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Effects of melatonin foliar application on hot pepper growth and stress tolerance

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ABSTRACT

Purpose: Drought and waterlogging are one of the most severe abiotic stresses towards plant growth and development. The experiment was implemented in the Isfahan University of Technology's research greenhouse to examine the effect of melatonin foliar spraying in hot pepper plants under waterlogging and drought stresses.
Methods: This factorial experiment was performed as a complete randomized design (CRD) in triplicate. The treatments included controls, waterlogging, drought stress, and melatonin foliar spraying at 0 and 500 μM concentrations.
Results: The results demonstrated that the foliar melatonin spraying was more advantageous under drought and waterlogging stress conditions *via* increasing ammonium content, NR (nitrate reductase activity), chlorophyll content, proline, protein, transpiration, shoot length, root water content, amino acid alterations, and PIP gene expression.
Conclusions: Melatonin influenced the hot pepper's growth by affecting enzyme activity NR hormonal changes (ABA), amino acid changes, and PIP gene expression.

Introduction

In nature, plants are subjected to a variety of abiotic stresses, such as waterlogging, drought, and salinity; indeed all have impacts on crop growth, development, and production (Agnihotri, 2013). Drought stress reduces crop productivity by altering physiological and biochemical processes such as leaf stomatal activity (Paudel et al., 2021), photosynthesis activity (Ahmad et al., 2019), translocation, respiration, changes of ABA (abscisic acid) and ethylene (Jaspers and Kangasjarvi, 2010; Maksup et al., 2012), cellular redox immune function (M. Zhang et al., 2019), and metabolism (M. Zhang et al., 2019; Pinheiro and Chaves, 2011). Moreover, drought stress generates abscisic acid (ABA), which can induce stomatal closure and lower the internal carbon dioxide concentration (Ci) at high levels (Kong et al., 2016). In response to drought stress, plants have developed numerous metabolic adaptation strategies to preserve themselves against the harmful effects (Wang et al., 2021).

Carbon (C) and nitrogen (N) metabolism are two of the most important metabolic procedures in plants and they are inextricably linked (Cui et al., 2019). N absorption is critical in the adaptation of plant photosynthesis to drought stress. C metabolism provides energy as well as natural carbon skeletons for N assimilation and amino acid production (Q. Zhang et al., 2019); consequently, C and N metabolism stability is pivotal for drought resistance (Ren et al., 2020; Liu et al., 2013).

Waterlogging is a major abiotic stressor that reduces plant growth and development as well as agricultural crop productivity (Goyal et al., 2020; Zheng et al., 2017). Excess water causes saturated soil and a lack of oxygen which plants avoid by shifting plant metabolism toward anaerobic, glycolytic, and fermentative metabolism. Two most vital enzymes in fermentative metabolism are alcohol dehydrogenase and pyruvate decarboxylase, while ethanol as the main procedure byproduct is toxic to plant roots (Yamauchi et al., 2018).

Under waterlogging stress, the nitrogen uptake is reduced (Jitsuyama, 2017). Plants frequently exhibit N deficit after waterlogging deu to effecting on N metabolism enzymes, resulting in lower chlorophyll content and limiting plant photosynthetic ability, ultimately impeding plant growth and development (Jitsuyama, 2017). While crops are unable to withstand rhizosphere extra water, their metabolism suffers and changes. Excess water in the soil limits the amount of oxygen available for plant roots, consequently they suffer from a shortage of soil oxygen for aerobic metabolism (Drew, 1997; Alam et al., 2010).

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Damaging impacts of anoxia and hypoxia include cytoplasmic pH reduction, reactive oxygen species storage, and toxic metabolites, which are responsible for reducing growth and yield (Subbaiah and Sachs, 2003). The decrease in oxygen availability for the roots during floods has an impact on respiration and electron transportation. Furthermore, waterlogging causes leaf water shortages and photosynthetic restriction (Fiedler and Vepraskas, 2007). Waterlogging in plants has resulted in the development of a few adaptive processes in anaerobiosis. Waterlogging accelerates metabolic and structural changes such as the production of adventitious roots and aerenchyma (Drew, 1997). Alanine fermentation, ethanol, lactic acid sulfides, soluble Fe and Mn, acetaldehyde, and acetic and formic acids are produced under waterlogging circumstances (Fiedler and Vepraskas, 2007). Understanding how *Arabidopsis* (Klok et al., 2002), maize (Chang et al., 2000), and rice (Dubey et al., 2003) respond to low oxygen has mostly been achieved through genomic and proteomic approaches. It has been discovered that the process of protein synthesis in plant roots is significantly changed during anaerobiosis. These proteins are known as glycolysis enzymes or sugar-phosphate metabolic enzymes (Alam et al., 2010; Ahsan et al., 2007).

Some reports showed that the leaves' water potential did not change during the waterlogging treatment, confirming stomata's adaptive role in preventing leaf dehydration caused by decrease in root hydraulic conductivity. The stomata remain open and the leaf water remains constant if the relative water content does not change (Fiedler and Vepraskas, 2007). The soil reduction-oxidation potential decreases as waterlogging time increases, and hazardous materials such as sulfides, acetic and lactic acid, ethanol, acetaldehyde, formic acid, and soluble Fe and Mn are formed in cells (Fiedler and Vepraskas, 2007); accordingly, lack of oxygen is the most serious issue that plants face in waterlogged conditions, which is followed by the accumulation of poisonous substances. Furthermore, as a stress-responsive hormone, ABA aids in the coordination of plant growth under stress *via* signaling between the root and shoot systems, as well as influencing the expression of numerous aquaporins such as plasma membrane intrinsic proteins (PIPs). Aquaporins (AQPs), also known as MIPs (major intrinsic proteins), are membrane proteins that increase water and tiny uncharged molecule permeance. PIPs are divided into two categories: PIP1 and PIP2 (Danielson and Johanson, 2008). PIP2 proteins have high water-channel activity, whereas PIP1 proteins have low water permeability (Suga and Maeshima, 2004). AQPs control water transport, thus play a critical role in drought stress tolerance. The response of PIP genes to water restriction and ABA concentration differs between plants and organs. PIP genes have been discovered to participate in both ABA-dependent and -independent signaling pathways. Furthermore, the expression of specific PIPs was up- or down-regulated depending on the duration and severity of the stress, while others remained unaltered (Lian et al., 2006).

Hot peppers (*Capsicum* spp.) are one of the world's most important vegetables and spices, while consumed fresh, dried, or powdered (Perry et al., 2007). This plant has a variety of nutritious substances including antioxidants, vitamins A and C, and neutral and acidic phenolic compounds, whereas all may detract from the risk of degenerative, mutagenic, and chronic diseases (Sung et al., 2005). Pepper is a water-stress-sensitive plant, and drought stress reduces crop production. Fruit set is one of the most delicate stages (Ferrara et al., 2010). Studies indicated that drought stress lowered stomatal conductance and photosynthesis during hot pepper growth (Delfine et al., 2001), while waterlogging reduces photosynthetic rate, Fv/Fm ratio, and damage PSII, transpiration, and stomatal resistance, asides from crop yield in general (Masoumi et al., 2021).

Melatonin (N-acetyl-5-methoxytryptamine) is a new phytohormone that responds to abiotic stressors including drought (Ahmad et al., 2019). In abiotic stress conditions such as the presence of heavy metals, pathogen infections, drought, salinity, high temperature, besides UV radiation, melatonin acts as an anti-stress agent (Yao et al., 2021; Wang et al., 2020; Zhang et al., 2020). MzASMT overexpression in transgenic *Arabidopsis thaliana* increased melatonin synthesis and drought tolerance (Arnao and Hernandez-Ruiz, 2015). Previous studies demonstrated that melatonin improves plant stress resistance in response to abiotic stress by modulating C or N metabolism (Hu et al., 2016). It is able to increase stomatal conductance, photosynthetic rate, transpiration rate, mineral uptake, exudation of organic acid anions and phenolic compounds, hormonal regulation, sugar metabolism, and ROS scavenging during stress, and regulates antioxidant enzyme activity, which can alleviate oxidative damage to lipids, proteins, and nucleic acids (Ahmad et al., 2019). Although, the positive effect of melatonin on some plants has already been reported under various stresses, the present study was aimed to investigate the possible effects of melatonin on the growth and physiological aspects of hot pepper seedlings to alleviate drought and waterlogging stresses.

Material and methods

Plant materials and experimental design

The experiment was carried out in the research greenhouse of the Isfahan University of Technology to evaluate the effect of melatonin foliar spraying on hot pepper plants under waterlogging and drought stress. This factorial experiment was accomplished as a complete randomized design (CRD) with three replications. The treatment groups were involved as controls, waterlogging and drought stress, and melatonin foliar spraying at concentrations 0 and 500 μM. Water status treatments included optimum irrigation based on field capacity (C), and waterlogging was achieved by waterlogging the pot. Water vaporing was prevented by covering the pot's surface. Drought stress was applied by irrigating at 50% field capacity (D), which was considered as drought condition. The irrigation volume was calculated by estimating the crop's evapotranspiration (ETC/mm) using the formula ETC = ETO KC, where ETO is the Penman-Monteith reference evapotranspiration (mmd^{-1}) and KC is the crop coefficient recommended by FAO (Allen et al., 1998). Soil's water content was constantly monitored by putting densitometry probe tubes around the roots of the control well-watered plants. Irrigation was carried out whenever 40% of the available water was depleted, and the amount of the water required to bring each soil to FC was calculated according to Olberz et al. (2018) (Olberz et al., 2018).

Before each irrigation, the soil's moisture content within the top 90 cm of the profile was assessed using the gravimetric method. This full irrigation control system used all the water used. In the I100 treatment, irrigation water was used to raise the field's capacity moisture content to 90 cm deep, and the volume of applied water was measured using water meters set up in each zone (Beyhan and H, 2023; Singh et al., 2023).

