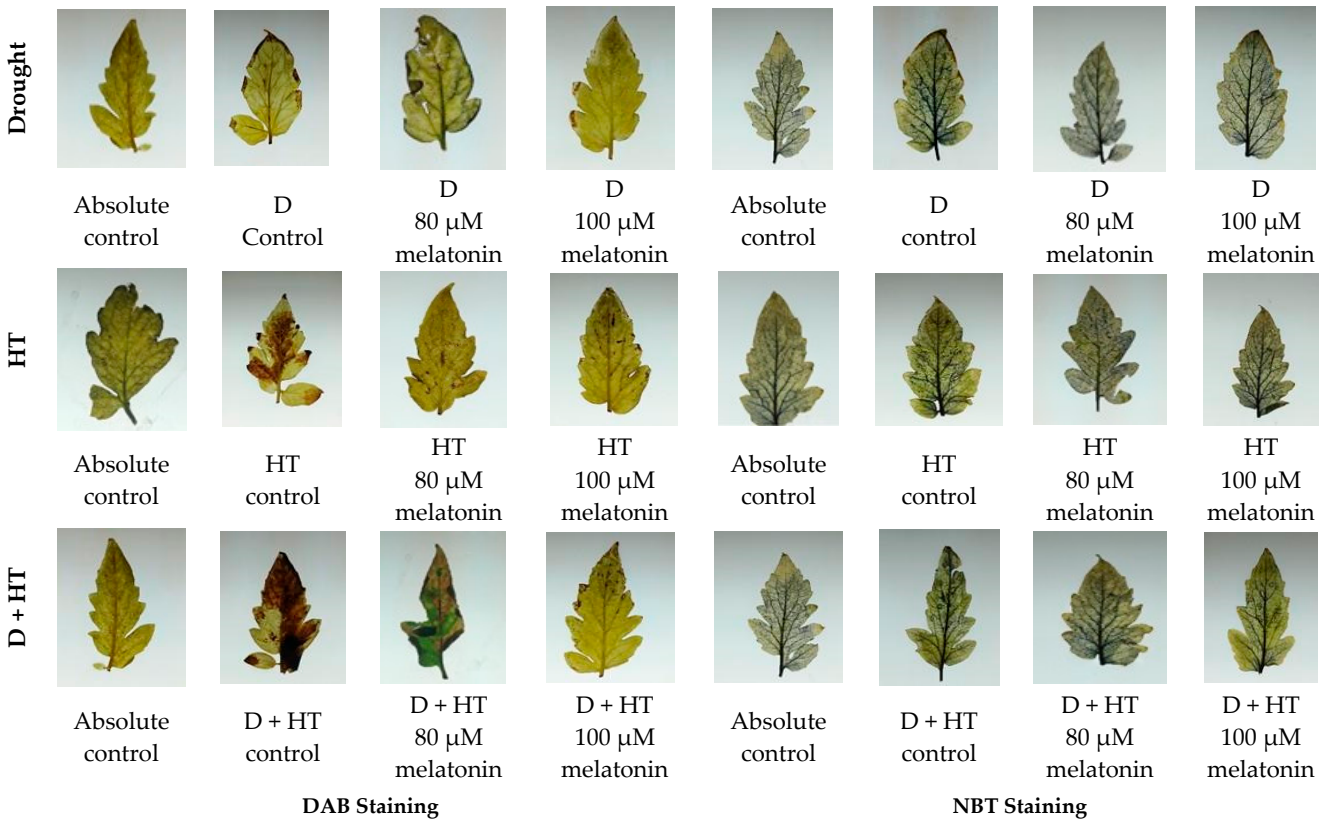
(**B**) Transpiration rate

(**C**) Stomatal conductance

(**D**) Intercellular CO_2 concentration

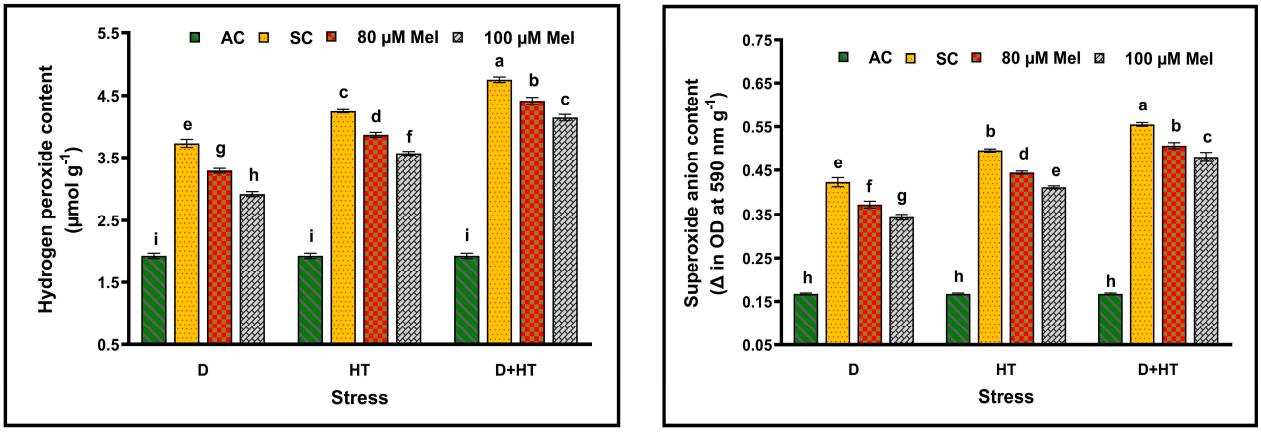
Figure 4. Effect of stress (drought—D, high temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM melatonin—80 µM Mel, and 100 µM melatonin—100 µM Mel) on (**A**) photosynthetic rate, (**B**) transpiration rate, (**C**) stomatal conductance, and (**D**) intercellular CO_2 concentration in tomato on 10th day of stress. The results were presented as mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted by small letters, given that the means with same letters are not statistically significant at $p = 0.05$.

The effect of stress, foliar spray, and the interaction of stress and foliar spray was significant ($p < 0.05$) for staining (Figure 5A,B) and hydrogen peroxide and superoxide anion contents (Figure 6A,B). Among the stresses, D + HT-stressed plants had a higher free radical content and staining than D or HT stress (Figures 5A,B and 6A,B). Among the foliar sprays, decreased H_2O_2 and O_2^- content and staining were observed in 100 µM melatonin-treated plants than in other treatments (Figures 5A,B and 6A,B). Drought-stressed plants sprayed with 100 µM melatonin had decreased free radical content and staining to a higher level than HT + 100 µM melatonin and D + HT + 100 µM melatonin-sprayed plants (Figures 5A,B and 6A,B).



