



Exogenous melatonin enhances heat tolerance in buckwheat seedlings by modulating physiological response mechanisms

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ABSTRACT

Melatonin (MT) serves as a potent antioxidant in plant organisms, bolstering their resilience to temperature stress. In this study, the impact of MT on various buckwheat varieties under high-temperature stress conditions (40 °C) was investigated. Specifically, five buckwheat seedling varieties, comprising three sweet buckwheat variants (*Fagopyrum esculentum*) and two bitter buckwheat types (*Fagopyrum tataricum*), were subjected to foliar sprays of melatonin at concentrations of 50, 100 and 200 µM, with water at 25 °C employed as a control. Results demonstrated that exogenous MT at different concentrations improved the growth and physiological parameters of buckwheats, ameliorating damage induced by high-temperature stress. Notably, the application of 100 µM MT significantly augmented shoot biomasses of buckwheat seedlings under high-temperature conditions. Furthermore, the MT significantly increased the levels of osmotic adjustment substances and chlorophyll concentrations, enhanced antioxidant enzyme activities, chlorophyll fluorescence parameters, and improved photosynthetic gas exchange parameters in five different varieties of buckwheat. This led to the alleviation of damage to buckwheat seedlings subjected to high-temperature stress. Subsequently, five advanced statistical analysis methods: Principal Component Analysis, Grey Relational Analysis, Path Coefficient Analysis, Membership Function Method, and Coupling Coordination Analysis were employed to delve deeper into the existing data indicators. To summarize, the beneficial effect of exogenous melatonin on seedling growth is primarily achieved through the coordination and regulation of the antioxidant enzyme system and osmotic regulatory substances, ensuring the growth and development of buckwheat seedlings while also improving their heat tolerance. The treatment with a concentration of 100 µM of MT was the most effective.

1. Introduction

Buckwheat is both a valuable food crop and a decorative plant. The seeds are gluten-free and high in dietary fiber, rutin, and protein. Buckwheat's amino acid profile is noticeably balanced, with a particularly high lysine concentration, which enhances both its nutritional value and unique flavor (Christa and Soral-Śmietana, 2008; Halbrech et al., 2005). Buckwheat plays a vital role in environmental conservation besides its human health benefits. It safeguards soil from erosion, efficiently absorbs nitrogen and phosphorus, displays robust resistance to

pests and diseases, and flourishes in diverse soil conditions (Kopeć et al., 2021). However, being a crop that prefers cooler climates, buckwheat is highly vulnerable to increased temperatures. The optimum temperature for buckwheat growth was 21–25 °C (Aubert et al., 2020).

With the escalation of global warming, the heightened frequency and severity of extreme weather events present a significant threat (Kumari et al., 2020). Heat stress emerges as a critical environmental challenge impacting agricultural growth and productivity by disturbing cellular balance in crops, leading to impaired growth and potential death under severe conditions (Prasad et al., 2023; Sajid et al., 2022; Zhu et al.,

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2022). Studies show that heat stress can hinder leaf expansion, reduce photosynthesis, harm cellular and chloroplast structures, diminish pollination rates, and trigger oxidative stress responses in buckwheat, consequently impacting its growth rate and yield, consequently impacting its growth rate and yield (Aubert et al., 2020; Hornyák et al., 2020; Pláček et al., 2019). These physiological changes significantly hinder buckwheat's growth and productivity, limiting its cultivation in various ecological zones due to its thermal sensitivity. Therefore, it is imperative to identify strategies to mitigate high-temperature stress.

Melatonin (MT), also known as pineal gland secretion, is an indole compound widely distributed in both animals and plants (Arnao, 2014; Arnao and Hernández-Ruiz, 2006). In the plant kingdom, melatonin is revered as a crucial biological defense molecule, enhancing plant resilience against environmental stressors through various mechanisms (Bose and Howlader, 2020; Khan et al., 2020). Studies have shown melatonin's ability to effectively counteract hydroxyl radicals ($\cdot\text{OH}$), peroxynitrite radicals ($\cdot\text{ONOO}^-$), and radicals responsible for lipid peroxidation, highlighting its role as a potent endogenous antioxidant. Additionally, melatonin scavenges other harmful species like lipid peroxyl radicals ($\text{LOO}^\cdot$), superoxide anion radicals ($\text{O}_2^\cdot$), hydrogen peroxide (H_2O_2), and nitric oxide radicals ($\text{NO}^\cdot$) (Zhang and Zhang, 2014). Research reveals that the application of exogenous melatonin can effectively alleviate high-temperature stress by protecting cell membranes, enhancing photosynthetic efficiency, preserving the photosystem, and preventing heat-induced disruption of photosynthesis (Hu et al., 2023; Sehar et al., 2023). These findings not only elucidate the diverse roles of melatonin in regulating plant responses to thermal stress but also present a novel approach of utilizing exogenous melatonin as a protective agent for plants to mitigate the detrimental effects of high-temperature stress.

Although the influence of melatonin on plant growth and development is well-documented, its specific mode of action in buckwheat under high-temperature stress remains elusive. Investigating the effects of exogenous melatonin on buckwheat's growth and development under such conditions is poised to enhance our understanding of melatonin's operational mechanisms and may reveal buckwheat's unique mechanisms for heat tolerance. Therefore, this study aims to bridge this gap by examining how exogenous melatonin alleviates high-temperature stress in buckwheat, comprehensively evaluating the impacts of melatonin treatment on growth parameters, photosynthetic efficiency, and antioxidant defense systems across five buckwheat varieties exposed to thermal stress. By elucidating the growth-inhibitory effects, this research seeks to establish a scientific basis for utilizing plant growth regulators to improve crop heat tolerance and provide valuable insights for studying and implementing heat resistance in other agricultural species.