Green sweet pepper seeds (*Capsicum frutescence* var. Longum) germinated in vermiculite/perlite (2:1, v/v). A week after transplanting, the drought stress and melatonin foliar spraying treatment was applied two- to four-leaf seedlings in a black plastic container with soil and lasted for 2 months. Every 10 days 5/1000 mg/L of chemical fertilizer NPK (20, 20, 20) was applied. Plants were tied to a wire above the greenhouse, and no pesticide was used. The following variables were assessed one week after the last treatment application.

Measured parameters

Plant growth parameters

Plants were harvested and washed at the end of the experiment. Shoots were separated from roots with a steel blade and dried for two days in a conventional oven at 70 °C to achieve a constant weight. The fresh weight (FW) and dry weight (DW) of shoots and roots in addition to their ratio were calculated. The root's and shoot's length was determined by a ruler. A change in water volume was used to calculate root volume (Haghighi et al., 2012). The number of flower abscissions per

plant was recorded during the experiment.

Relative water content

Weighing leaves before and after 24 h rehydration with distilled water was performed to determine relative water content (RWC). After 24 h, the samples were dried at 65 °C for 72 h before being measured again. The equation RWC = (FW - DW)/(TW - DW) × 100 was used to calculate RWC, where FW, DW, and TW are defined as fresh weight, dry weight, and turgid weight, respectively (Lopez-Serrano et al., 2019).

SPAD value and photosynthetic attributes

A nondestructive dual-wavelength chlorophyll meter was used to calculate the SPAD value. Per replicate, five measurements were taken. Photosynthetic rate (Pn) (μmol $CO_2m^{-2}s^{-1}$) and transpiration (mmol $H_2Om^{-2}s^{-1}$) were determined with a portable unit (Li-Cor, Li-3000, USA). They were determined on three fully expanded leaves.

Ammonium content of leaves

Ammonium was measured using the method developed by Husted et al. (2000) (Husted et al., 2000) by using 1 mL of Nessler reagent. The NH_4^+ content was determined using a standard curve and represented as mmol $NH_4^+ g^{-1}$FW. Nitrate extraction was performed following the procedure suggested by (Cataldo et al., 2008), and a spectrophotometer (BMG LABTECH SPECTROstar® Nano, Ortenberg, Germany) readings at 410 nm were obtained.

Nitrate reductase of leaves

The identical leaves were used in each replication for each treatment, which was mixed and three replications were randomly selected for measurements at the end of the experiment. The nitrate reductase enzyme activity was determined using the technique provided by Cazetta and Villela (2004) (Cazetta and Villela, 2004). In brief, 400 mg of leaf samples were immersed in phosphate solution (100 mg, pH = 7.5) containing 4% propanol and potassium nitrate for 1 h in the dark at 30 °C. Afterwards, the sulfanilic acid solution was dissolved in 2 mL chloride acid and 1 mL naphthylethylene diamide (0.02%). The absorbance was measured at 540 nm after 20 min. The enzyme activity was estimated using mol.g^{-1}FW after the standard solution was produced with sodium nitrite ($NaNO_3$).

The protein content of leaves

Twelve leaves from each treatment were chosen. Samples (1 g) were homogenized with 4 mL sodium phosphate buffer (pH = 7.2) and centrifuged at 4 °C. The protein level was measured and compared to the standard curve using the Bradford technique (1976) (Bradford, 1976), and the absorption capacity of leaves was determined using a UV–Vis spectrophotometer at 595 nm wavelength. The protein content was stated in $mg.mL^{-1}$.

Nitrogen concentration of leaves

After ashing the plant material at 470 °C, an acid solution of the ash (HCl = 10 mL, 2 N) was produced. According to Wiled et al., (1972) (Wild et al., 1972) the N content was evaluated using the macro-Kjeldahl technique (model PEP7, Minolta, Japan).

ABA content of leaves

One g of fresh leaves was mixed with 10 mL of 80% methanol and 0.1 g of polyvinyl pyrrolidone at 4 °C. Then the material was centrifuged at 4000 g for 15 min. Once the pH reached 8, the supernatant was collected. Methanol was evaporated and 5 mL of deionized water was added and dissolved finally, ethyl acetate was added and the mixture was re-evaporated. A 0.45 mm filter was used to inject ABA into an HPLC-DAD (high-performance liquid chromatography-diode array detector) using a reverse-phase column (Diamondsic, C18;5 μm; 25 cm × 4.6 mm). A gradient solvent system of methanol in water (acetic acid 3%) at a flowrate of 4 mL/min. To calibrate the output peak to assess the sample's extraction level, an ABA standard with a purity rate of 99.97% (Sigma Aldrich) was used.

The extracted sample's value was calculated using the area under the curve and its success in the output peak. The approach devised by employing an HPLC (Unicam Crystal 200 HPLC system, England) that was instantly coupled to a PDA (photodiode array) detector, employed for ABA measurement. A reverse-phase column (C18) (Zorbax SB-C18 100A; 3.5 μm; 150 mm × 2.1 mm) was loaded with 10 μL of the extract. The column's temperature was set at 25 °C. The HPLC column's initial operating conditions were established as follows: first, methanol-water (formic acid) 10:90 was used for 5 min; next, a linear gradient to methanol-water (formic acid) 30:70 was used for 5 min. This condition was kept for 10 min before moving to a linear gradient system. In the following 35 min, a methanol-water (formic acid) 45:55 was applied and the column was kept for 15 min. All chemicals were satisfactorily separated within 45 min, and the column was washed for 5 min each time using a methanol-water (formic acid) 95:5. After the above mentioned process, the column was re-equilibrated with methanol-water (formic acid) 10:90 for 30 min. The peak area of the standard curve was employed as a criterion to determine sample concentrations.

PIP1- expression

The RNA from the leaves and roots was extracted using the Iraizol Kit (Iraizol, RNA Biotech Co, Iran), and the total RNA was processed with DNase to eliminate contaminating DNA. RNA quantity and quality were measured using the Picodrop P200 instrument and absorbance at 260 and 260/280 nm. Revert Aid M-MuLV reverse transcriptase was used to create first-strand cDNA from 1 μg of DNase I-treated RNA and oligo. The cDNA was tested for the aquaporin (PIP1) gene using real-time PCR. The primer pairs were used to amplify the aquaporin gene (PIP1) and the actin gene (Actin 1) as internal control which is provided in Table 1. The ABI StepOne Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) and SYBR Green qPCR Master Mix were used to run all qPCR reactions in triplicate. Thermal cycling conditions were used for all reactions: 95 °C for 10 min, 40 cycles of 94 °C for 15 s, optimum temperatures of primer pairs for 30 s, and 72 °C for 30 s. Finally, for each pair of primers, the specificity of the amplified product was validated using melt curve analysis and gel electrophoresis. The PIP1-expression was demonstrated using the $2-\Delta\Delta Ct$ technique (Okunlola et al., 2017).

Gas exchange parameters

A portable photosynthesis meter (Li-Cor Li-3000, USA) was used to

Table 1
The sequences of the primers used in qRT-PCR.

Primer name		Primer sequence (5′–3′)	Annealing temperature (°C)	Size Band (bp)
PIP1	F	5′-AGGGATTCATGCAAGGACCA-3′	55	228
	R	5′-TGGTGGCCAAATGAACCAAG-3′		
Actin 1	F	5′-GTCCTCTTCCAACCATCCAT−3′	55	231
	R	5′-TACTTTCTCTCTGGTGGTGC−3′		

measure gas exchange parameters (photosynthesis rate, transpiration) from the youngest completely expanded leaf for three replications per treatment from 10:00 to 11:00 a.m. on a clear day. The observations were carried out with a photosynthetically active radiation (PAR) intensity of 1000 molm-^{2}s-^{1} and a CO_2 concentration of 350 mol^{-}1 mol.

Total phenolic

The Folin-Ciocalteu method was used to determine the total phenolic content. A spectrophotometer was used to measure the absorbance at 725 nm. Using the gallic acid (0–0.1 mg/mL) standard curve, the data were represented in gallic acid equivalents (mg/100 g fresh weight). If the absorbance value measured exceeded the standard curve's linear range, more dilution was performed (Singleton and Rossi, 1965).

Chlorophyll fluorescence

The fluorescence of chlorophyll was measured in dark and light-adapted leaves between 9:00–11:00 am using a portable fluorometer. Fv/Fm was determined after 30 min of dark adaption. The same leaves were also tested for chlorophyll index using a chlorophyll meter (SPAD-502 plus, Minolta, Japan).

Identification and quantification of amino acids, phenolic, and flavonoids

Citrate buffer was used to extract amino acids from leaves, which were then put into an HPLC system equipped with an MD-1510 PDA detector and tuned at 263 nm (max) (Unicam Crystal 200 HPLC system (England)). For injection, a 20 μL loop with 7125 valves was employed. At 25 °C (1.0 mL/min flowrate), the RP-18 column was employed, and eluent A was water (50 mM acetate buffer, pH = 4.2), while eluent B was acetonitrile (Lisiewska et al., 2008). Subsequently, 100 mg of leaves were combined with HPLC-grade methanol (80%; 10 mL) and shacked (8 h; 110 rpm; 25 °C) to make leaf extracts. The extract samples were then filtered using a nylon acro disk (0.22 m). The HPLC analysis was performed using the Li (2015) technique (Li et al., 2015). The stationary phase was injected using a Symmetry C18 column (5 μm, 250 × 4.6 mm) (Waters Crop., Milford, MA, USA), formic acid (0.1%), and acetonitrile (injection rate was 0.8 mL/min).