(**A**) Hydrogen peroxide generation rate (**B**) Superoxide anion generation rate

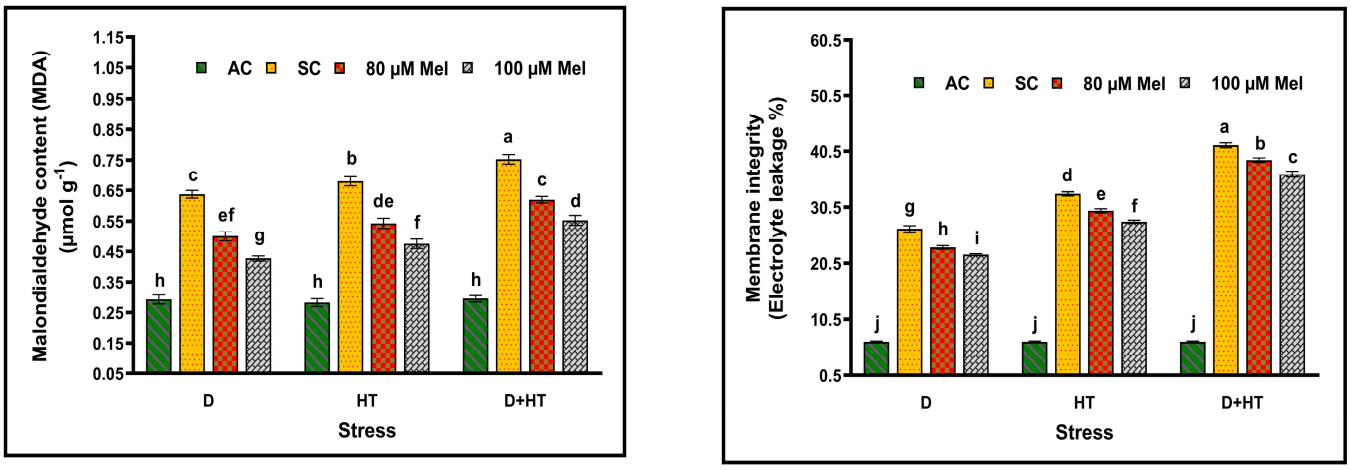
Figure 5. Effect of stress (drought—D, high temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM melatonin—80 µM Mel, and 100 µM melatonin—100 µM Mel) on (**A**) histochemical detection of hydrogen peroxide generation rate via DAB staining and (**B**) histochemical detection of superoxide anion generation rate via NBT staining in tomato on 10th day of stress.



(**A**) Hydrogen peroxide content (**B**) Superoxide anion content

Figure 6. Effect of stress (drought—D, high -temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM mMelatonin—80 µM Mel, and 100 µM mMelatonin—100 µM Mel) on (**A**) hydrogen peroxide content, and (**B**) superoxide anion content in tomato on 10th day of stress. The results were presented as mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted by small letters, given that the means with same letters are not statistically significant at $p = 0.05$.

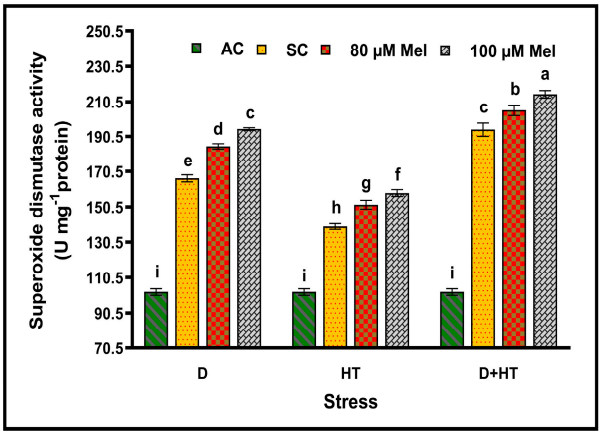
The effect of stress, foliar spray, and the interaction of stress and foliar spray was significant ($p < 0.05$) for malondialdehyde (MDA) content and electrolyte leakage (EL) (Figure 7A,B). Among the stresses, D + HT-stressed plants showed increased MDA contents and electrolyte leakage to a higher level than HT or D stresses (Figure 7A,B). Among the foliar sprays, 100 µM melatonin-treated plants had decreased MDA content and electrolyte leakage level than in other treatments (Figure 7A,B). A greater decrease in MDA and electrolyte leakage was observed under D + 100 µM melatonin-sprayed plants than HT + 100 µM melatonin and D + HT + 100 µM melatonin-sprayed plants (Figure 7A,B).



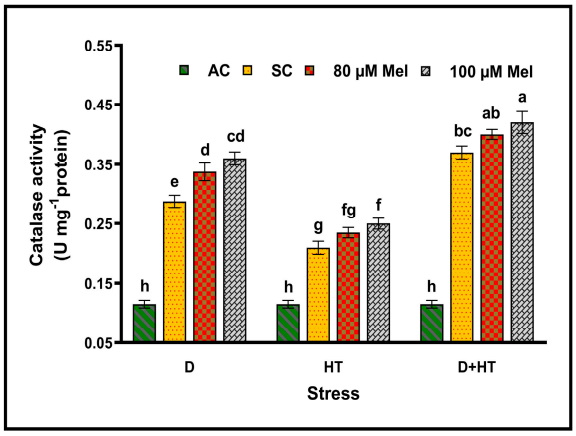
(**A**) Malondialdehyde content (**B**) Electrolyte leakage

Figure 7. Effect of stress (drought—D, high temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM melatonin—80 µM Mel, and 100 µM melatonin—100 µM Mel) on (**A**) malondialdehyde content and (**B**) electrolyte leakage in tomato on 10th day of stress. The results were presented as mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted by small letters, given that the means with same letters are not statistically significant at $p = 0.05$.

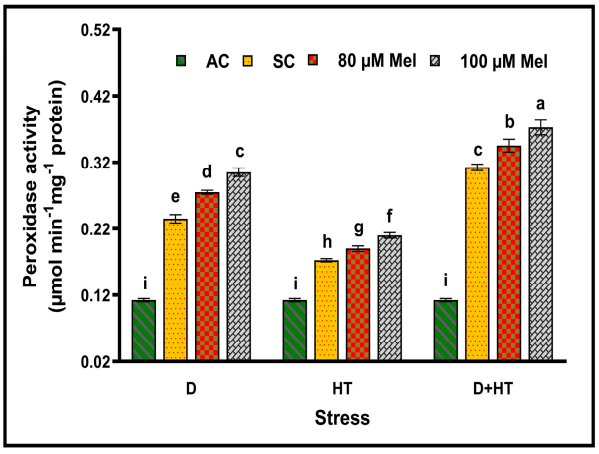
The antioxidant enzymes, viz., SOD (Figure 8A), CAT (Figure 8B), POD (Figure 8C), APX (Figure 8D), and GR (Figure 8E), were significantly ($p < 0.05$) influenced by stress, foliar spray, and the interaction of stress and foliar spray (Figure 8A–E). Among the stresses, SOD, CAT, and POD activity was higher under D + HT stress than under D or HT stress. In contrast, the same treatment showed less activity of APX and GR (Figure 8A–E). Among the foliar sprays, increased SOD, CAT, and POD enzyme activity was recorded in 100 µM melatonin-treated plants compared to other foliar spray treatments (Figure 8A–C). D + 100 µM melatonin-sprayed plants had an increased SOD (17%), CAT (24%), and POD (27%) activity than HT + −100 µM melatonin-treated plants and D + HT +100 µM melatonin-treated plants (Figure 8A–C). A similar trend was observed for APX and GR enzyme activity (Figure 8D,E).