2. Materials and methods

2.1. Plant material and reagents

From October 2022 until June 2023, the experiment was conducted at the Laboratory of Plant Physiology and Biochemistry, College of Landscape and Tourism, Hebei Agricultural University, located in Baoding City, Hebei Province, China (38.827222°N, 115.453389°E). The testing materials included three common buckwheat varieties: Fenghuangtong ('Fhth'), Kanbaotianqiao ('Kbtq'), and 'Xn9978', two Tartary buckwheat varieties: Zhongku NO. 3 ('Zk3h'), Chuanqiao NO. 8 ('Cq8h'). These buckwheat seeds were obtained from the Buckwheat Gene Resources Innovation Research Group at the Institute of Crop Science, Chinese Academy of Sciences, a component of China's agricultural business. melatonin of 99% purity was received from Sigma Company in the United States.

2.2. Experimental setup

This study examined the growth of five buckwheat varieties: 'Fhth', 'Kbtq', 'Zk3h', 'Cq8h', and 'Xn9978'. Seedlings were grown in plastic pots measuring 15 cm in diameter and 12 cm in height. Watering was initially performed to establish a soil moisture level of around 80%, followed by the planting of disinfected, high-quality seeds. The germination substrate, including $\geq 45\%$ organic matter, reduced the requirement for fertilizer application for 20 days after germination. Following that, a water-soluble fertilizer high in macronutrients was administered biweekly by root soaking.

A subset of consistently growing seedlings was selected for melatonin therapy 30 days after germination, when they had established 2 to 3 leaves. Melatonin concentrations of 0 μM , 50 μM , 100 μM , and 200 μM were delivered at 20:00 for five consecutive nights. Spray on both sides of the leaves at a time to stop the melatonin drip (Jiang et al., 2021).

Following the last treatment, the seedlings were moved to an artificial climate room set to 40 °C for the high-temperature treatment and 25 °C for the control. To achieve consistent light dispersion, the pots' positions within the chamber were frequently modified. The experimental design contained three duplicates of each treatment, each containing ten plants. The treatments were as follows: W (leaf spraying with water at 25 °C), W + H (leaf spraying with water at 40 °C), MT50+H (leaf spraying with 50 μM melatonin at 40 °C), MT100+H (leaf spraying with 100 μM melatonin at 40 °C), and MT200+H. Physiological measures were taken at 0, 12, 24, 48, and 72 h post-treatment to fully examine the seedlings' physiological responses to the treatments.

2.3. Measurement of physiological and biochemical indicators

After 72 h of high-temperature stress, the growth responses of buckwheat seedlings were measured. Seedling height was measured from the soil surface to the apex with a centimeter ruler, and leaf area was calculated using the length-width coefficient technique reported by Mao et al. (2014). Seedlings were then divided into stems and roots to determine fresh biomass. For dry biomass, the seedlings were dried at 70 °C until a consistent weight was reached, and then weighed.

The approach given by Borna et al. (2019) was slightly modified to measure soluble sugars (Ss) concentration. 0.3 g of seed sample was added to 9 mL of distilled water in a boiling water bath for 30 min. 1 mL of the supernatant was combined with 5 mL of sulfuric acid-anthrone reagent, heated for 10 min, and then cooled. The absorption value was determined using a spectrophotometer at 620 nm.

The proline (Pro) concentration was measured using the approach provided by Subramanyam et al. (2019). Specifically, 0.3 g of the material was homogenized in 3 mL of 3% aqueous sulfosalicylic acid, then centrifuged. Then, 2 mL of the supernatant was combined with an equal volume of glacial acetic acid and ninhydrin reagent. The mixture was cooked in boiling water for 30 min. After cooling, the sample was centrifuged at 10,000 rpm for 5 min. The absorbance was measured at 520 nm using a spectrophotometer.

The soluble protein (Sp) concentration was determined using the Coomassie Brilliant Blue technique, as described by Yasmeen et al. (2013). To make the Coomassie Brilliant Blue G-250 solution, first dissolve 0.1 g of dye in 50 mL of 90% ethanol, then add 100 mL of 85% phosphoric acid. The volume was subsequently increased to 1000 mL using distilled water, and the solution was kept in an amber glass container for one month. In the test process, 0.1 mL of the enzyme solution was mixed with 0.9 mL of distilled water, then 5 mL of Coomassie Brilliant Blue G-250 reagent was added. The mixture was thoroughly homogenized and allowed to stand for 2 min. Absorbance readings were then taken at a wavelength of 595 nm.

The relative electrical conductivity (REC) was measured using a conductivity meter, as reported by Lim et al. (2022). Fresh ornamental buckwheat samples were washed with deionized water and then dried to eliminate surface moisture. Following cutting, the samples were

properly weighed, and 0.1 g of each was deposited in centrifuge tubes (triplicates). The samples were immersed in deionized water for 24 h. First, conductivity measurements were obtained (R1), and then the samples were cooled in boiling water for 20 min before being measured again (R2).

Catalase (CAT) activity was determined using the technique published by Baureder et al. (2014). For each test, a 50 mL Erlenmeyer flask was used (the second as the control). The test flask got 2.5 mL of enzyme extract, whereas the control flask had 2.5 mL of boiling enzyme extract. Each received 2.5 mL of H₂O₂. The mixes were then incubated in a 30 °C water bath for 10 min. The reaction was then terminated by adding 2.5 mL of 10% H₂SO₄. Titrated with 0.1 mol/L KMnO₄ till pink (30 s remaining).

Malonaldehyde (MDA) was measured using the acid ninhydrin technique (Landi, 2017). A 0.1g tissue sample was weighed and homogenized with 2 mL of 10% trichloroacetic acid and a little quantity of quartz sand. The homogenate was then mixed with an additional 8 mL of 10% trichloroacetic acid before being transferred to a 10 mL centrifuge tube and centrifuged at 2000 rpm for 10 min. The supernatant served as the sample extract. In a centrifuge tube, combine 3 mL of 0.5% thiobarbituric acid and 1 mL of enzyme extract. The mixture was then incubated in a boiling water bath for 30 min, cooled quickly, then centrifuged at 9000 rpm for 15 min. The absorbance of the supernatant was measured at 450 nm, 532 nm, and 600 nm wavelengths to determine the absorbance value, with thiobarbituric acid serving as a blank.