Plant material was air dried at room temperature and then ground into powder for the extraction and quantitative determination of phenolic acids. Using 80% aqueous methanol, the extraction was carried out under continual shaking for 48 h. Filtration was used to remove plant debris, and raw extracts were evaporated then redissolved in DMSO to a final concentration of 200 mg/mL. To achieve a final concentration of 2 mg/mL, extracts were diluted with mobile phase solvents A (0.05% aqueous formic acid) and B (methanol), which were premixed in a 1:1 ratio (Orcic et al., 2014). The Unicam-crystal-200 series high-performance liquid chromatograph was used to examine samples and standards. Ten microliters were introduced into the device, and chemicals were separated on a quick resolution column kept at 50 °C called the Zorbax Eclipse XDBC18 (50 mm 4.6 mm, 1.8 lm). Photo-diode array detector (Model 966) was employed for detection. At 300 nm, the detection was seen. The mobile phase was administered in gradient mode at a flow rate of 1 ml/min (0 min 30% B, 6 min 70% B, 9 min 100% B, and 12 min 100% B, with a 3 min re-equilibration interval).

Statistical analysis

Software Statistix 8 was used to analyze the data using two-way ANOVA with three replications. The treatments' means were separated using the least significant difference (LSD). Statgraphics Centurion Version XVI was used to perform principal component analysis (PCA).

Results

The interaction effect of drought and waterlogging stress when melatonin was applied on PIP expression and biochemical changes

The ANOVA and main effect of measured parameters are presented as supplementary in Tables S1 and S2. According to the findings, drought stress reduced PIP1 expression in roots and leaves, which reduced in waterlogging more than drought stress. Melatonin increased the expression of PIP1 in leaves. In all treatments, PIP1 expression was lower in the roots than the leaves (Table 2).

The variations of amino acid content under drought and waterlogging stresses, when melatonin was applied, were presented in supplementary Table 3. Table 3 displays total sulfur, aromatic, essential, non-essential, and total amino acids. In the non-melatonin treatment, these amino acids are almost similar in control, drought stress, and waterlogging. Melatonin applications increased all these amino acids, especially in drought stress. Furthermore, the melatonin treatment enhanced total amino acid levels in drought stress (Table 3).

The interaction effects of melatonin, drought, and waterlogging stress on growth parameters

The present study indicated that waterlogging and drought stress reduced shoot fresh and dry weight of hot peppers; whereas the melatonin application increased the fresh and dry weight of shoots (Fig 1A, B) and roots (Fig 1C, D) in control and drought-stressed plants.

Melatonin application further increased shoot length in non-stress (17.43%), waterlogging (7.16%), and drought-stressed (7.71%) plants (Fig 2A). Moreover, melatonin treatment showed the ability to elevate the root length in plants stressed by drought (16.6%) and waterlogging (9.21%). The drought-stressed plants had the shortest root length (Fig 2B); indeed the melatonin application was able to increase 10, 20, and 30% the root volume in non-stress, drought, and waterlogging stressed plants, respectively (Fig 2C); while application of melatonin reduced flower abscission per plant under drought (79.31%), waterlogging (75.67%) stresses, and control (69.75%) (Fig 2D).

Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$)

The current study demonstrated that the melatonin treatment improved shoot water content under drought and waterlogging stress, as well as control plants, although the difference was not statistically significant (Fig 3A). Melatonin application increased root water content in plants under drought stress, and this increase was more pronounced in plants under drought stress (Fig 3B).

The interaction effects of melatonin, drought, and waterlogging stress on some photosynthesis traits, ABA content

When plants were stressed by drought or waterlogging, their chlorophyll fluorescence were increased compared to non-stressed plants.

Table. 2
Real-time quantitative PCR analysis of PIP1 mRNA levels in leaf and root after the effect of drought and waterlogging stress and melatonin application.

Stress	PIP1 expression in root	PIP1 expression in leaves
Control	0.798a	2.077a
Drought	0.721ab	1.582b
Waterlogging	0.470c	0.978c
Melatonin application		
Non- Melatonin	0.682a	1.138b
Melatonin	0.592a	1.962a

Within a column of stress and melatonin application, the means followed by the same letter are not significantly different at $P<5\%$ according to the least significant difference test. Expression values are normalized by actin-expressed transcripts.

Table. 3
The effect of foliar application of melatonin on pepper under drought and waterlogging stress on total sulfur, aromatic, essential, non-essential, and total amino acids.

	Non-melatonin			Melatonin		
	Control	Drought	Waterlogging	Control	Drought	Waterlogging
Total sulfur AA	1.538±0.523	1.691±0.524	1.538±0.542	3.686±0.513	4.450±0.514	3.686±0.551
Total aromatic AA	2.472±0.575	2.767±0.558	2.472±0.556	6.550±0.547	8.021±0.578	6.550±0.598
Total essential AA	2.502±0.512	2.812±0.516	2.502±0.574	6.698±0.641	8.250±0.681	6.698±0.687
Total non-essential AA	9.681±0.714	9.946±0.814	9.681±0.846	28.614±1.21	29.939±1.52	28.614±1.42
Total amino acids	18.404±0.981	19.568±0.854	18.404±0.875	50.250±1.945	56.063±1.947	50.250±1.741

Data are means of three replications ± standard errors according to LSD test.

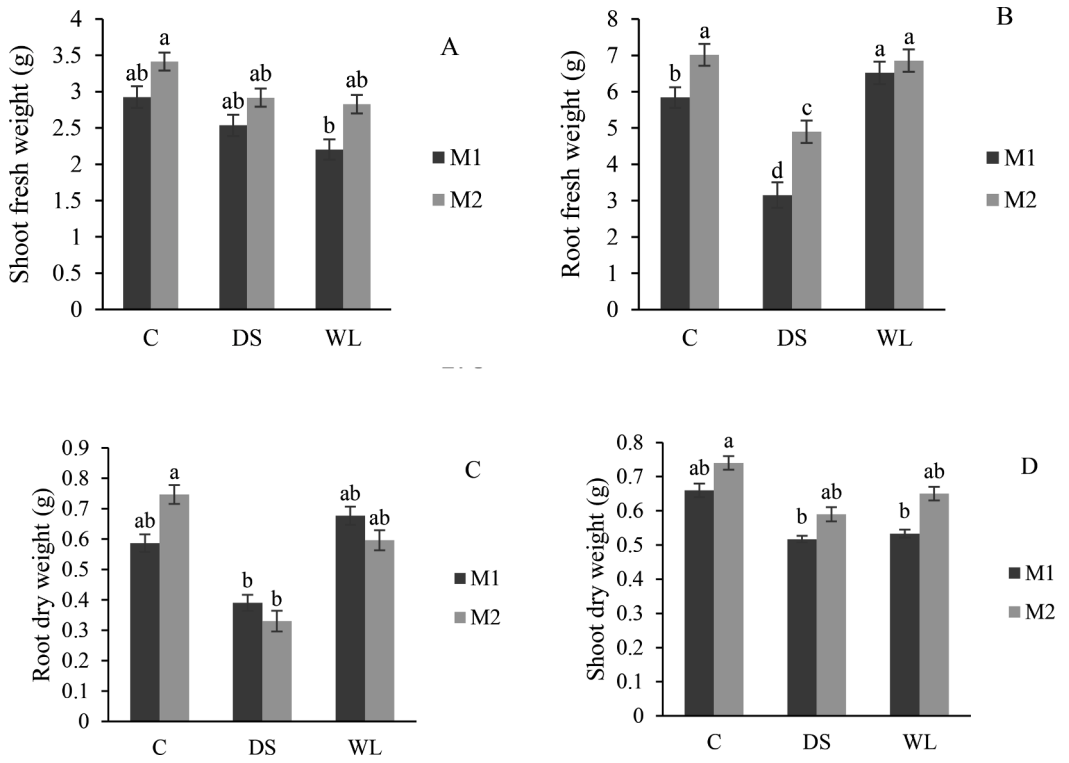

Fig. 1. The interaction effects of melatonin (M1 and M2), drought (DS), and waterlogging (WL) stress on shoot fresh weight (A), root fresh weight (B), root dry weight (C), and shoot dry weight (D). Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$).

Melatonin foliar spray reduced the amount of chlorophyll fluorescence compared to the plants was not exposed to melatonin (Fig 4A). Melatonin increased chlorophyll content by 17.13, 18.21, and 26.07% in non-stressed, drought-stressed, and waterlogged plants, respectively (Fig 4B). Besides melatonin increased photosynthesis by 26.89, 16.11, and 59.15%, respectively, and transpiration by 41.55, 69.12, and 91.61% in plants without stress, drought stress, and waterlogging, respectively (Fig 4C, D).

Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$)

Compared to the non-stressed plants, the amount of proline was increased in plants under drought and waterlogging stresses. Additionally, the use of melatonin in drought-stressed and waterlogged plants increased the proline level by 47.14 and 53.84%, respectively, compared to the control plants (Fig 5A). Moreover, an increase in protein content was observed in the non-stressed plants treated with melatonin (13.02%), plants under drought stress (8.85%), and plants under waterlogging stress (26.69%), respectively (Fig 5B). ABA levels were increased in drought- and waterlogging-stressed plants in comparison with the non-stressed plants. The application of melatonin further amplified the amount of ABA in the non-stressed plants by 18.04%, while was decreased by 21.54% and 9.74% in plants experiencing drought and waterlogging, respectively (Fig 5C).

The interaction effects of melatonin, drought, and waterlogging stress on nitrogen metabolism

Our finding showed that the drought stress decreased nitrate reductase and ammonium uptake, compared to the peppers grown without stress. Melatonin foliar spraying increased nitrate reductase by 29.25, 82.5, and 51.27% and ammonium content by 20.03, 50.90, and 51.37% in plants without stress, plants under drought stress, and plants subjected to waterlogging, respectively (Fig 6A, B, C). Compared to the non-stressed plants, nitrate levels were increased in drought- and waterlogging-stressed plants.