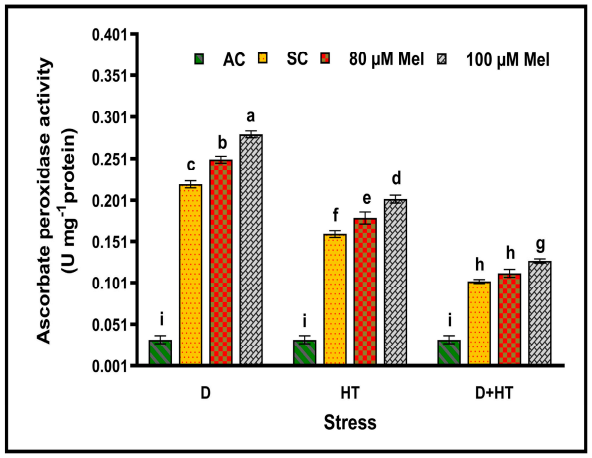
(**A**) Superoxide dismutase



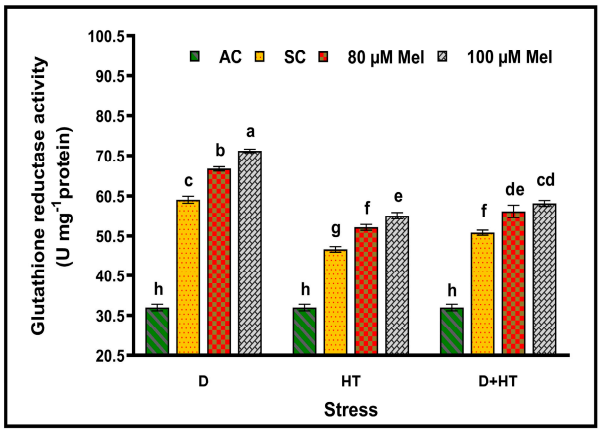
(**B**) Catalase



(**C**) Peroxidase



(**D**) Ascorbate peroxidase



(**E**) Glutathione reductase

Figure 8. Effect of stress (drought—D, high temperature—HT, and D + HT) and foliar spray (irrigated control—AC, stress control—SC, 80 µM melatonin—80 µM Mel, and 100 µM melatonin—100 µM Mel) on (**A**) superoxide dismutase (SOD), (**B**) catalase (CAT), (**C**) peroxidase (POD), (**D**) ascorbate peroxidase (APX), and (**E**) glutathione reductase (GR) enzyme activity in tomato on 10th day of stress. The results were presented as mean of four replications and standard error of means (SEM). Based on analysis of variance (ANOVA), the least significant difference test ($LSD_{5\%}$) was used for means comparison. The significance was denoted by small letters, given that the means with same letters are not statistically significant at $p = 0.05$.

The effect of stress, foliar spray, and the interaction of stress and foliar spray was significant ($p < 0.05$) for the relative tolerance index and fruit yield (Table 1). Among the stresses, a greater decrease in relative tolerance index was observed in D + HT-stressed plants than for individual stresses (Table 1). Among the foliar sprays, a higher relative tolerance index was observed in 100 µM melatonin-treated plants than for other foliar spray treatments (Table 1). The HT + 100 µM melatonin-treated plants had an increased (86%) relative tolerance index (Table 1) compared to the D + 100 µM melatonin (74%) and D + HT and 100 µM melatonin (56%) groups.

Table 1. Effect of different stress and melatonin treatment on relative tolerance index and fruit yield in tomato.

Parameters	Treatments	D	HT	D + HT
Relative tolerance index (%)	Stress Control	55.7 ± 1.56 ^{e}	70.1 ± 2.16 ^{cd}	45.9 ± 2.69 ^{f}
	80 µM melatonin	65.9 ± 2.61 ^{d}	79.4 ± 2.63 ^{b}	51.5 ± 1.57 ^{ef}
	100 µM melatonin	73.6 ± 1.65 ^{bc}	85.9 ± 3.36 ^{a}	55.9 ± 2.49 ^{e}
Yield (kg plant^{-1})	Absolute Control	3.84 ± 0.08 ^{a}	3.84 ± 0.08 ^{a}	3.84 ± 0.08 ^{a}
	Stress Control	2.22 ± 0.06 ^{d}	1.65 ± 0.03 ^{g}	1.07 ± 0.04 ^{i}
	80 µM melatonin	2.55 ± 0.02 ^{c}	1.82 ± 0.05 ^{f}	1.15 ± 0.03 ^{i}
	100 µM melatonin	2.84 ± 0.05 ^{b}	2.04 ± 0.04 ^{e}	1.35 ± 0.03 ^{h}

The data represent the mean of four replications and the error bars represent SEM. The means with different letters are significantly different at $p = 0.05$. The stress treatments represented as drought (D), high temperature (HT), and combined drought and high temperature (D + HT); foliar treatments represented as irrigated control (AC), stress control (SC), 80 µM melatonin (80 µM Mel), and 100 µM melatonin (100 µM Mel).

Among the stresses, compared to HT and D + HT stress, D-stressed plants had increased fruit yield (Table 1). Among the foliar sprays, 100 µM melatonin-treated plants showed increased fruit yield compared to other foliar spray treatments (Table 1). The plants treated with D + 100 µM melatonin had an increased fruit yield (32%) compared to plants treated with HT + 100 µM melatonin (23%) and D + HT + 100 µM melatonin (16%) (Table 1).

4. Discussion

Abiotic stress, viz., drought or high temperature, affects the productivity of horticultural crops to a greater extent ranging from 50% to 70% [64]. The effect of drought (D) or high temperature (HT) either individually or in combination triggers ROS production that impairs the photosynthetic membrane and thylakoid membrane due to imbalanced antioxidant activity that results in increased levels of lipid peroxidation and ion leakage [65]. As an antioxidant booster, exogenous melatonin is used in the current study to decrease the stress-induced oxidative damage [66]. Similarly, previous findings on tomatoes revealed that exogenous melatonin (100 µM) has a prominent effect on mitigating ROS-induced oxidative damage [67,68]. In addition, many investigators have reported that the individual effects of D or HT stress can be mitigated via exogenous melatonin application in maize [17], soyabean [18], tomato [20], and strawberry [21], but little information is available on the effect of melatonin under combined drought or HT stress.

The chlorophyll index measures the chlorophyll content and is directly associated with photosynthetic efficiency [69]. This study suggested that D, HT, or D + HT stress decreased the chlorophyll index, and it could be associated with thylakoid membrane damage or decreased 5-aminolevulinate dehydratase enzyme activity. Our research results were similar to the findings of Din et al. [70]. Moreover, D + HT stress-treated plants showed a more decreased chlorophyll index, which evidenced that the effects of combined stress are predominant over individual D or HT stress [71,72]. However, the findings of our study resulted that the exogenous melatonin spray under D or HT stress, individually or in combination, increased the chlorophyll index over the stress-control group, which could be associated with reduced activity of chlorophyll degradation enzymes. These results agree with Yang et al. [15].