Superoxide dismutase (SOD) activity was evaluated using the nitroblue tetrazolium reduction method (Stephenie et al., 2020). The assay involved two sets of test tubes: one for treatment and the other for sample analysis. The reaction mixture contained the following: 1.5 mL of pH 7.8 phosphate buffer (only for test tubes), 0.05 mL of enzyme solution (only for control), 0.3 mL of methionine, 0.3 mL of nitroblue tetrazolium, 20.3 mL of EDTA-Na, 0.25 mL of distilled water, 0.05 mL of enzyme solution, 0.3 mL of riboflavin. Two test tubes were used pH 7.8 phosphate buffer instead of enzyme solution. For the two test tubes, one tube was placed in the dark, while the other was reacted in 4000 LX (25 °C light incubator, level 6 light) sunlight for 20 min. After the reaction was over, they were covered with black plastic bags to terminate the reaction. The absorbance of the samples was measured at 560 nm, using the unilluminated tube as a blank.

Peroxidase (POD) was measured using the guaiacol technique (Stephenie et al., 2020). The reaction mixture included 2.9 mL of phosphate buffer, 1 mL of 2% H₂O₂, 1 mL of guaiacol solution, and 0.1 mL of enzyme solution (added at the end). The mixture was put in a 37 °C water bath for 15 min before rapidly chilling in an ice bath. To stop the process, 2 mL of trichloroacetic acid was added. The absorbance was measured at 470 nm with a control of 2.5 mL of heated and boiling enzyme solutions.

The net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), transpiration rate (*Tr*), and ambient CO₂ concentration (*Ci*) were measured using an XT LI-6400 portable photosynthesis system equipped with a leaf chamber fluorometer (Li-Cor Inc., Lincoln, NE, USA). The fully expanded leaves from each plant's middle to upper parts were randomly selected and measured at 12h. Before measurements, the leaves were acclimated for 2 min at each PPFD, including 0, 20, 40, 60, 80, 100, 300, 500, 800, 1200, 1500, and 2000 μmol mol⁻¹. The *Ci* was set up as 380 μmol mol⁻¹ and 2.0 ± 0.4 kPa, respectively (Lee et al., 2023).

Fv is calculated by the difference of Fm and Fo, and it reflects the reduction of electron acceptors of PSII(QA). The maximal PSII efficiency of dark-adapted leaves (Fv/Fm), maximum primary photochemical yield (Fv/Fo) of PSII, a crucial indicator for assessing the extent of light inhibition in plant photosynthesis and directly correlated with photosynthetic intensity, was calculated using various formulas and the relative variable fluorescence(Vj)index reflects the degree of damage to plant leaves in adverse environments and is an important parameter for evaluating the ability of plants to cope with stress were calculated following Wan et al. (2020).

The chlorophyll content was determined using the method of He et al. (2020) with some modification. chlorophyll α (Ca), chlorophyll β (Cb), total chlorophyll and carotenoid (Cc) concentrations in the leaves after 72h were determined quantitatively. The fresh leaves (0.1 g) of *P. indicus* seedlings were sampled at each time point and placed in a glass tube containing 10 mL of 80% acetone. The tube was stored in a refrigerator at 4 °C for seven days, and the extract was measured at three different wavelengths (663, 645, and 480 nm) with a UV spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Japan).

2.4. Statistical analysis

Data were recorded in Microsoft Excel and analyzed statistically with SPSS 26.0. Duncan's test was employed for comparing average values and performing the least significant difference test. Correlation, path, and principal component analyses were conducted. Graphs were generated using Prism 9.0 statistical software. Additionally, cluster analysis was performed using Tbtools (<https://www.Tbtools.com>) and Chplot (<https://www.chplot.online/>) online platforms.

(1) Correlation analysis (Adler and Parmryd, 2010)

Correlation analysis (Pearson correlation) assesses the degree of connection between variables. The value ranges from −1 to 1. A value of 1 represents a perfect positive correlation, −1 shows a complete negative correlation, and zero indicates no linear association.

The relevant calculation formula is:

$$R = \frac{\sum (X_i - X_{av}) \times (Y_i - Y_{av})}{\sqrt{\sum (X_i - X_{av})^2 \times \sum (Y_i - Y_{av})^2}}$$

Where X_i , Y_i represents different values of the measurement index, X_{av} , Y_{av} represents the corresponding average number of the measured indicators.

(2) Path analysis (Lu et al., 2022)

The independent variables in this study include SOD, POD, CAT, Sp, Ss, Pro, and MDA, with REC serving as the dependent variable (Y). We initially performed a Y normalcy test. The linear regression equation was then obtained by doing a stepwise regression analysis. The indirect route coefficient (n) of each variable to the dependent variable is determined using the direct path coefficient and linear regression equation acquired through stepwise regression analysis. The relevant calculating formula is:

$$n = r_{ij} \times P_{jy}$$

In the formula, r_{ij} is the correlation coefficient between X_i and X_j ; P_{jy} is the direct path coefficient of X_j ; P_i is the direct path coefficient of the independent variable.

(3) Membership function analysis

The method of Schroeter-Zakrzewska and Pradita (2021) was used to calculate the membership function value (Um) of each measurement indicator. The calculation formula is:

$$U_m = (C_{im} - C_{imin}) \div (C_{imax} - C_{imin})$$

where C_{imax} represents the maximum value of the mth measurement index; C_{imin} represents the minimum value of the mth measurement index; the greater the average value of the membership function, the stronger the high temperature resistance of the variety.