Foliar application of melatonin decreased the amount of nitrate in plants under drought stress (13.48%) and waterlogging (11.46%) compared to the plants without melatonin application, whereas the opposite trend was observed in non-stressed plants, where increasing nitrate levels (17.91%) were reported.

According to PCA analysis, spraying foliar melatonin in both drought and waterlogging stresses indicated to be more effective in ammonium, nitrate reductase, chlorophyll content, proline, protein, transpiration, shoot length, and root water content because they are near and have

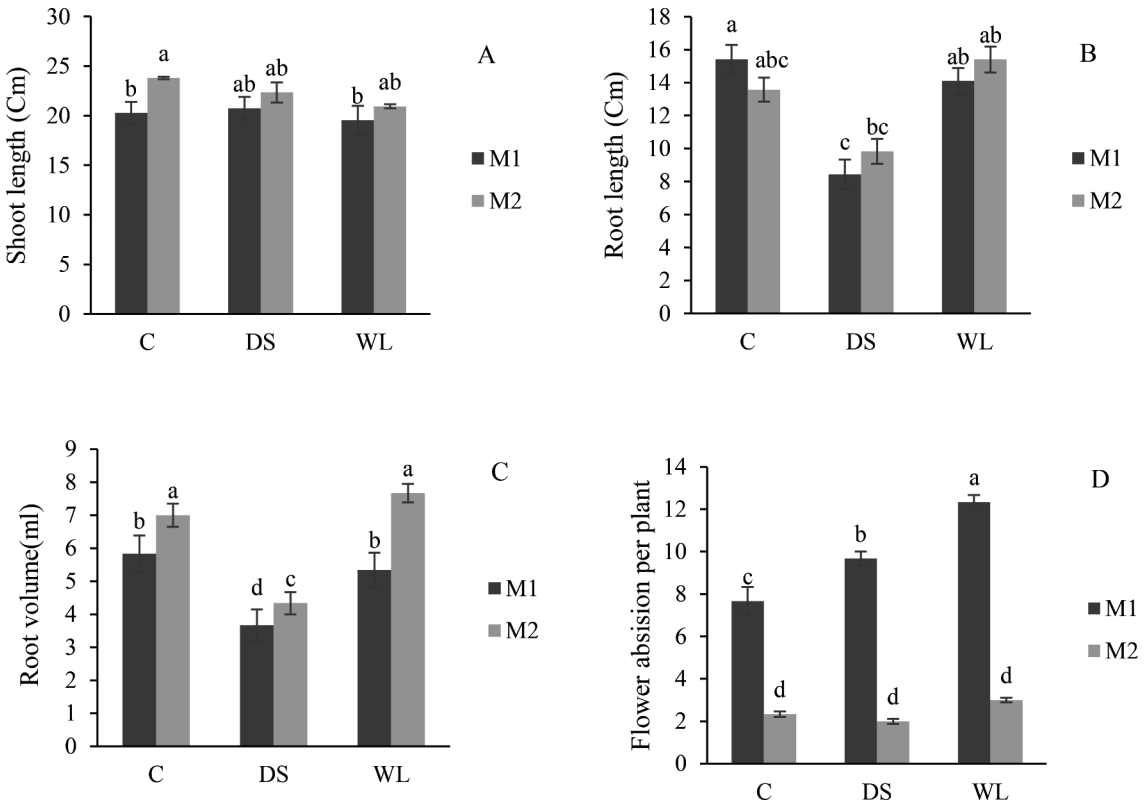

Fig. 2. The interaction effects of melatonin (M1 and M2), drought (DS), and waterlogging (WL) stress on shoot length (A), root length (B), root volume (C), and flower abscission (D).

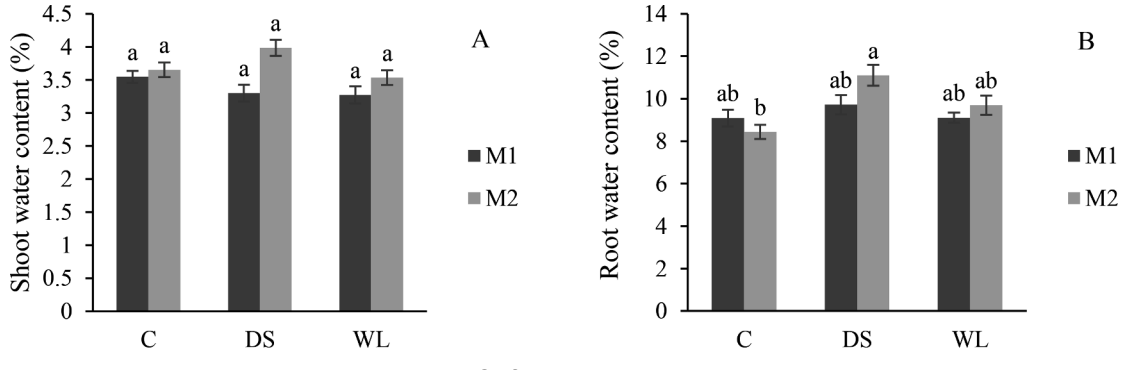

Fig. 3. The interaction effects of melatonin (M1 and M2), drought (DS) and waterlogging (WL) stress on shoot water content (A), root water content (B). Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$).

lower degree to each other. Melatonin foliar spraying had the greatest effect on shoot and root fresh weights, shoot and root dry weights, root length, and root volume in the non-stressed plants placed in the right-down part of the figure. ABA and nitrogen were more affected by drought stress, in non-melatonin (Fig. 7). Conclusively, stress indicators such as chlorophyll fluorescence, ABA content, and enzyme modifications like NR were more visible in the left portion of the figure where no melatonin was treated (M1), while growth attributes were more visible in the right hand of the figure when melatonin was applied (M2) (Fig. 7).

Non melatonin. Optimum irrigation (M1C), Non. melatonin. Drought stress (M1D), Non melatonon. Waterlogging stress (M1F), Melatonin. Optimum irrigation (M2C), Melatonin. Drought stress (M2D), Melatonin. Waterlogging stress (M2F).

Discussion

Water stress (drought and waterlogging) is one of the major environmental factors that severely inhibits plant growth and development (Zheng et al., 2017; Guo et al., 2020; Gupta et al., 2020) that is generated by affecting different aspects of plant morphology, physiology, and biochemistry (Liu et al., 2020). The current study aimed to study the effect the applications of exogenous melatonin on tolerance pepper under water stress.

Our finding showed that some growth characteristics with the application of melatonin improved than control. External application of melatonin resulted in a considerable increase in the length of roots and shoots and biomass of soybean plants (Imran et al., 2021). It could be due to increased auxin and ethylene production in roots, since auxin was increased with waterlogging in sunflowers and stimulated ethylene production in roots, root initiation increased by raising the auxin and

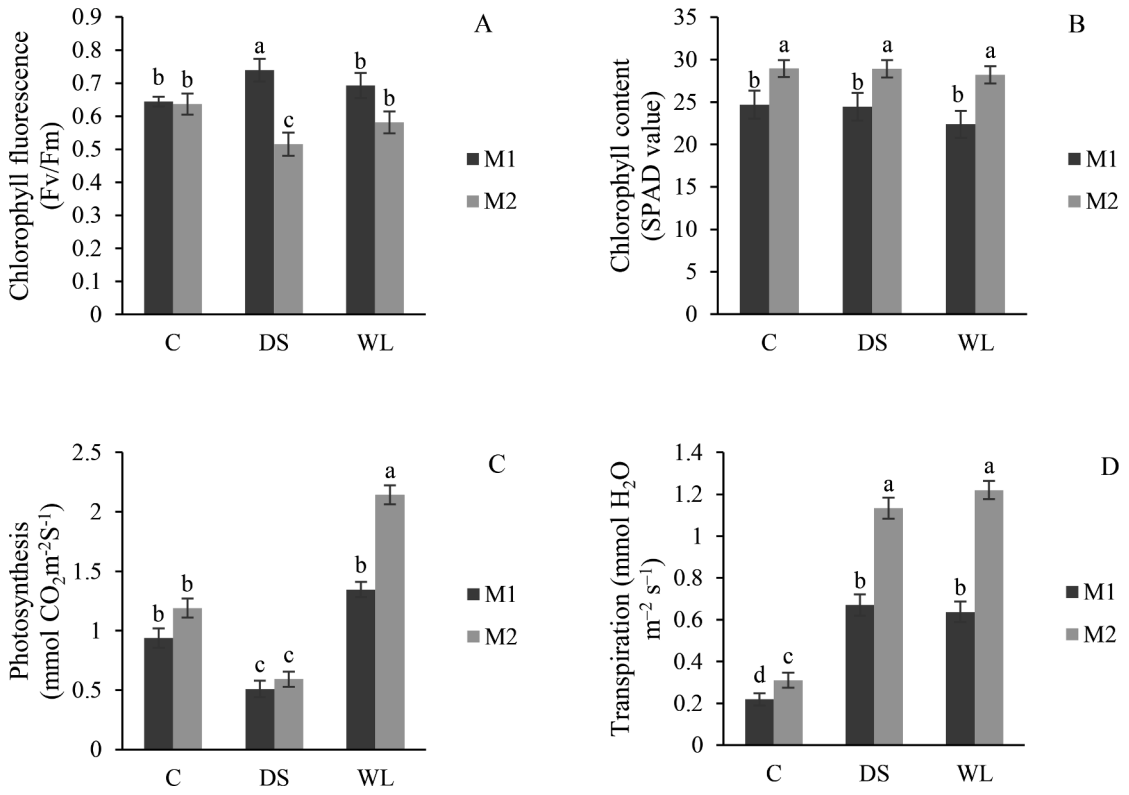

Fig. 4. The interaction effects of melatonin (M1 and M2), drought (DS) and waterlogging (WL) stress on chlorophyll fluorescence (A), chlorophyll content (B), photosynthesis (C), transpiration (D).