The decreased P_n under abiotic stress could be due to damage in the site of light reaction situated in the thylakoid membrane and carbon metabolism [73]. Drought decreased P_n is mediated by a turgor-loss-induced stomatal closure mechanism, which resulted in a decrease in g_s [74]. In contrast, decreased P_n under HT stress occurs due to biochemical changes of photosynthetic enzymes [75]. Similarly, the process of photosynthesis is examined in the present study, which results in decreased stomatal conductance and photosynthetic rate and increased intercellular CO_2 concentration and transpiration rate under D, HT, or D + HT stress. Our results were corroborated by the reports of Benavides et al. [63]. However, melatonin spray under D-stressed plants increased the g_s and P_n rate more than in stress-control plants, proving that melatonin could acclimate the tomato plants to withstand the stress. Similar findings of Altaf et al. [20] reported that melatonin pretreatment in tomato restored the gas exchange parameters through reducing the negative effects of stress. The results of this study also imply that melatonin could regulate the balanced flow of electrons in PSII, which prevents chlorophyll pigment degradation and decreases thylakoid membrane damage (F_0/F_m), which could upregulate the PSII photochemistry and therefore enhance photosynthesis. Similarly, the results of Arena et al. [76] follow the same trend.

Free radical production is significantly higher under D or HT stress; in particular, increased ROS production was found to have more adverse effects under combined stress [77]. Our present study revealed that the ROS content was enhanced under D, HT, or D + HT stress, which could result in oxidative damage. Among the individual stresses, plants exposed to HT stress showed increased membrane damage, indicating that HT is more deleterious than D stress. The severity of oxidative damage caused by H_2O_2 and O_2^- was assessed via histochemical staining, and the result indicated that D + HT stress showed a tremendous increase in ROS production. The results of our study agree with the report of Hussain et al. [78] on maize. In contrast, the foliar spray of melatonin decreased ROS production more than the stress-control. Decreased ROS production would reduce the levels of MDA content and electrolyte leakage that improve membrane integrity. The results were supported by Fahad et al. [79]. Also, few results convinced that increased membrane integrity under stress could be due to increased antioxidants enzymes activity in peach [80], and pepper [81,82].

The antioxidant enzymes, viz., superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase, were increased under individual and combined D or HT stress [83,84]. The results of this study indicate that under HT and D + HT stress, the activity of antioxidant enzymes, viz., SOD, CAT, and POD, was found to be increased, while APX and GR activity was found to be insufficient to scavenge free radicals. Our results are similar to Ayidin et al. [85] and Duan et al. [86] for tomato. Therefore, foliar spray of melatonin (100 µM) supplementation increased the SOD, CAT, and POD activity compared to stress-control plants. This trend is similar to the findings of Zandalinas et al. [87], suggesting that activation of antioxidant enzymes might be the reason for decreased membrane damage in citrus. In addition, APX removes H_2O_2 similar to CAT and POD, which cope to withstand combined drought and high-temperature stress [88]. In our study, APX and GR activity showed higher increases in D + 100 µM melatonin than HT + 100 µM melatonin and D + HT + 100 µM melatonin-treated plants. Although melatonin spray is effective under all stress, D + 100 µM predominantly mitigates the negative effects through increasing the antioxidant enzymes over increased ROS production, thereby maintaining redox homeostasis [89]. The results were comparable to Huang et al. [73] for maize and Raja et al. [90] for tomato, as melatonin keeps the equilibrium between ROS generation and antioxidant enzyme activity under stress.

To determine whether melatonin's foliar application could help mitigate stress, we calculated the relative tolerance index (RTI) based on the stomatal conductance in stressed and unstressed plants [63]. Plants normally depend on transpiration, a cooling mechanism, to escape drought and high-temperature stress [91]. In such conditions, responses of stomatal opening and closing under D or HT that depend on g_s were studied in detail [92,93]. Our

results showed an increased RTI (70%) under HT stress compared to D (56%) and D + HT stress (46%). The trend of RTI is similar to g_s. However, the RTIs of D + 100µM melatonin, HT + 100µM melatonin, and D + HT + 100µM melatonin-sprayed plants were 74%, 86%, and 56%, respectively. The above finding proves that foliar application of melatonin can be the best crop management strategy to increase crop stress tolerance [7]. In addition, abiotic stress, viz., D or HT, adversely affects crop productivity in horticultural crops [94] and, therefore, intensive efforts were taken to improve stress tolerance to meet global food demand [95]. In recent years, melatonin-related studies also reported on the detrimental effects of D, HT, or D + HT stress on crop yield for lentil [96], moringa [97], and tomato [34]. Our study showed that foliar application of melatonin under all stresses increased the fruit yield, and this could be due to sustained photosynthesis under stressful environments through efficient activation of the antioxidant defense system.

5. Conclusions

In summary, D, HT, or D + HT stress can increase the production of ROS, which could increase membrane damage due to poor antioxidant activity. Among the stresses, D + HT stress is more detrimental than HT and D stress alone. The foliar spray of 100 µM melatonin under all stress decreased the ROS more than stress-control, proving its antioxidant potential, resulting in lower thylakoid membrane damage and increased photosynthetic rate and fruit yield in tomato. Therefore, exogenous melatonin application effectively mitigates the negative effects of D, HT, or D + HT stress through increasing the antioxidant activity which protects the photosynthetic system from oxidative damage. The current study on melatonin will help the researchers to understand how plants cope to withstand D, HT, or D + HT stress. Since a few years, melatonin is gaining interest among the researchers, although topics related to mitigation of combined stresses were recently under progress. There is a lack of ideas on how melatonin functions effectively in plant systems and how its mechanisms related to foliar uptake and translocation overcome stress Amidst difficulties, the pathways involved in melatonin biosynthesis and its associated genes, melatonin signaling and its regulation, and crosstalk with other hormones under abiotic stress need to be explored in future. Future research may also aim to focus on unexplored parts of the anisotropic or isotropic stomatal behavior and its mechanisms under stress to understand the photosynthetic process in depth, which could also be an effective strategy to improve crop productivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9060673/s1, Table S1: Role of melatonin in drought and high-temperature stress on crop yield; Tables S2–S16: Mean and ANOVA for physiological and yield traits.