(4) Coupling Coordination Analysis

Coupling coordination analysis adopts the method proposed by Dong

Table 1
Standard classification of coupling coordination levels.

Coupling Coordination D Value Range	Coordination Level	Coupling Coordination Degree
[0.0–0.1)	1	Extreme Imbalance
[0.1–0.2)	2	Serious Imbalance
[0.2–0.3)	3	Moderate Imbalance
[0.3–0.4)	4	Slight Imbalance
[0.4–0.5)	5	Near Imbalance
[0.5–0.6)	6	Barely Coordinated
[0.6–0.7)	7	Primary Coordination
[0.7–0.8)	8	Intermediate Coordination
[0.8–0.9)	9	Good Coordination
[0.9–1.0]	10	Excellent Coordination

et al. (2023). The coupling coordination model involves the calculation of three indices, namely, the coupling.
Coordination index T value:

$$T = \alpha U + \beta A$$

Coupling coordination D value: degree C value, coordination index T value, and coupling coordination D value. Their calculation formulas are as follows:

Coupling degree C value:

$$C = 2 \times \left(\frac{U \times A}{(U + A)^2} \right)^{1/2}$$

$$D = \sqrt{C \times T}$$

Where C represents the coupling degree of the antioxidant enzyme system and osmotic regulating substances, T represents the comprehensive level of the antioxidant enzyme system and osmotic regulating substances, and D represents the coupling coordination of the antioxidant enzyme system and osmotic regulating substances; U and A are the comprehensive scores of the antioxidant enzyme system and osmotic regulating substances, and α and β are the contribution coefficients of

the antioxidant enzyme system and osmotic regulating substances. It is generally considered that the antioxidant enzyme system and osmotic regulating substances are equally important, i.e., $\alpha = \beta = 0.5$.

The classification standard of coupling coordination levels is shown in Table 1.

3. Results

3.1. Effects of exogenous melatonin on biomass accumulation in buckwheat seedlings under high temperature stress

The administration of melatonin significantly influenced biomass accumulation in buckwheat seedlings, as depicted in Table 2. In comparison with the W treatment, both the fresh and dry weights of leaves and stems of the five buckwheat varieties were notably reduced in the W + H treatment. Notably, among these varieties, the fresh and dry weights of leaves of ‘Zk3h’ were most impacted, experiencing a decrease of 26.95% and 54.55%, respectively. Additionally, the fresh and dry weights of stems of ‘Kbtq’ exhibited the most significant changes, decreasing by 37.18% and 36.11%, respectively. Conversely, the MT50+H, MT100+H, and MT200+H treatments, when compared with the W + H treatment, facilitated seedling growth, with the MT100+H treatment demonstrating the most pronounced effect. Specifically, under this treatment, both the fresh and dry weights of leaves and stems of the five buckwheat varieties increased. Notably, the impact on ‘Zk3h’ was most substantial, with fresh weights of leaves and stems increasing by 26.70% and 32.74%, and dry weights of leaves and stems increasing by 60.00% and 33.33%, respectively.

3.2. Effects of exogenous melatonin on the concentration of chlorophyll in buckwheat leaves under high temperature stress

The concentration of chlorophyll α , chlorophyll β , carotenoids, and total chlorophyll in the leaves of five kinds of buckwheat seedlings was significantly lower than that in the W treatment (Fig. 1). Notably, ‘Cq8h’ was the most susceptible to high temperatures, with drops of 27.54%,

Table 2
Effect of different concentrations of melatonin and high temperature on the fresh and dry weight of five varieties of buckwheat. Data (means $\pm$ SD, n = 3) followed by different letters among treatments within the same varieties indicate significant differences at the 5% level. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