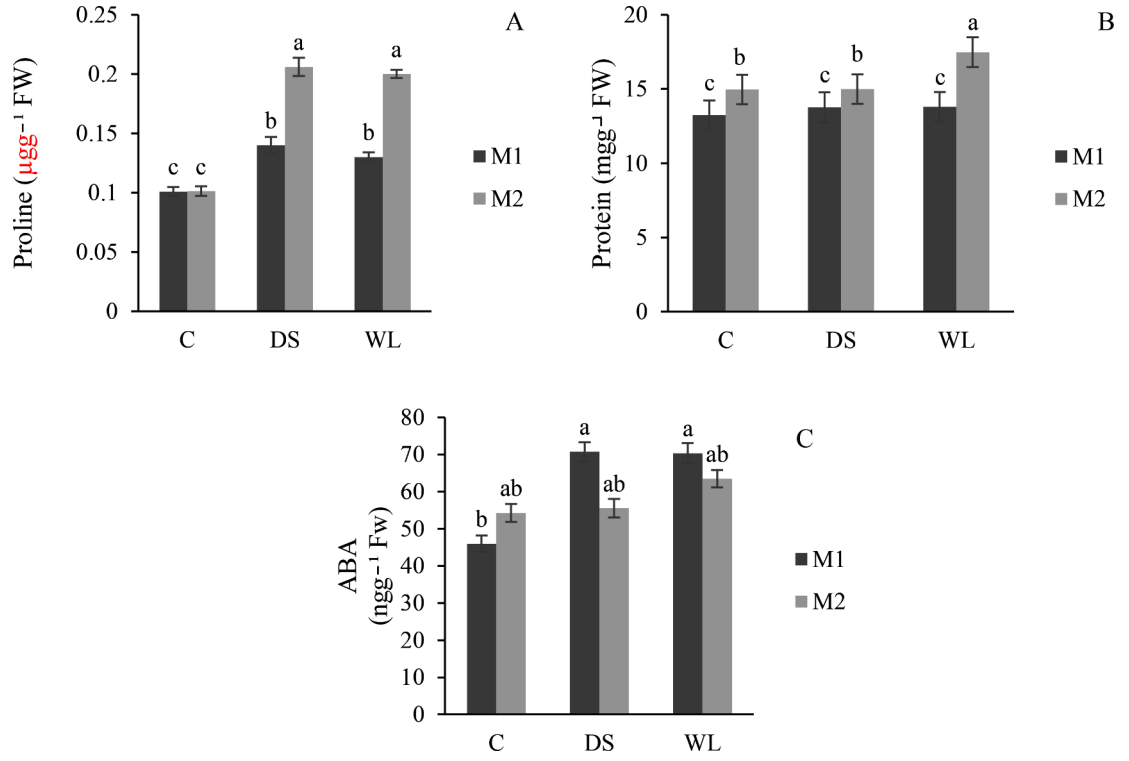

Fig. 5. The interaction effects of melatonin (M1 and M2), drought (DS) and waterlogging (WL) stress on proline (A), protein (B), and ABA (C). Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$).

ethylene levels (Visser et al., 1996)

The chlorophyll content in plants under drought stress was reduced in the current study. But melatonin increased chlorophyll concentration, photosynthesis, and aerobic respiration. The drought-stressed chlorophyll content of soybean was enhanced *via* melatonin applied (Imran et al., 2021). Exogenous melatonin treatment considerably improved

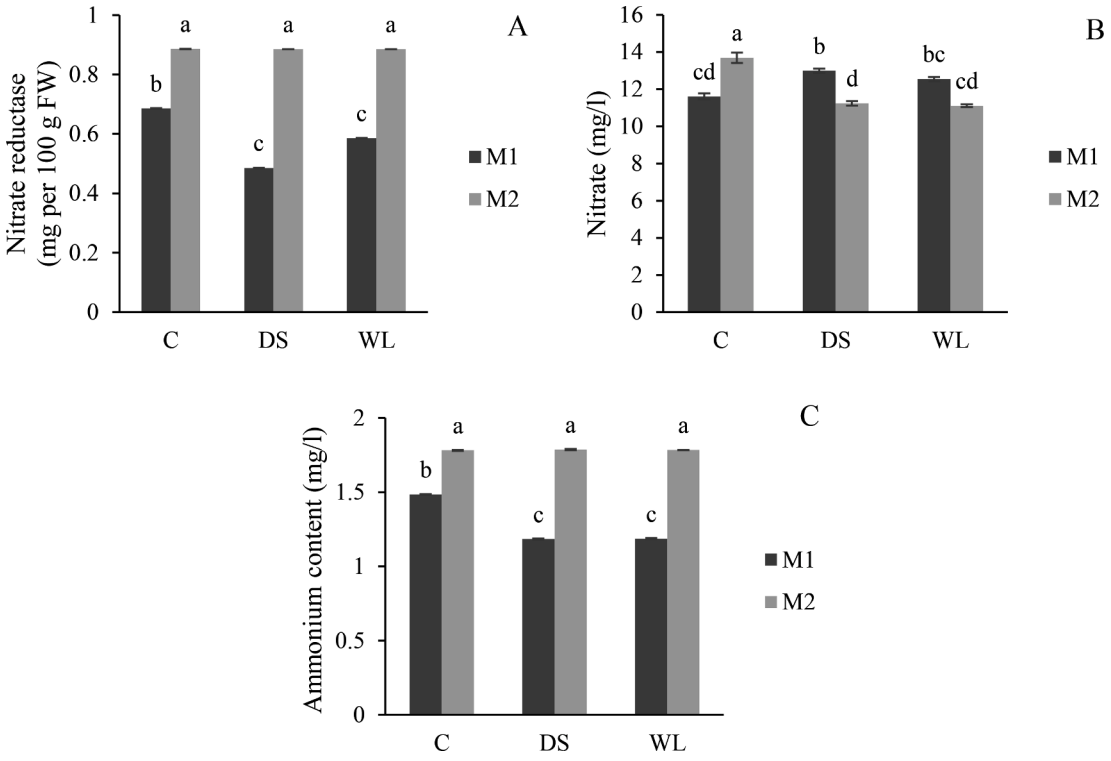

Fig. 6. The interaction effects of melatonin (M1 and M2), drought (DS) and waterlogging (WL) stress on nitrate reductase (A), nitrate (B), and ammonium content (C). Bars show the mean standard error. Means followed by the same letter are not statistically different based on LSD test ($P < 0.05$).

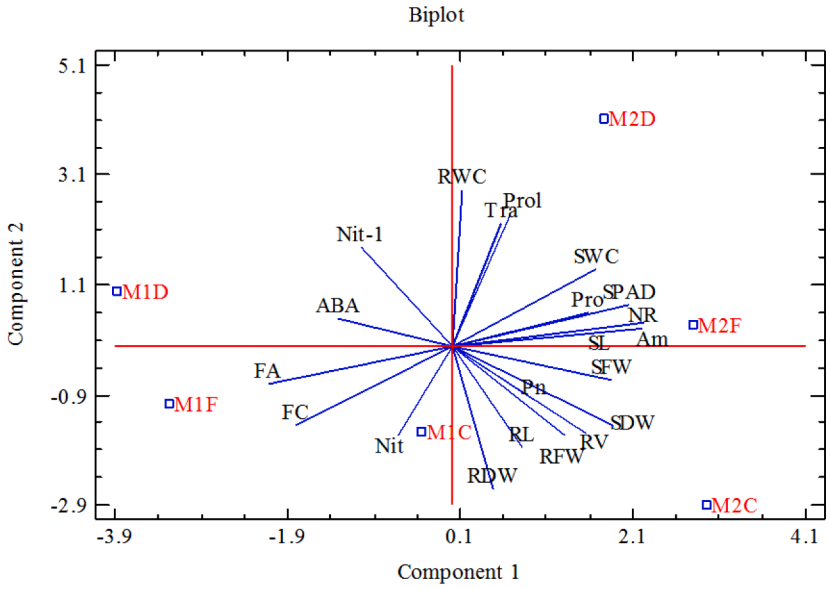

Fig. 7. Biplot analysis of interaction effect of foliar application of melatonin on pepper under drought and waterlogging stress. Shoot length (SL), Root length (RL), Root volume (RV), Shoot fresh weight (SFW), Root fresh weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW), Chlorophyll fluorescence (FC), Chlorophyll content (SPAD), Photosynthesis (Pn), Transpiration (Tra), Shoot water content (SWC), Root water content (RWC), Flower abscission (FA), Proline (Prol), Nitrogen (Nit-1), Ammonium (Am), Nitrate (Nit), Nitrate reductase (NR), ABA (ABA), protein (Pro). Control (C), Drought stress (D), Waterlogging stress (F), 0 (M1), 500 (M2) μM Melatonin concentration.

rice seedling growth and development, chlorophyll content, photosynthesis rate, and photosystem II activity (Han et al., 2017). It seems that foliar spraying with melatonin prevented chlorophyll degradation.

Transpiration in our study in melatonin application was decreased under waterlogging stress. waterlogging persistent reduced the formation of active oxygen species, lowering the activity of defensive enzymes. Since CO_2 loss through stomata was less than through transpiration, it can be concluded that the treated plants can manage CO_2 loss by decreasing stomata conductance in comparison with H_2O loss; indeed, it can help plants maintain more efficient photosynthesis by maintaining water potential and hydraulic conductance.