Author Contributions: Conceptualization, M.K.K.A., S.A., K.M.K., S.R. (Swarnapriya Ramakrishnan), M.S., R.M., G.V. and P.R.B.V.; methodology, M.K.K.A., S.A., K.M.K., P.R.B.V., V.D., R.V., B.P. and S.R. (Sivakumar Rathinavelu); software, M.K.K.A.; validation, M.K.K.A., S.A., K.M.K., S.R. (Swarnapriya Ramakrishnan) and M.S.; statistical analysis, M.K.K.A. and S.A.; endnote, M.K.K.A.; investigation, S.A. and K.M.K.; data curation, M.K.K.A. and S.A.; writing—original draft preparation, M.K.K.A., S.A. and D.M.; review and editing, M.K.K.A., S.A., D.M., K.M.K., S.R. (Swarnapriya Ramakrishnan) and M.S.; visualization, M.K.K.A., S.A., K.M.K., S.R. (Swarnapriya Ramakrishnan) and M.S.; supervision, S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

D	Drought
HT	High-temperature
D + HT	Combined drought and high-temperature
AT	Ambient temperature
SOD	Superoxide dismutase
CAT	Catalase
POD	Peroxidase
APX	Ascorbic peroxidase
GR	Glutathione reductase
ROS	Reactive oxygen species
PSII	Photosystem II
OTC	Open top chamber
PEG	Polyethylene glycol
SPAD	Soil plant analysis development
P_n	Photosynthetic rate
E	Transpiration rate
g_s	Stomatal conductance
Ci	Intercellular CO_2 concentration
H_2O_2	Hydrogen peroxide
O_2^-	Superoxide anion
NBT	Nitroblue tetrazolium
DAB	3,3- diaminobenzidine
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid
EC	Electrical conductivity
EL	Electrolyte leakage
PVP	Poly vinyl pyrrolidone
EDTA	Ethylene diamine tetraacetic acid
RTI	Relative tolerance index

References

1. Bouabdelli, S.; Zeroual, A.; Meddi, M.; Assani, A. Impact of temperature on agricultural drought occurrence under the effects of climate change. *Theor. Appl. Climatol.* **2022**, *148*, 191–209. [CrossRef]
2. Dos Santos, T.B.; Ribas, A.F.; De Souza, S.G.H.; Budzinski, I.G.F.; Domingues, D.S. Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. *Stresses* **2022**, *2*, 113–135. [CrossRef]
3. Portner, H.O.; Roberts, D.C.; Adams, H.; Adler, C.; Aldunce, P.; Ali, E.; Begum, R.A.; Betts, R.; Kerr, R.B.; Biesbroek, R. *Climate Change 2022: Impacts, Adaptation and Vulnerability*; IPCC: Geneva, Switzerland, 2022; p. 3056.
4. Basavaraj, P.; Rane, J. Avenues to realize potential of phenomics to accelerate crop breeding for heat tolerance. *Plant Physiol. Rep.* **2020**, *25*, 594–610. [CrossRef]
5. Goswami, M.; Suresh, D. Plant growth-promoting rhizobacteria-alleviators of abiotic stresses in soil: A review. *Pedosphere* **2020**, *30*, 40–61. [CrossRef]
6. Islam, M.T. Applications of nanomaterials for future food security: Challenges and prospects. *Malays. J. Halal Res.* **2019**, *2*, 6–9. [CrossRef]
7. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S. Crop production under drought and heat stress: Plant responses and management options. *Front. Plant Sci.* **2017**, *8*, 1147. [CrossRef] [PubMed]
8. Zia, R.; Nawaz, M.S.; Siddique, M.J.; Hakim, S.; Imran, A. Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiol. Res.* **2021**, *242*, 126626. [CrossRef] [PubMed]
9. Muhammed, M.A.; Mohamed, A.K.S.; Qayyum, M.F.; Haider, G.; Ali, H.A. Physiological response of mango transplants to phytohormones under salinity stress. *Sci. Hortic.* **2022**, *296*, 110918. [CrossRef]
10. Barickman, T.C.; Adhikari, B.; Sehgal, A.; Walne, C.H.; Reddy, K.R.; Gao, W. Drought and elevated CO_2 impacts on photosynthesis and biochemicals of basil (*Ocimum basilicum* L.). *Stresses* **2021**, *1*, 223–237. [CrossRef]
11. Mathur, S.; Agrawal, D.; Jajoo, A. Photosynthesis: Response to high temperature stress. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 116–126. [CrossRef]

12. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
13. Cen, H.; Wang, T.; Liu, H.; Tian, D.; Zhang, Y. Melatonin application improves salt tolerance of alfalfa (*Medicago sativa* L.) by enhancing antioxidant capacity. *Plants* **2020**, *9*, 220. [CrossRef]
14. Khan, T.A.; Fariduddin, Q.; Nazir, F.; Saleem, M. Melatonin in business with abiotic stresses in plants. *Physiol. Mol. Biol. Plants* **2020**, *26*, 1931–1944. [CrossRef]
15. Yang, S.J.; Huang, B.; Zhao, Y.Q.; Hu, D.; Chen, T.; Ding, C.-B.; Chen, Y.-E.; Yuan, S.; Yuan, M. Melatonin enhanced the tolerance of *Arabidopsis thaliana* to high light through improving anti-oxidative system and photosynthesis. *Front. Plant Sci.* **2021**, *12*, 752584. [CrossRef]
16. Yu, R.; Zuo, T.; Diao, P.; Fu, J.; Fan, Y.; Wang, Y.; Zhao, Q.; Ma, X.; Lu, W.; Li, A.; et al. Melatonin enhances seed germination and seedling growth of *Medicago sativa* under salinity via a putative melatonin receptor MsPMTR1. *Front. Plant Sci.* **2021**, *12*, 702875. [CrossRef]
17. Ahmad, S.; Muhammad, I.; Wang, G.Y.; Zeeshan, M.; Yang, L.; Ali, I.; Zhou, X.B. Ameliorative effect of melatonin improves drought tolerance by regulating growth, photosynthetic traits and leaf ultrastructure of maize seedlings. *BMC Plant Biol.* **2021**, *21*, 368. [CrossRef]
18. Imran, M.; Aaqil Khan, M.; Shahzad, R.; Bilal, S.; Khan, M.; Yun, B.-W.; Khan, A.L.; Lee, I.-J. Melatonin ameliorates thermotolerance in soybean seedling through balancing redox homeostasis and modulating antioxidant defense, phytohormones and polyamines biosynthesis. *Molecules* **2021**, *26*, 5116. [CrossRef] [PubMed]
19. Li, J.; Liu, Y.; Zhang, M.; Xu, H.; Ning, K.; Wang, B.; Chen, M. Melatonin increases growth and salt tolerance of *Limonium bicolor* by improving photosynthetic and antioxidant capacity. *BMC Plant Biol.* **2022**, *22*, 16. [CrossRef] [PubMed]
20. Altaf, M.A.; Shahid, R.; Ren, M.X.; Naz, S.; Altaf, M.M.; Khan, L.U.; Tiwari, R.K.; Lal, M.K.; Shahid, M.A.; Kumar, R.; et al. Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. *Antioxidants* **2022**, *11*, 309. [CrossRef]
21. Okatan, V.; Askın, M.A.; Polat, M.; Bulduk, I.; Colak, A.M.; Guclu, S.F.; Kahramanoglu, İ.; Tallarita, A.V.; Caruso, G. Effects of melatonin dose on fruit yield, quality, and antioxidants of strawberry cultivars grown in different crop systems. *Agriculture* **2023**, *13*, 71. [CrossRef]
22. Cano, A.; Giraldo-Acosta, M.; Garcia-Sanchez, S.; Hernandez-Ruiz, J.; Arnao, M.B. Effect of melatonin in broccoli post-harvest and possible melatonin ingestion level. *Plants* **2022**, *11*, 2000. [CrossRef]
23. Wang, K.; Cai, S.; Xing, Q.; Qi, Z.; Fotopoulos, V.; Yu, J.; Zhou, J. Melatonin delays dark induced leaf senescence by inducing miR171b expression in tomato. *J. Pineal Res.* **2022**, *72*, e12792. [CrossRef]
24. Zeng, W.; Mostafa, S.; Lu, Z.; Jin, B. Melatonin-mediated abiotic stress tolerance in plants. *Front. Plant Sci.* **2022**, *13*, 847175. [CrossRef]
25. Tripathi, A.; Tripathi, D.K.; Chauhan, D.; Kumar, N.; Singh, G. Paradigms of climate change impacts on some major food sources of the world: A review on current knowledge and future prospects. *Agric. Ecosyst. Environ.* **2016**, *216*, 356–373. [CrossRef]
26. Ahammed, G.J.; Xu, W.; Liu, A.; Chen, S. Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* L. *Environ. Exp. Bot.* **2019**, *161*, 303–311. [CrossRef]
27. Yang, L.; Bu, S.; Zhao, S.; Wang, N.; Xiao, J.; He, F.; Gao, X. Transcriptome and physiological analysis of increase in drought stress tolerance by melatonin in tomato. *PLoS ONE* **2022**, *17*, e0267594. [CrossRef] [PubMed]
28. Raiola, A.; Rigano, M.M.; Calafiore, R.; Frusciante, L.; Barone, A. Enhancing the health-promoting effects of tomato fruit for biofortified food. *Mediat. Inflamm.* **2014**, *2014*, 16. [CrossRef]
29. Zhou, R.; Yu, X.; Ottosen, C.O.; Rosenqvist, E.; Zhao, L.; Wang, Y.; Yu, W.; Zhao, T.; Wu, Z. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biol.* **2017**, *17*, 24. [CrossRef] [PubMed]
30. Tiwari, R.K.; Lal, M.K.; Naga, K.C.; Kumar, R.; Chourasia, K.N.; Subhash, S.; Kumar, D.; Sharma, S. Emerging roles of melatonin in mitigating abiotic and biotic stresses of horticultural crops. *Sci. Hortic.* **2020**, *272*, 109592. [CrossRef]
31. Martinez, V.; Nieves-Cordones, M.; Lopez-Delacalle, M.; Rodenas, R.; Mestre, T.C.; Garcia-Sanchez, F.; Rubio, F.; Nortes, P.A.; Mittler, R.; Rivero, R.M. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules* **2018**, *23*, 535. [CrossRef]
32. Ahammed, G.J.; Xu, W.; Liu, A.; Chen, S. COMT1 silencing aggravates heat stress-induced reduction in photosynthesis by decreasing chlorophyll content, photosystem II activity, and electron transport efficiency in tomato. *Front. Plant Sci.* **2018**, *9*, 998. [CrossRef]
33. Zhou, R.; Yu, X.; Ottosen, C.O.; Zhang, T.; Wu, Z.; Zhao, T. Unique miRNAs and their targets in tomato leaf responding to combined drought and heat stress. *BMC Plant Biol.* **2020**, *20*, 107. [CrossRef]
34. Ibrahim, M.F.M.; Elbar, O.H.A.; Farag, R.; Hikal, M.; El-Kelish, A.; El-Yazied, A.A.; Alkahtani, J.; El-Gawad, H.G.A. Melatonin Counteracts Drought Induced Oxidative Damage and Stimulates Growth, Productivity and Fruit Quality Properties of Tomato Plants. *Plants* **2020**, *9*, 1276. [CrossRef] [PubMed]
35. Sadak, M.S.; Bakry, B.A. Alleviation of drought stress by melatonin foliar treatment on two flax varieties under sandy soil. *Physiol. Mol. Biol. Plants* **2020**, *26*, 907–919. [CrossRef] [PubMed]
36. Wei, W.; Li, Q.T.; Chu, Y.N.; Reiter, R.J.; Yu, X.-M.; Zhu, D.H.; Zhang, W.K.; Ma, B.; Lin, Q.; Zhang, J.S. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J. Exp. Bot.* **2015**, *66*, 695–707. [CrossRef]