Varieties	Treatment	Leaf		Stem	
		Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
‘Fhth’	W	0.450 $\pm$ 0.036 a	0.064 $\pm$ 0.002 a	0.837 $\pm$ 0.043 a	0.067 $\pm$ 0.002 a
	W + H	0.384 $\pm$ 0.021 b	0.045 $\pm$ 0.002 c	0.695 $\pm$ 0.087 b	0.046 $\pm$ 0.003 c
	MT50+H	0.428 $\pm$ 0.028 ab	0.051 $\pm$ 0.002 b	0.773 $\pm$ 0.041 ab	0.051 $\pm$ 0.004 bc
	MT100+H	0.444 $\pm$ 0.016 a	0.058 $\pm$ 0.004 a	0.805 $\pm$ 0.028 a	0.053 $\pm$ 0.003 b
	MT200+H	0.410 $\pm$ 0.026 ab	0.049 $\pm$ 0.004 bc	0.837 $\pm$ 0.043 ab	0.048 $\pm$ 0.003 bc
‘Kbtq’	W	0.419 $\pm$ 0.022 a	0.064 $\pm$ 0.003 a	0.694 $\pm$ 0.011 a	0.036 $\pm$ 0.002 a
	W + H	0.354 $\pm$ 0.026 c	0.061 $\pm$ 0.002 c	0.436 $\pm$ 0.020 c	0.023 $\pm$ 0.003 c
	MT50+H	0.380 $\pm$ 0.016 abc	0.040 $\pm$ 0.004 bc	0.484 $\pm$ 0.034 bc	0.027 $\pm$ 0.003 bc
	MT100+H	0.410 $\pm$ 0.028 ab	0.044 $\pm$ 0.004 b	0.504 $\pm$ 0.025 b	0.031 $\pm$ 0.002 ab
	MT200+H	0.364 $\pm$ 0.029 bc	0.041 $\pm$ 0.002 c	0.452 $\pm$ 0.032 c	0.025 $\pm$ 0.004 c
‘Zk3h’	W	0.282 $\pm$ 0.031 a	0.033 $\pm$ 0.002 a	0.304 $\pm$ 0.025 a	0.016 $\pm$ 0.001 a
	W + H	0.206 $\pm$ 0.021 b	0.015 $\pm$ 0.001 c	0.223 $\pm$ 0.013 c	0.012 $\pm$ 0.002 b
	MT50+H	0.234 $\pm$ 0.022 ab	0.020 $\pm$ 0.002 b	0.253 $\pm$ 0.022 bc	0.015 $\pm$ 0.002 ab
	MT100+H	0.261 $\pm$ 0.036 a	0.024 $\pm$ 0.003 b	0.296 $\pm$ 0.020 a	0.016 $\pm$ 0.001 a
	MT200+H	0.247 $\pm$ 0.018 ab	0.022 $\pm$ 0.002 b	0.276 $\pm$ 0.015 ab	0.015 $\pm$ 0.002 ab
‘Cq8h’	W	0.377 $\pm$ 0.018 a	0.044 $\pm$ 0.002 a	0.481 $\pm$ 0.012 a	0.028 $\pm$ 0.003 a
	W + H	0.307 $\pm$ 0.029 b	0.034 $\pm$ 0.002 c	0.395 $\pm$ 0.011 c	0.021 $\pm$ 0.001 b
	MT50+H	0.340 $\pm$ 0.036 ab	0.036 $\pm$ 0.002 bc	0.425 $\pm$ 0.028 bc	0.024 $\pm$ 0.003 ab
	MT100+H	0.369 $\pm$ 0.015 a	0.039 $\pm$ 0.002 b	0.455 $\pm$ 0.033 ab	0.027 $\pm$ 0.002 a
	MT200+H	0.330 $\pm$ 0.014 ab	0.034 $\pm$ 0.002 c	0.422 $\pm$ 0.018 bc	0.022 $\pm$ 0.002 b
‘Xn9978’	W	0.402 $\pm$ 0.022 a	0.055 $\pm$ 0.002 a	0.648 $\pm$ 0.024 a	0.044 $\pm$ 0.002 a
	W + H	0.333 $\pm$ 0.035 b	0.045 $\pm$ 0.004 c	0.482 $\pm$ 0.016 c	0.034 $\pm$ 0.002 c
	MT50+H	0.363 $\pm$ 0.023 ab	0.049 $\pm$ 0.002 bc	0.508 $\pm$ 0.027 bc	0.037 $\pm$ 0.002 bc
	MT100+H	0.392 $\pm$ 0.014 a	0.051 $\pm$ 0.002 ab	0.570 $\pm$ 0.046 b	0.042 $\pm$ 0.003 ab
	MT200+H	0.381 $\pm$ 0.018 a	0.047 $\pm$ 0.002 bc	0.532 $\pm$ 0.049 bc	0.040 $\pm$ 0.002 ab

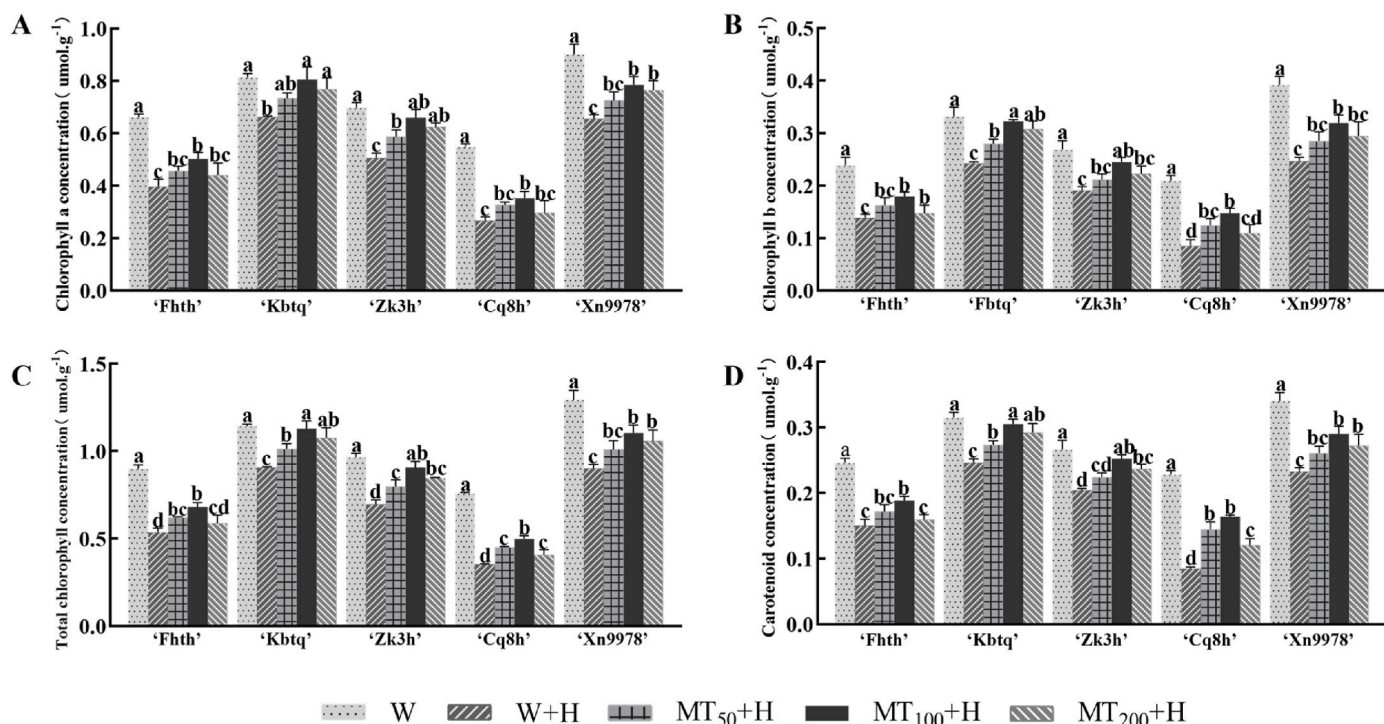


Fig. 1. Effect of different concentrations of melatonin and high temperature on the concentrations of chlorophyll α (A), chlorophyll β (B), carotenoids (C), and total chlorophyll (D) in leaves of five varieties of buckwheat. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