Drought stress raises ABA levels, which causes stomatal closure and contributes to leaf senescence (Burgess and Huang, 2016), whereas melatonin lowers ABA levels *via* favorably regulating biosynthetic genes while negatively regulating regulatory genes (Sharma et al., 2020). In our study, the lowest amount of ABA was observed under drought stress in the treatment with melatonin.

Melatonin is a crucial component in the hormonal system that promotes plant tolerance to drought stress by regulating the levels of phytohormones such as ABA, auxins (Auxs), cytokinins (CKs), and gibberellins (GAs) (Burgess and Huang, 2016). During drought stress, plant hormones impact antioxidant metabolism, carbohydrate synthesis

(carbon metabolism), stomatal movement, and leaf senescence. ABA accumulates on leaves and hinders K absorption in stomata, stomata closure reduces their water potential and closing them (Pessarakli, 2010). However, because of the sluggish diffusion of O_2 in saturated soil, a little amount of accessible O_2 is quickly consumed by root and microbial respiration; consequently, excess methane and carbon dioxide (CO_2) accumulation in the soil increases ethylene production in the submerged section of plants (Greenway et al., 2006).

Our study showed drought stress reduced nitrate absorption and nitrate reductase activity and increased ammonium, however, melatonin reduced these negative effects. Melatonin raised the nitrate reductase level by increasing the expression of the NR and NiR enzyme encoding genes (M. Zhang et al., 2019). Drought-induced NH_4^+ accumulation in plant leaves is toxic to plants because high levels of NH_4^+ cause protein extrusion and cytosolic pH disturbances (Ren et al., 2020). Melatonin reduced ammonium toxicity by increasing photosynthesis and the TCA cycle. The water deficiency stress typically disrupts nitrogen metabolism by activating nitrogen absorption enzymes and causing the synthesis of N-containing compounds (Ren et al., 2020). Previous research demonstrated that drought stress can reduce NO_3 uptake and thus inhibit NR activity (Miranda-Apodaca et al., 2020).

According to our findings and those suggested in the literature, unchangeable photosynthesis in peppers under stressful circumstances can be caused by the following factors:

1) increasing ABA in the root and transferring to the leaves, which can close stomata (Pessarakli, 2010)
2) decreasing the root hydraulic conductivity, resulting in decreased water potential and stomata closing (Pessarakli, 2010)
3) a reduction in Rubisco activity, which may occur under waterlogging and drought stress circumstances and result in a loss in photosynthetic rate, which is also conceivable in pepper but was not addressed here (Pessarakli, 2010)

The effect of waterlogging and drought stress on the growth and water relation of pepper

Pepper is a plant that suffers from drought stress (González-Dugo et al., 2007). In our study, the growth indices of pepper during drought stress are lower than the control. Several studies demonstrated that water stress reduces pepper production (Ferrara et al., 2010). Both the vegetative and reproductive phases of pepper growth were slowed down in *C. chinense* and *C. annuum* under extreme drought circumstances (Widuri et al., 2017). Other studies confirm that drought stress encourages root growth (for instance the chili pepper), particularly when it is applied to plants for a longer period of time (Pessarakli, 2010). But this does not match the results of our study. According to Kpyoarissis et al. (1995), exhibiting that drought stress restricts water availability by lowering root water potential, which affects leaf water potential and causes pepper fresh shoot weight to exceed shoot dry weight (Kyparissis et al., 1995).

In our study, root growth of pepper during waterlogging stress are lower than the control some researchers discovered that waterlogging inhibited root growth because it depleted the soil's oxygen supply. They revealed that waterlogging limited respiration and electron transportation by reducing oxygen availability in the rhizosphere (Fiedler and Vepraskas, 2007); therefore, roots die and growth is hindered, however, it appears that in pepper, the detrimental effects of waterlogging on shoot growth were more pronounced than on roots. By limiting oxygen accessibility, waterlogging has a major negative effect on the roots, which affects respiration and electron transportation (Fiedler and Vepraskas, 2007). . Linkemer et al. (1998) demonstrated that plants responded differently to waterlogging at different phases of development, demonstrating the most susceptible in the initial stage (Linkemer et al., 1998)

It appears that lowering transpiration and stomata conductance can cause plants to retain more water and hence increase the fresh weight of the treated plants, while waterlogging stresses have a greater impact on the dry weight. Excess water in the soil immediately affects plant roots and indirectly affects shoots (Henshaw et al., 2007). On the other hand, waterlogging decreases shoot and root weights through a different method because the water potential did not considerably change. These discrepancies may be explained by PIP expression, as PIP expression in the root and shoot greatly increased under stress when melatonin was used to improve the fresh and dry weights of the root and dry weight of the shoot in pepper. Furthermore, melatonin improved RWC and shoot fresh weight during waterlogging via increasing PIP expression in the leaf. Higher PIP expression in the pepper leaf does not seem to have the same impact on water status, root weight, and dry weight of the shoot under drought stress the same as PIP expression effect in the root. The increased PIP expression in the roots was unsuccessful for both fresh and dry weights, according to one assertion, while it was effective on fresh weight in the leaves.

The effect of waterlogging and drought stress on the pigment of pepper leaves

A decrease in chlorophyll content is the first sign of oxidative stress and growth reduction during drought stress (Mansfield and Jones, 1971).

Previously other species have reported decreased or unchanged chlorophyll levels depending on the length and severity of the drought. Our findings supported previous research on pepper, which demonstrated that *C. chinense* and *C. frutesense* can withstand drought by preventing chlorophyll decomposition. Chlorophyll stores the light needed for photosynthesis (Farooq and al., 2009). They presented that the ability of *Capsicum* spp. against drought stress is related to maintaining chlorophyll and carotenoid content as well as the peppers' photosynthetic apparatus in drought stress (Farooq and al., 2009).

According to previous studies, waterlogging lowers chlorophyll fluorescence, resulting in PSII degradation and a decrease in photosynthesis (Sharma et al., 2020). Waterlogging stress lowers water and nutrient intake, reduces chlorophyll content, (Arnao and Hernandez--Ruiz, 2015), nitrate reductase (Chen et al., 2021), and causes chlorosis and mortality (M. Zhang et al., 2019). Moreover, waterlogging reduced the stability index of the membrane and the chlorophyll concentration in pigeon pea leaves (Keatinge and al., 2014); this agrees with the findings of this study. However, in our investigation, the waterlogging treatment produced more photosynthesis than the other treatments. According to Yamauchi et al. (2014), photosynthetic ability and tolerance to waterlogging are positively associated. It would seem that peppers' great waterlogging tolerance has increase their potential for photosynthesis (Yamauchi et al., 2014).

Pepper leaves were thinned as a consequence of waterlogging, while the cell walls of epithelial layer cells in the root were thickened. Oxidative free radicals disrupt the enzymatic protective system, and lipid peroxidation degrades the cell membrane (Broughton et al., 2015).

The effect of waterlogging and drought stress on some stress indices and PIP gen expression of pepper

Previous studies demonstrated that chlorophyll fluorescence, which harms PSII and lessens photosynthesis, is decreased by waterlogging; subsequently, the photosystems' ability to generate chemical energy is lost, by lowering Fv/Fm and increasing the stress-related formation of ROS, since the chemical energy is not being used for CO_2 fixation. In conclusion, the ROS generation and total antioxidant enzyme activity both increased when waterlogging was prolonged (Guidi and Soldatini, 1997). In terms of a reduction in chlorophyll fluorescence, pepper showed a similar outcome. Stress indices were risen independent of the type of stress since there was no discernible difference between

waterlogging and drought stress. However, as previously stated, proline, ABA content showed more significant changes by stress than other indices. Plants can withstand mild drought stress by storing osmolytes such as proline and amino acids (Rose, 1988). Proteins and proline levels were risen in water logging and drought-stressed especially when melatonin was used, allowing the plants to maintain tissue water status and withstand stress (Chiang and Dandekar, 1995). The melatonin applied externally maintains membrane integrity and regulates osmolarity and cell turgor. (Georgiadou et al., 2018).

Moreover, drought stress reduced total protein content in plants (Chen and Zhang, 2000). The results of the recent investigations were opposite to the notion that osmotic adjustment is influenced by total protein concentration. It seems that proline and amino acids in pepper had a greater impact on osmotic adjustment during drought stress than the overall protein composition of pepper. Proline content increased in pepper species under waterlogging stress, which is one of the plants' self-defense responses (Wu et al., 1997).

Waterlogging or deficits, which are initially exposed to the roots, have an impact on the control of water movement in the shoots. Drought and waterlogging stress cause ABA synthesis in roots, which then accumulate in leaf tissues, particularly the guard cells (Olivella et al., 2000). In other words, ABA regulates plant growth during stress by inducing long-distance signaling between roots and shoots and modulating the expression of several PIPs. Thus, during waterlogging and drought stress, ABA acts as a messenger between roots and leaves (Laur and Hacke, 2013). Additionally, it implied that the leaves as well as the roots can be the source of ABA (Li et al., 2010). Contrarily, ABA encourages stomatal closure *via* a number of processes including a biochemical impact on guard cells, a reduction in water permeability within leaf vascular tissue, and elevated root aquaporin activity (Yue et al., 2014). Aquaporin activity is a long-lasting effect of ABA on plant hydraulic characteristics, which improves plant water status. Important function of ABA signals in Arabidopsis is PIP1 overexpression (Jang et al., 2004).