37. Hu, W.; Zhang, J.; Wu, Z.; Loka, D.A.; Zhao, W.; Chen, B.; Wang, Y.; Meng, Y.; Zhou, Z.; Gao, L. Effects of single and combined exogenous application of abscisic acid and melatonin on cotton carbohydrate metabolism and yield under drought stress. *Ind. Crops Prod.* **2022**, *176*, 114302. [CrossRef]
38. Hosseini, M.S.; Samsampour, D.; Zahedi, S.M.; Zamanian, K.; Rahman, M.M.; Mostofa, M.G.; Tran, L.P. Melatonin alleviates drought impact on growth and essential oil yield of lemon verbena by enhancing antioxidant responses, mineral balance, and abscisic acid content. *Physiol. Plant* **2021**, *172*, 1363–1375. [CrossRef]
39. Jafari, M.; Shahsavar, A. The Effect of Foliar Application of Melatonin on Changes in Secondary Metabolite Contents in Two Citrus Species under Drought Stress Conditions. *Front. Plant Sci.* **2021**, *12*, 692735. [CrossRef]
40. Cao, L.; Kou, F.; Zhang, M.; Jin, X.; Ren, C.; Yu, G.; Zhang, Y.; Wang, M. Effect of Exogenous Melatonin on the Quality of Soybean and Natto Products under Drought Stress. *J. Chem.* **2021**, *2021*, 8847698. [CrossRef]
41. Zou, J.; Jin, X.; Zhang, Y.; Ren, C.; Zhang, M.; Wang, M. Effects of melatonin on photosynthesis and soybean seed growth during grain filling under drought stress. *Photosynthetica* **2019**, *57*, 512–520. [CrossRef]
42. Fan, X.; Zhao, J.; Sun, X.; Zhu, Y.; Li, Q.; Zhang, L.; Zhao, D.; Huang, L.; Zhang, C.; Liu, Q. Exogenous Melatonin Improves the Quality Performance of Rice under High Temperature during Grain Filling. *Agronomy* **2022**, *12*, 949. [CrossRef]
43. Khan, M.N.; Khan, Z.; Luo, T.; Liu, J.; Rizwan, M.; Zhang, J.; Xu, Z.; Wu, H.; Hu, L. Seed priming with gibberellic acid and melatonin in rapeseed: Consequences for improving yield and seed quality under drought and non-stress conditions. *Ind. Crops Prod.* **2020**, *156*, 112850. [CrossRef]
44. Zou, J.; Yu, H.; Yu, Q.; Jin, X.; Cao, L.; Wang, M.; Wang, M.; Ren, C.; Zhang, Y. Physiological and UPLC-MS/MS widely targeted metabolites mechanisms of alleviation of drought stress-induced soybean growth inhibition by melatonin. *Ind. Crops Prod.* **2021**, *163*, 113323. [CrossRef]
45. Iman Khesali, L.; Saeed, P.; Sanam Safaei, C.; Behrooz, S. Evaluating drought stress tolerance in different *Camellia sinensis* L. cultivars and effect of melatonin on strengthening antioxidant system. *Sci. Hortic.* **2023**, *307*, 111517. [CrossRef]
46. Gholami, R.; Hoveizeh, N.F.; Zahedi, S.M.; Gholami, H.; Carillo, P. Melatonin alleviates the adverse effects of water stress in adult olive cultivars (*Olea europea* cv. Sevillana & Roughani) in field condition. *Agric. Water Manag.* **2022**, *269*, 107681. [CrossRef]
47. Hojjati, M.; Ghanbari Jahromi, M.; Abdosi, V.; Mohammadi Torkashvanda, A. Exogenous Melatonin Improved Water Status, Antioxidant Capacity, Fruit Quality, and Altered Fatty Acid Profile of Sweet Cherry (*Prunus avium* L.) under Different Irrigation Regimes. Available online: https://ssrn.com/abstract=4257898 (accessed on 25 October 2022).
48. Crop Production Guide of Horticulture Crops. Directorate of Horticulture and Plantation Crops and TNAU. Available online: https://agritech.tnau.ac.in/pdf/HORTICULTURE.pdf (accessed on 24 March 2023).
49. Liu, J.; Wang, W.; Wang, L.; Sun, Y. Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul.* **2015**, *77*, 317–326. [CrossRef]
50. Dalal, V.K.; Tripathy, B.C. Water-stress induced downsizing of light-harvesting antenna complex protects developing rice seedlings from photo-oxidative damage. *Sci. Rep.* **2018**, *8*, 5955. [CrossRef]
51. Lei, C.; Ye, M.; Li, C.; Gong, M. H_2O_2 Participates in the Induction and Formation of Potato Tubers by Activating Tuberization-Related Signal Transduction Pathways. *Agronomy* **2023**, *13*, 1398. [CrossRef]
52. Velikova, V.; Loreto, F. On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant Cell Environ.* **2005**, *28*, 318–327. [CrossRef]
53. Doke, N. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiol. Plant Pathol.* **1983**, *23*, 345–357. [CrossRef]
54. Heath, R.L.; Packer, L. Effect of light on lipid peroxidation in chloroplasts. *Biochem. Biophys. Res. Commun.* **1965**, *19*, 716–720. [CrossRef]
55. Sullivan, C.Y.; Ross, W.M. Selecting for Drought and Heat Resistance in Grain Sorghum. In *Stress Physiology in Crop Plants*; Mussell, H., Staples, R.C., Eds.; John Wiley and Sons: New York, NY, USA, 1979; Volume 21, pp. 263–281.
56. Camejo, D.; Jimenez, A.; Alarcon, J.J.; Torres, W.; Gomez, J.M.; Sevilla, F. Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. *Funct. Plant Biol.* **2006**, *33*, 177–187. [CrossRef]
57. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
58. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef] [PubMed]
59. Aebi, H. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126. [CrossRef] [PubMed]
60. Kumar, K.B.; Khan, P.A. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv PR 202) leaves during senescence. *Indian J. Exp. Biol.* **1982**, *20*, 412–416.
61. Chen, G.-X.; Asada, K. Ascorbate peroxidase in tea leaves: Occurrence of two isozymes and the differences in their enzymatic and molecular properties. *Plant Cell Physiol.* **1989**, *30*, 987–998. [CrossRef]
62. Smith, I.K.; Vierheller, T.L.; Thorne, C.A. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis (2-nitrobenzoic acid). *Anal. Biochem.* **1988**, *175*, 408–413. [CrossRef]
63. Pantoja-Benavides, A.D.; Garces-Varon, G.; Restrepo-Díaz, H. Foliar growth regulator sprays induced tolerance to combined heat stress by enhancing physiological and biochemical responses in rice. *Front. Plant Sci.* **2021**, *12*, 702892. [CrossRef]
64. Boyer, J.S. Plant productivity and environment. *Science* **1982**, *218*, 443–448. [CrossRef]