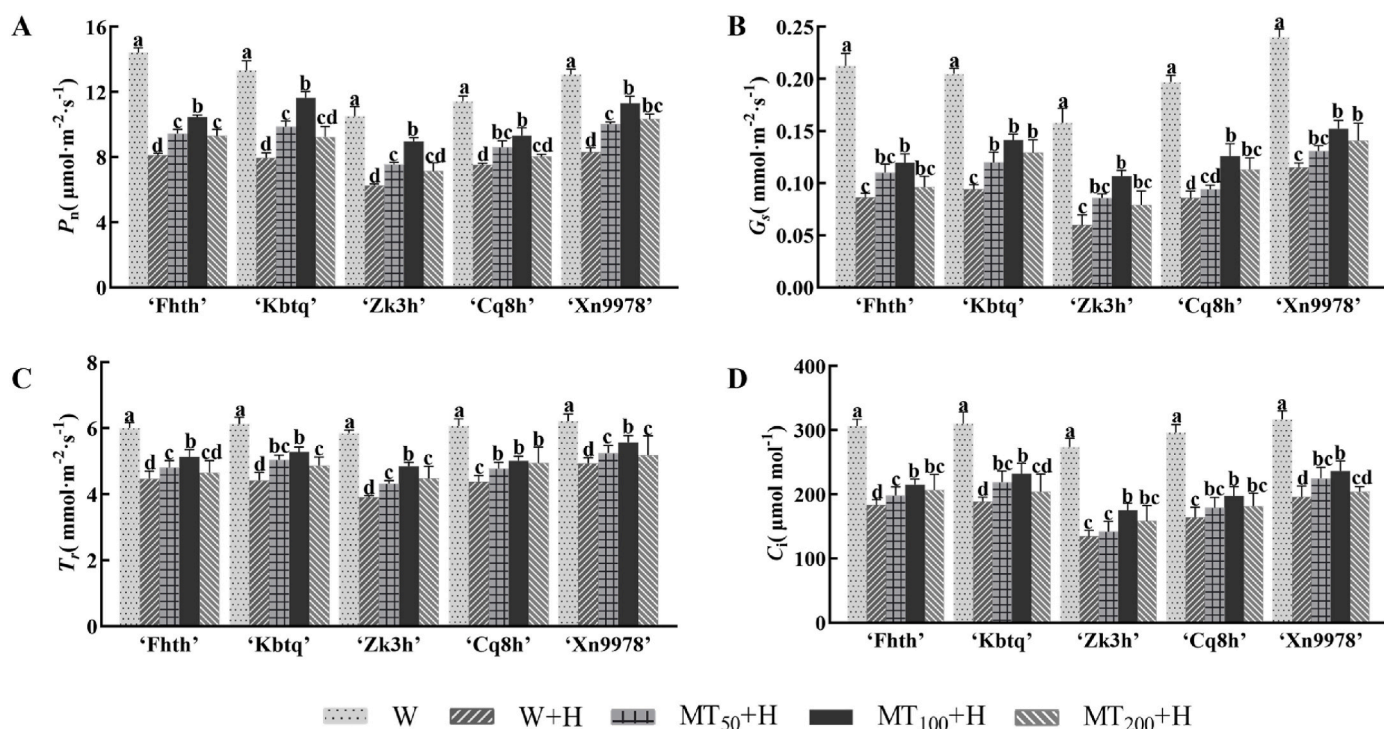


Fig. 2. Effect of different concentrations of melatonin and high temperature on the photosynthetic parameters P_n (A), G_s (B), T_r (C), and C_i (D) in leaves of five varieties of buckwheat. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