Amino acids contents

In several plants, amino acids (AA) are used as a natural plant growth booster. In a normal setting, AAs are recognized as stimulants with quantitative and qualitative roles in plant growth. These chemicals are required for the formation of hormones and secondary metabolites (Rouphael and Colla, 2020). AAs improve plant efficiency through certain metabolic processes and coenzyme activities, and they play an important role in plant growth pathways (Nouraei et al., 2018). Plant growth was increased by the use of amino acids during drought stress (Güneş et al., 1996). In this investigation, glutamic acid had the largest proportion in both control and stress conditions (Atanasova, 2008). Melatonin raised the overall non-essential amino acid while decreasing the total essential amino acid. The proportions of total sulfur amino acids, total aromatic amino acids, total essential amino acids, and total non-essential amino acids were decreased with drought stress, ranging from 1 to 4, 2–8, 2–8, and 9–29%, respectively, implying that total essential amino acids were decreased more than other AAs. When the plant was stressed, the total non-essential amino acids were dropped to the least (Table 3).

There have been reports of an increase in amino acids other than proline during drought stress. Asparagine and alanine in arginine in *Cryptomeria*, for example, and glutamic acid, aspartic acid, and glutamine in cotton Under stress, the most abundant amino acids in pepper were glutamic acid, glutamine, and asparagine, which were equivalent to cotton (Hanower and Brzozowska, 1975) and rice (Yang et al., 2000). Although proline concentration was risen in drought and waterlogging stresses compared to the control, while glutamic acid, glutamine, and asparagine were the most abundant amino acids in stress, particularly in drought stress. It has been claimed that these amino acids function by inhibiting the role of Abscisic Acid (ABA) in stomata closure, leading to gaseous exchange regulation and a reduction in the deleterious effects of photosynthesis. The increased transpiration and stomatal conductivity suggest that this effect is due to the increased glutamic acid, glutamine, and asparagine, which induce stomatal opening for gaseous exchange, lowering photosynthetic decrease in cabbage during drought.

Since amino acids are the precursors of many proteins and other amino acids, increasing the amino acid content of plants will be led to increasing their nutritional worth (Güneş et al., 1996). Furthermore, when compared to controls, AA reduced nitrate levels in pepper leaves, showing that exogenously delivered AA limits nitrate uptake (Fig. 5). This conclusion is consistent with a previous study, which found that partially substituting amino acids for nitrate fertilization reduced nitrate content in bulbs, cabbage (Fabiani et al., 2002), and radish while boosting N content in their leaves (Gonzalez et al., 2010). Nitrate accumulation decreased with melatonin during waterlogging and drought stresses, implying that melatonin at waterlogging and drought stresses decreased nitrate metabolism by an unknown mechanism, and so more research is needed to examine this effect.

Conclusion

In conclusion, when melatonin was not employed, stress markers such as chlorophyll fluorescence, ABA concentration, and enzyme changes such as NR were more influenced. Melatonin promoted growth characteristics when used under stress. So it seems that melatonin can alleviate the deleterious effect of stress. On the other hand, Foliar melatonin spraying was more beneficial in drought and waterlogging stresses in terms of ammonium, nitrate reductase, chlorophyll content, proline, protein, transpiration, shoot length, and root water content. So melatonin through improving growth and N metabolism affects photosynthesis traits and maintenance of growth even under stress. Moreover, Melatonin affected growth *via* influencing enzyme activity (NR), hormonal changes (ABA), amino acid changes, and PIP gene expression. Conclusively application of melatonin (500 μM) on pepper under drought and waterlogging stress can be recommended to decrease the deleterious effect of the stresses.

Contributions

All of the authors contribute to the design, managing, analyzing, and writing of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2023.100192.

References

Agnihotri, S., 2013. Impact of silicon on various agro-morphological and physiological
parameters in maize and revealing its role in enhancing water stress
tolerance. Emir. J. Food Agric. 25 (2).

Paudel, I., et al., 2021. Intraspecific plasticity in hydraulic and stomatal regulation under drought is linked to aridity at the seed source in a wild pear species. Tree Physiol. 41 (6), 960–973.

Ahmad, S., et al., 2019. Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic capacity and antioxidant defense system of maize seedlings. PeerJ 7, e7793.

Jaspers, P., Kangasjarvi, J., 2010. Reactive oxygen species in abiotic stress signaling. Physiol. Plant. 138 (4), 405–413.

Maksup, S., Roytrakul, S., Supaibulwatana, K., 2012. Physiological and comparative proteomic analyses of Thai jasmine rice and two check cultivars in response to drought stress. J. Plant Interact. 9 (1), 43–55.

Zhang, M., et al., 2019a. Exogenous melatonin reduces the inhibitory effect of osmotic stress on photosynthesis in soybean. PLoS ONE 14 (12), e0226542.

Pinheiro, C., Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data? J. Exp. Bot. 62 (3), 869–882.

Kong, X., et al., 2016. Stress-inducible expression of an F-box Gene TaFBA1 from wheat enhanced the drought tolerance in transgenic tobacco plants without impacting growth and development. Front. Plant Sci. 7, 1295.

Wang, Y., et al., 2021. Influence of different nitrogen sources on carbon and nitrogen metabolism and gene expression in tea plants (Camellia sinensis L.). Plant Physiol. Biochem. 167, 561–566.

Cui, G., et al., 2019. Response of carbon and nitrogen metabolism and secondary metabolites to drought stress and salt stress in plants. J. Plant Biol. 62 (6), 387–399.

Zhang, Q., et al., 2019b. Melatonin improved waterlogging tolerance in Alfalfa (Medicago sativa) by reprogramming polyamine and ethylene metabolism. Front. Plant Sci. 10, 44.

Ren, J., et al., 2020. Coordinated regulation of carbon and nitrogen assimilation confers drought tolerance in maize (Zea mays L.). Environ. Exp. Bot. 176.

Liu, C., et al., 2013. Carbon and nitrogen metabolism in leaves and roots of dwarf bamboo (Fargesia denudata Yi) subjected to drought for two consecutive years during sprouting period. J. Plant Growth Regul. 33 (2), 243–255.

Goyal, K., Kaur, K., Kaur, G., 2020. Foliar treatment of potassium nitrate modulates the fermentative and sucrose metabolizing pathways in contrasting maize genotypes under water logging stress. Physiol. Mol. Biol. Plants 26 (5), 899–906.

Zheng, X., et al., 2017. Melatonin improves waterlogging tolerance of malus baccata (Linn.) Borkh. Seedlings by maintaining aerobic respiration, photosynthesis and ROS migration. Front. Plant Sci. 8, 483.

Yamauchi, T., et al., 2018. Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. Plant Physiol. 176 (2), 1118–1130.

Jitsuyama, Y., 2017. Hypoxia-responsive root hydraulic conductivity influences soybean cultivar-specific waterlogging tolerance. Am. J. Plant Sci. 8, 770–790.

Drew, M.C., 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 223–250.

Alam, I., et al., 2010. Proteome analysis of soybean roots under waterlogging stress at an early vegetative stage. J. Biosci. 35 (1), 49–62.

Subbaiah, C.C., Sachs, M.M., 2003. Molecular and cellular adaptations of maize to flooding stress. Ann. Bot. 91 (2), 119–127. Spec No.

Fiedler, S., Vepraskas, M.J.R., 2007. Soil redox potential: importance, field measurements, and observations. Adv. Agronomy 94, 1–54.

Klok, E.J., et al., 2002. Expression profile analysis of the low-oxygen response in Arabidopsis root cultures. Plant Cell 14 (10), 2481–2494.

Chang, W.W., et al., 2000. Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. Plant Physiol. 122 (2), 295–318.

Dubey, H., et al., 2003. Proteome maps of flood-tolerant fr 13a and flood-sensitive ir 54 rice types depicting proteins associated with O2 deprivation stress and recovery regimes. Curr. Sci. Assoc. 84, 83–89.

Ahsan, N., et al., 2007. A proteomic screen and identification of waterlogging-regulated proteins in tomato roots. Plant Soil 295 (1–2), 37–51.

Danielson, J.A., Johanson, U., 2008. Unexpected complexity of the aquaporin gene family in the moss Physcomitrella patens. BMC Plant Biol. 8, 45.

Suga, S., Maeshima, M., 2004. Water channel activity of radish plasma membrane aquaporins heterologously expressed in yeast and their modification by site-directed mutagenesis. Plant Cell Physiol. 45 (7), 823–830.

Lian, H.L., et al., 2006. Upland rice and lowland rice exhibited different PIP expression under water deficit and ABA treatment. Cell Res. 16 (7), 651–660.

Perry, L., et al., 2007. Starch fossils and the domestication and dispersal of chili peppers (Capsicum spp. L.) in the Americas. Science 315 (5814), 986–988.

Sung, Y., Chang, Y.Y., Ting, N., 2005. Capsaicin Biosynthesis in Water-stressed Hot Pepper Fruits. Botanic. Bull. Academia Sinica 46, 35–42.

Ferrara, A., et al., 2010. Flowering, growth and fruit setting in greenhouse bell pepper under water stress. J. Agron. 10 (1), 12–19.

Delfine, S., Loreto, F., Alvino, A., 2001. Drought-stress effects on physiology, growth and biomass production of rainfed and irrigated bell pepper plants in the mediterranean region. J. Am. Soc. Hortic. Sci. 126 (3), 297–304.

Masoumi, Z., Haghighi, M., Jalali, S.A.H., 2021. Flooding or drought which one is more offensive on pepper physiology and growth? Mol. Biol. Rep. 48 (5), 4233–4245.

Yao, J.W., et al., 2021. Role of melatonin in UV-B signaling pathway and UV-B stress resistance in Arabidopsis thaliana. Plant Cell Environ. 44 (1), 114–129.

Wang, M., Zhang, S., Ding, F., 2020. Melatonin mitigates chilling-induced oxidative stress and photosynthesis inhibition in tomato plants. Antioxidants (Basel) 9 (3).

Zhang, T., et al., 2020. Alleviating effects of exogenous melatonin on salt stress in cucumber. Sci. Hortic. 262.

Arnao, M.B., Hernandez-Ruiz, J., 2015. Functions of melatonin in plants: a review. J. Pineal Res. 59 (2), 133–150.