65. Hasanuzzaman, M.; Bhuyan, M.; Parvin, K.; Bhuiyan, T.F.; Anee, T.I.; Nahar, K.; Hossen, M.S.; Zulfiqar, F.; Alam, M.M.; Fujita, M. Regulation of ROS Metabolism in Plants under Environmental Stress: A Review of Recent Experimental Evidence. *Int. J. Mol. Sci.* **2020**, *21*, 8695. [CrossRef]
66. Sachdev, S.; Ansari, S.A.; Ansari, M.I.; Fujita, M.; Hasanuzzaman, M. Abiotic Stress and Reactive Oxygen Species: Generation, Signaling, and Defense Mechanisms. *Antioxidants* **2021**, *10*, 277. [CrossRef] [PubMed]
67. Mushtaq, N.; Iqbal, S.; Hayat, F.; Raziq, A.; Ayaz, A.; Zaman, W. Melatonin in Micro-Tom Tomato: Improved Drought Tolerance via the Regulation of the Photosynthetic Apparatus, Membrane Stability, Osmoprotectants, and Root System. *Life* **2022**, *12*, 1922. [CrossRef]
68. Jahan, M.S.; Shu, S.; Wang, Y.; Chen, Z.; He, M.; Tao, M.; Sun, J.; Guo, S. Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biol.* **2019**, *19*, 414. [CrossRef]
69. Liu, C.; Liu, Y.; Lu, Y.; Liao, Y.; Nie, J.; Yuan, X.; Chen, F. Use of a leaf chlorophyll content index to improve the prediction of above-ground biomass and productivity. *PeerJ* **2019**, *6*, e6240. [CrossRef]
70. Din, J.; Khan, S.; Ali, I.; Gurmani, A.L. Physiological and agronomic response of canola varieties to drought stress. *J. Anim. Plant Sci.* **2011**, *21*, 78–82.
71. Dreesen, F.E.; De Boeck, H.J.; Janssens, I.A.; Nijs, I. Summer heat and drought extremes trigger unexpected changes in productivity of a temperate annual/biannual plant community. *Environ. Exp. Bot.* **2012**, *79*, 21–30. [CrossRef]
72. Yousaf, M.I.; Riaz, M.W.; Jiang, Y.; Yasir, M.; Aslam, M.Z.; Hussain, S.; Sajid Shah, S.A.; Shehzad, A.; Riasat, G.; Manzoor, M.A.; et al. Concurrent Effects of Drought and Heat Stresses on Physio-Chemical Attributes, Antioxidant Status and Kernel Quality Traits in Maize (*Zea mays* L.) Hybrids. *Front. Plant Sci.* **2022**, *13*, 898823. [CrossRef]
73. Huang, B.; Chen, Y.-E.; Zhao, Y.-Q.; Ding, C.-B.; Liao, J.-Q.; Hu, C.; Zhou, L.-J.; Zhang, Z.-W.; Yuan, S.; Yuan, M. Exogenous Melatonin Alleviates Oxidative Damages and Protects Photosystem II in Maize Seedlings under Drought Stress. *Front. Plant Sci.* **2019**, *10*, 677. [CrossRef]
74. Chaves, M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Ann. Bot.* **2009**, *103*, 551–560. [CrossRef]
75. Havaux, M. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant Cell Environ.* **1993**, *16*, 461–467. [CrossRef]
76. Arena, C.; Conti, S.; Francesca, S.; Melchionna, G.; Hajek, J.; Bartak, M.; Barone, A.; Rigano, M.M. Eco-Physiological Screening of Different Tomato Genotypes in Response to High Temperatures: A Combined Field-to-Laboratory Approach. *Plants* **2020**, *9*, 508. [CrossRef]
77. Zandalinas, S.I.; Fichman, Y.; Devireddy, A.R.; Sengupta, S.; Azad, R.K.; Mittler, R. Systemic signaling during abiotic stress combination in plants. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 13810–13820. [CrossRef] [PubMed]
78. Hussain, H.A.; Men, S.; Hussain, S.; Chen, Y.; Ali, S.; Zhang, S.; Zhang, K.; Li, Y.; Xu, Q.; Liao, C. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. *Sci. Rep.* **2019**, *9*, 3890. [CrossRef] [PubMed]
79. Fahad, S.; Hussain, S.; Saud, S.; Hassan, S.; Tanveer, M.; Ihsan, M.Z.; Shah, A.N.; Ullah, A.; Nasrullah; Khan, F.; et al. A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. *Plant Physiol. Biochem.* **2016**, *103*, 191–198. [CrossRef]
80. Cao, S.; Shao, J.; Shi, L.; Xu, L.; Shen, Z.; Chen, W.; Yang, Z. Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative damage. *Sci. Rep.* **2018**, *8*, 806. [CrossRef]
81. Korkmaz, A.; Deger, O.; Szafranska, K.; Koklu, S.; Karaca, A.; Yakupoglu, G.; Kocacinar, F. Melatonin effects in enhancing chilling stress tolerance of pepper. *Sci. Hortic.* **2021**, *289*, 110434. [CrossRef]
82. Li, Y.; Fan, Y.; Ma, Y.; Zhang, Z.; Yue, H.; Wang, L.; Li, J.; Jiao, Y. Effects of exogenous γ-aminobutyric acid (GABA) on photosynthesis and antioxidant system in pepper (*Capsicum annuum* L.) seedlings under low light stress. *J. Plant Growth Regul.* **2017**, *36*, 436–449. [CrossRef]
83. Giordano, M.; Petropoulos, S.A.; Rouphael, Y. Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. *Agriculture* **2021**, *11*, 463. [CrossRef]
84. Zhang, N.; Sun, Q.; Zhang, H.; Cao, Y.; Weeda, S.; Ren, S.; Guo, Y.D. Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot.* **2015**, *66*, 647–656. [CrossRef]
85. Aydin, S.; Buyuk, I.; Aras, E.S. Expression of SOD gene and evaluating its role in stress tolerance in NaCl and PEG stressed *Lycopersicum esculentum*. *Turk. J. Bot.* **2014**, *38*, 89–98. [CrossRef]
86. Duan, M.; Feng, H.L.; Wang, L.Y.; Li, D.; Meng, Q.W. Overexpression of thylakoidal ascorbate peroxidase shows enhanced resistance to chilling stress in tomato. *J. Plant Physiol.* **2012**, *169*, 867–877. [CrossRef]
87. Zandalinas, S.I.; Balfagón, D.; Arbona, V.; Gómez-Cadenas, A. Modulation of antioxidant defense system is associated with combined drought and heat stress tolerance in citrus. *Front. Plant Sci.* **2017**, *8*, 953. [CrossRef]
88. Koussevitzky, S.; Suzuki, N.; Huntington, S.; Armijo, L.; Sha, W.; Cortes, D.; Shulaev, V.; Mittler, R. Ascorbate peroxidase 1 plays a key role in the response of *Arabidopsis thaliana* to stress combination. *J. Biol. Chem.* **2008**, *283*, 34197–34203. [CrossRef]
89. Rabiatul Basria, S.M.N.M.; Simon, I.O. Reactive Oxygen Species, Cellular Redox Homeostasis and Cancer. In *Homeostasis—An Integrated Vision*; IntechOpen: London, UK, 2018. [CrossRef]

90. Raja, V.; Qadir, S.U.; Alyemeni, M.N.; Ahmad, P. Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in *Solanum lycopersicum*. *Biotechnology* **2020**, *10*, 208. [CrossRef]
91. Marchin, R.M.; Backes, D.; Ossola, A.; Leishman, M.R.; Tjoelker, M.G.; Ellsworth, D.S. Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. *Glob. Chang. Biol.* **2022**, *28*, 1133–1146. [CrossRef]
92. Drake, J.E.; Tjoelker, M.G.; Vårhammar, A.; Medlyn, B.E.; Reich, P.B.; Leigh, A.; Pfautsch, S.; Blackman, C.J.; Lopez, R.; Aspinwall, M.J. Trees tolerate an extreme heatwave via sustained transpirational cooling and increased leaf thermal tolerance. *Glob. Chang. Biol.* **2018**, *24*, 2390–2402. [CrossRef] [PubMed]
93. Mott, K.A.; Peak, D. Stomatal responses to humidity and temperature in darkness. *Plant Cell Environ.* **2010**, *33*, 1084–1090. [CrossRef] [PubMed]
94. Zheng, Y.; Wang, X.; Cui, X.; Wang, K.; Wang, Y.; He, Y. Phytohormones regulate the abiotic stress: An overview of physiological, biochemical, and molecular responses in horticultural crops. *Front. Plant Sci.* **2022**, *13*, 1095363. [CrossRef] [PubMed]
95. Dhankher, O.P.; Foyer, C.H. Climate resilient crops for improving global food security and safety. *Plant Cell Environ.* **2018**, *41*, 877–884. [CrossRef]
96. Sehgal, A.; Sita, K.; Kumar, J.; Kumar, S.; Singh, S.; Siddique, K.H.M.; Nayyar, H. Effects of drought, heat and their interaction on the growth, yield and photosynthetic function of lentil (*Lens culinaris*) genotypes varying in heat and drought sensitivity. *Front. Plant Sci.* **2017**, *8*, 1776. [CrossRef] [PubMed]
97. Sadak, M.S.; Abdalla, A.M.; Abd Elhamid, E.M.; Ezzo, M.I. Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress. *Bull. Natl. Res. Cent.* **2020**, *44*, 18. [CrossRef]