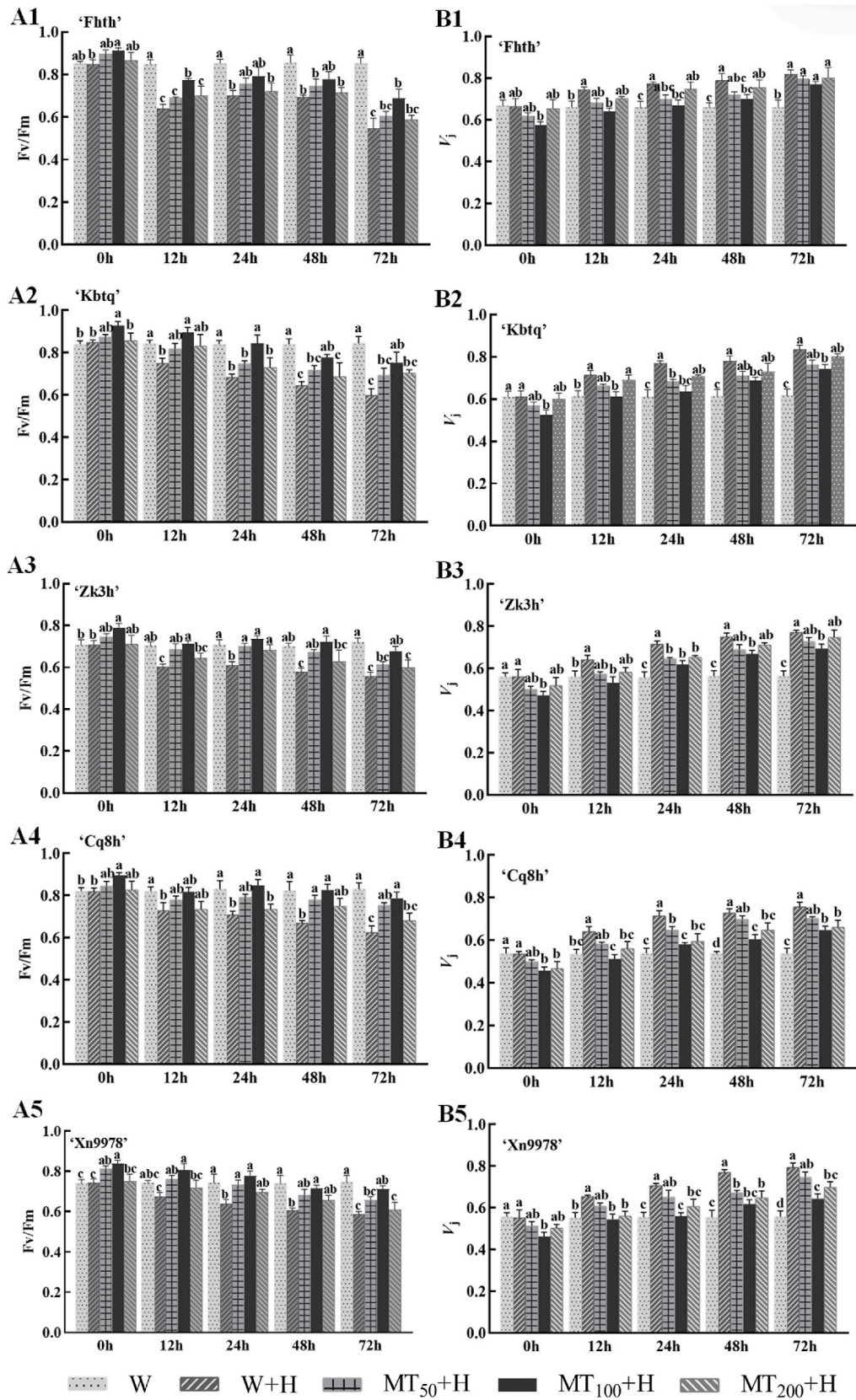


Fig. 3. Effect of different concentrations of melatonin and high temperature on the chlorophyll fluorescence parameters F_v/F_m (A1-A5) and V_j (B1-B5) in leaves of five varieties of buckwheat at 0, 12, 24, 48 and 72 h post-treatment. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

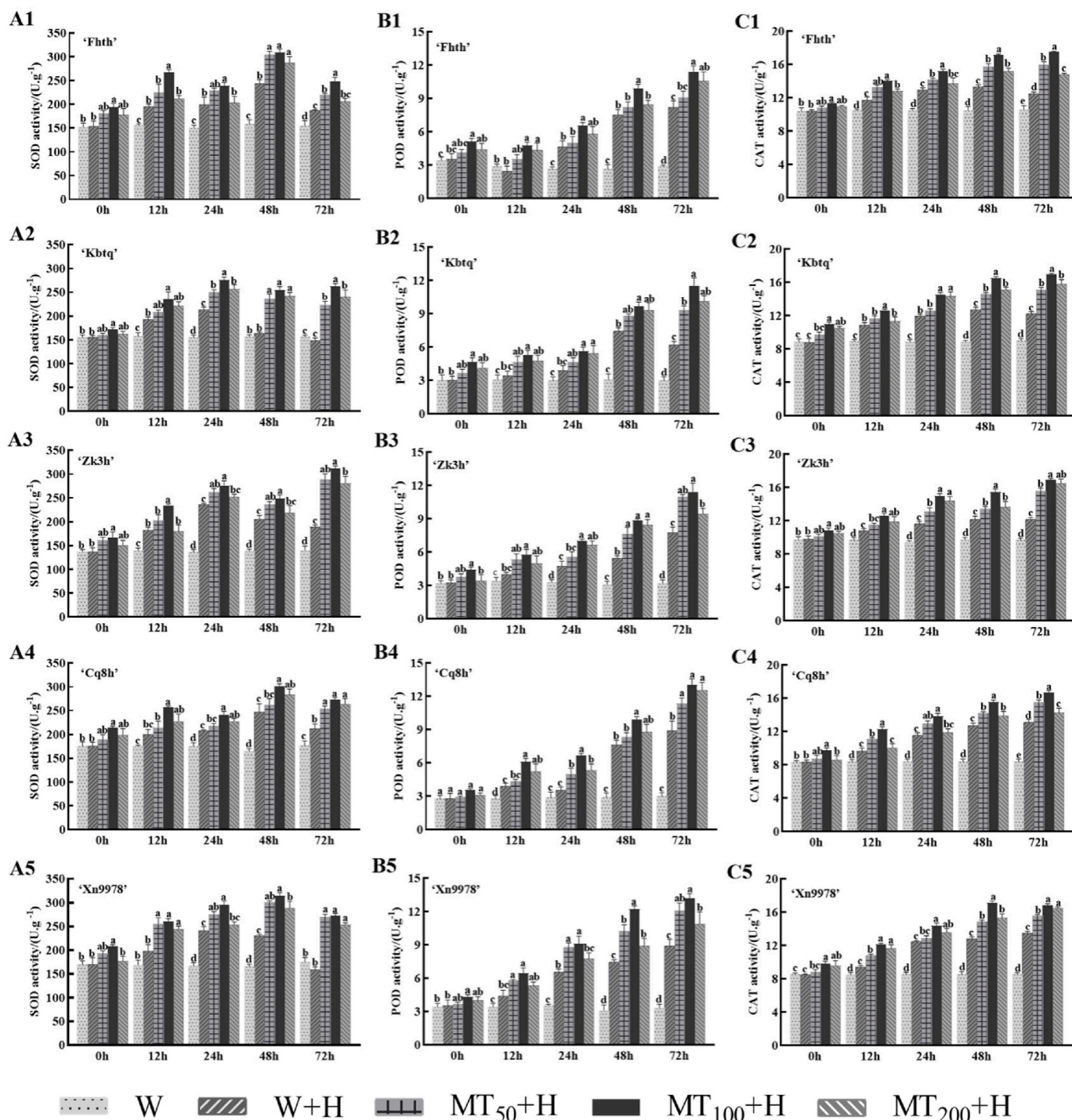


Fig. 4. Effect of different concentrations of melatonin and high temperature on the activities of SOD (A1-A5), POD (B1-B5) and CAT (C1-C5) in leaves of five varieties of buckwheat at 0, 12, 24, 48 and 72 h post-treatment. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

26.75%, 21.85%, and 20.87%, respectively, indicating its increased sensitivity to extreme temperatures. In contrast, 'Kbtq' showed very slight losses, with drops of 18.47%, 26.75%, 21.85%, and 27.96%, indicating its strong tolerance to high temperatures.