Hu, Z., et al., 2016. Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. Plant Physiol. Biochem. 100, 94–104.

Allen, R.G., et al., 1998. Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. F.a.A.O.o.t.U.N. FAO irrigation and drainage, Rome, Italy. Editor.

Olberz, M., Kahlen, K., Zinkernagel, J., 2018. Assessing the impact of reference evapotranspiration models on decision support systems for irrigation. Horticulturae 4 (4).

Beyhan, Ucak A, H, A., 2023. Drought stress resistance indicators of chickpea varieties grown under deficit irrigation conditions. Peer J 11 (e14818).

Singh, M., et al., 2023. Root distribution, soil water depletion, and water productivity of sweet corn under deficit irrigation and biochar application. Agric. Water Manag. 279.

Haghighi, M., Afifipour, Z., Mozafarian, M., 2012. The effect of N-Si on tomato seed germination under salinity levels. J. Biol. Environ. Sci. 6 (16), 87–90.

Lopez-Serrano, L., et al., 2019. Pepper rootstock and scion physiological responses under drought stress. Front. Plant Sci. 10, 38.

Husted, S., et al., 2000. A critical experimental evaluation of methods for determination of NH4 in plant tissue, xylem sap and apoplastic fluid. Physiol. Plant 109 (2), 167–179.

Cataldo, D.A., et al., 2008. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6 (1), 71–80.

Cazetta, J.O., Villela, L.C.V., 2004. Nitrate reductase activity in leaves and stems of tanner grass (Brachiaria radicans Napper). Sci. Agric. 61 (6), 640–648.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Wild, S.A., et al., 1972. Soil and Plant Analysis for Tree Culture. Oxford & IBH Publishing Co, New Delhi.

Okunlola, G.O., et al., 2017. Physiological response of the three most cultivated pepper species (Capsicum spp.) in Africa to drought stress imposed at three stages of growth and development. Sci. Hortic. 224, 198–205.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16 (3), 144–158.

Lisiewska, Z., Kmiecik, W., Korus, A., 2008. The amino acid composition of kale (Brassica oleracea L. var. acephala), fresh and after culinary and technological processing. Food Chem. 108 (2), 642–648.

Li, C., et al., 2015. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. J. Exp. Bot. 66 (3), 669–680.

Orcic, D., et al., 2014. Quantitative determination of plant phenolics in Urtica dioica extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chem. 143, 48–53.

Guo, Y.Y., et al., 2020. Melatonin alleviates drought-induced damage of photosynthetic apparatus in maize seedlings. Russian J. Plant Physiol. 67 (2), 312–322.

Gupta, A., Rico-Medina, A., Cano-Delgado, A.I., 2020. The physiology of plant responses to drought. Science 368 (6488), 266–269.

Liu, M., et al., 2020. Properties of root water transport in canola (Brassica napus) subjected to waterlogging at the seedling, flowering and podding growth stages. Plant Soil 454 (1–2), 431–445.

Imran, M., et al., 2021. Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. AoB Plants 13 (4), plab026.

Visser, E., et al., 1996. An ethylene-mediated increase in sensitivity to auxin induces adventitious root formation in Flooded Rumex palustris Sm. Plant Physiol. 112 (4), 1687–1692.

Han, Q.H., et al., 2017. Effects of melatonin on anti-oxidative systems and photosystem II in cold-stressed rice seedlings. Front. Plant Sci. 8, 785.

Burgess, P., Huang, B., et al., 2016. Mechanisms of hormone regulation for drought tolerance in plants. In: Hossain, M., et al. (Eds.), Drought Stress Tolerance in Plants, Editors.

Sharma, A., et al., 2020. Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted Carya cathayensis plants. Sci. Total Environ. 713, 136675.

Pessarakli, M., 2010. Handbook of Plant and Crop Stress. CRC Press, New York.

Greenway, H., Armstrong, W., Colmer, T.D., 2006. Conditions leading to high CO2 (>5kPa) in waterlogged-flooded soils and possible effects on root growth and metabolism. Ann. Bot. 98 (1), 9–32.

Miranda-Apodaca, J., et al., 2020. N metabolism performance in Chenopodium quinoa subjected to drought or salt stress conditions. Plant Physiol. Biochem. 155, 725–734.

González-Dugo, V., Orgaz, F., Fereres, E., 2007. Responses of pepper to deficit irrigation for paprika production. Sci. Hortic. 114 (2), 77–82.

Widuri, L.I., et al., 2017. Relative leaf expansion rate and other leaf-related indicators for detection of drought stress in chili pepper (Capsicum annuum L.). Aust. J. Crop. Sci. 11 (12), 1617–1625.

Kyparissis, A., Petropoulou, Y., Manetas, Y., 1995. Summer survival of leaves in a soft-leaved shrub (Phlomis fruticosaL., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot. 46 (12), 1825–1831.

Linkemer, G., Board, J.E., Musgrave, M.E., 1998. Waterlogging effects on growth and yield components in late-planted soybean. Crop. Sci. 38 (6), 1576–1584.

Henshaw, T.L., et al., 2007. Soya Bean (Glycine max L. Merr.) genotype response to early-season flooding: I. root and nodule development. J. Agron. Crop. Sci. 193 (3), 177–188.
Mansfield, T.A., Jones, R.J., 1971. Effects of abscisic acid on potassium uptake and starch content of stomatal guard cells. Planta 101 (2), 147–158.
Farooq, M., et al., 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29 (1), 185–212.
Chen, Z., Cao, X., Niu, J., 2021. Effects of melatonin on morphological characteristics, mineral nutrition, nitrogen metabolism, and energy status in alfalfa under high-nitrate stress. Front. Plant Sci. 12, 694179.
Keatinge, J.D.H., et al., 2014. Overcoming biotic and abiotic stresses in the Solanaceae through grafting: current status and future perspectives. Biol. Agric. Hortic. 30 (4), 272–287.
Yamauchi, T., et al., 2014. Ethylene and reactive oxygen species are involved in root aerenchyma formation and adaptation of wheat seedlings to oxygen-deficient conditions. J. Exp. Bot. 65 (1), 261–273.
Broughton, S., et al., 2015. Waterlogging tolerance is associated with root porosity in barley (Hordeum vulgare L.). Mol. Breed. 35 (1).
Guidi, L., Soldatini, F., 1997. Chlorophyll fluorescence and gas exchanges in flooded soybean and sunflower plants. Plant Physiol. Biochem. 35, 713–717.
Rose, I.A., 1988. Effects of moisture stress on the oil and protein components of soybean seeds. Aust. J. Agric. Res. 39 (2).
Chiang, H.H., Dandekar, A.M., 1995. Regulation of proline accumulation in Arabidopsis thaliana (L.) Heynh during development and in response to desiccation. Plant, Cell Environ. 18 (11), 1280–1290.
Georgiadou, E.C., et al., 2018. Influence of heavy metals (Ni, Cu, and Zn) on nitro-oxidative stress responses, proteome regulation and allergen production in basil (Ocimum basilicum L.). Plants. Front Plant Sci 9, 862.
Chen, K., Zhang, C., 2000. Polyamine contents in the spring wheat leaves and their relations to drought resistance. Acta Phytophysiologica Sinica 26, 381–386.
Wu, L., et al., 1997. A comparison of physiological and morphological reactions of three types of blueberries to flooding stresses. Acta Hortic. Sinica 24, 287–288.
Olivella, C., et al., 2000. Hormonal and physiological responses of gerbera jamesonii to flooding stress. HortScience 35 (2), 222–225.
Laur, J., Hacke, U.G., 2013. Transpirational demand affects aquaporin expression in poplar roots. J. Exp. Bot. 64 (8), 2283–2293.
Li, X.J., et al., 2010. Abscisic acid pretreatment enhances salt tolerance of rice seedlings: proteomic evidence. Biochim. Biophys. Acta 1804 (4), 929–940.
Yue, C., et al., 2014. Molecular cloning and expression analysis of tea plant aquaporin (AQP) gene family. Plant Physiol. Biochem. 83, 65–76.
Jang, J.Y., et al., 2004. An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in Arabidopsis thaliana. Plant Mol. Biol. 54 (5), 713–725.
Rouphael, Y., Colla, G., 2020. Editorial: biostimulants in agriculture. Front. Plant Sci. 11, 40.
Nouraei, S., Rahimmalek, M., Saeidi, G., 2018. Variation in polyphenolic composition, antioxidants and physiological characteristics of globe artichoke (Cynara cardunculus var. scolymus Hayek L.) as affected by drought stress. Sci. Hortic. 233, 378–385.
Güneş, A., İnal, A., Aktaş, M., 1996. Reducing nitrate content of NFT grown winter onion plants (Allium cepa L.) by partial replacement of NO3 with amino acid in nutrient solution. Sci. Hortic. 65 (2–3), 203–208.
Atanasova, E., 2008. Effect of nitrogen sources on the nitrogenous forms and accumulation of amino acid in head cabbage. Plant, Soil Environ. 54 (2), 66–71.
Hanower, P., Brzozowska, J., 1975. Effect of osmotic stress on the composition of free amino acid in cotton leaves. Phytochemistry 14, 1691–1694.
Yang, C.W., Lin, C.C., Kao, C.H., 2000. Proline, ornithine, arginine and glutamic acid contents in detached rice leaves. Biol. Plant. 43 (2), 305–307.
Fabiani, A., et al., 2002. High-performance liquid chromatographic analysis of free amino acids in fruit juices using derivatization with 9-fluorenylmethyl-chloroformate. J. Chromatogr. Sci. 40 (1), 14–18.
Gonzalez, C., Zheng, Y., Lovatt, C.J., 2010. Properly timed foliar fertilization can and should result in a yield benefit and net increase in grower income. Acta Hortic. (868), 273–286.