In compared to the W + H treatment, the MT50+H, MT100+H, and MT200+H treatments increased the concentrations of chlorophyll α , chlorophyll β , carotenoids, and total chlorophyll in the leaves of five buckwheat seedling kinds. Notably, the MT100+H therapy had the greatest impact, particularly in 'Cq8h', where the concentrations of

chlorophyll α , chlorophyll β , carotenoids, and total chlorophyll increased by 31.63%, 72.64%, 93.94%, and 41.55%, respectively.

3.3. Effect of exogenous melatonin on the photosynthetic performance of buckwheat leaves under high temperature stress

Results of the analysis indicated that under W + H circumstances, the photosynthetic parameters P_n , G_s , Tr , and C_i of buckwheat seedlings were considerably lower than under room temperature treatment (W).

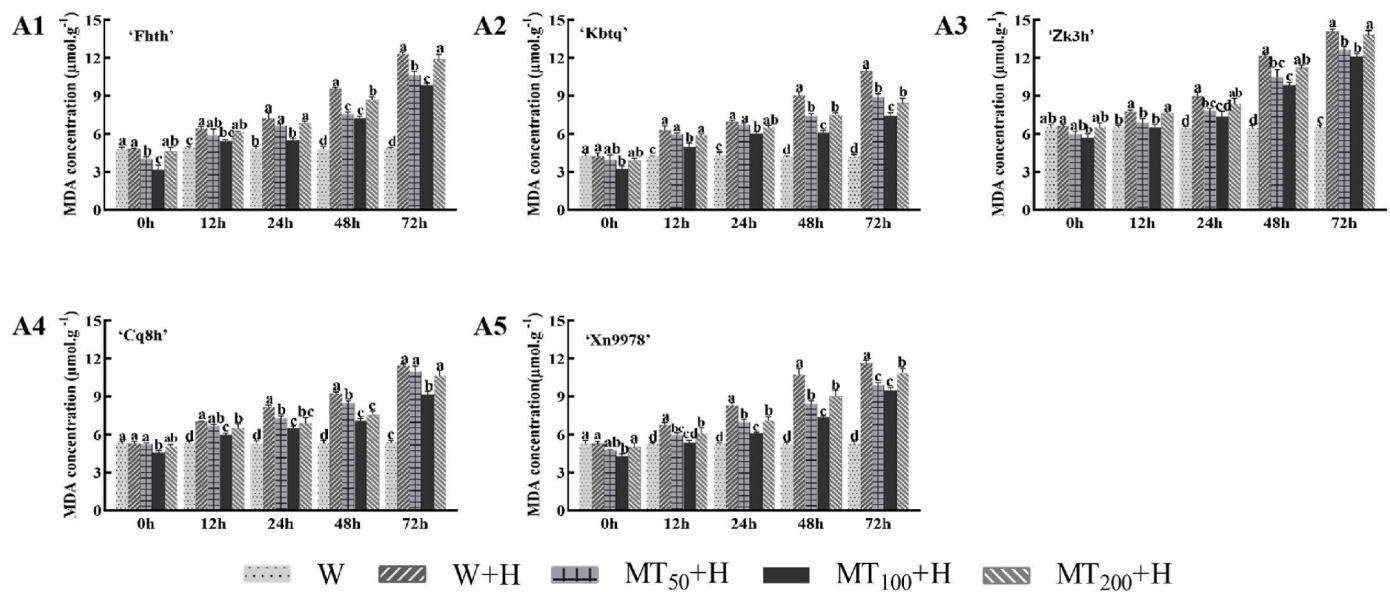


Fig. 5. Effect of different concentrations of melatonin and high temperature on the MDA concentration (A1–A5) in leaves of five varieties of buckwheat at 0, 12, 24, 48 and 72 h post-treatment. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

Notably, 'Zk3h' showed the most extreme decline, with decreases of 40.56%, 62.03%, 33.22%, and 50.91%, indicating that high temperatures had the most inhibitory effect on its photosynthesis (Fig. 2).

In contrast to the W + H treatment, the MT50+H, MT100+H, and MT200+H treatments significantly improved photosynthetic properties. The MT100+H treatment was especially interesting since it showed the greatest improvement in P_n , G_s , T_r , and C_i . Notably, 'Zk3h' and 'Kbtq' improved more significantly, with increases of 77.78%, 47.08%, 14.85%, 30.27% and 46.48%, 50.35%, 19.44%, and 22.97%, respectively (Fig. 2).

The Fv/Fm ratio of seedlings from five buckwheat types fell considerably when treated with W + H compared to W alone, indicating a severe suppression of photosynthesis owing to high temperature (Fig. 3A). Notably, the 'Fhth' and 'Kbtq' types showed particularly significant drops, with reductions of 36.01% and 22.69% at 72 h, respectively. In contrast, throughout varied treatment periods of MT50+H, MT100+H, and MT200+H, the Fv/Fm ratio considerably improved for all kinds save the 'Fhth' variety as compared to the W + H treatment. The MT100+H therapy, in particular, significantly improved the recovery of the Fv/Fm ratio, demonstrating that exogenous melatonin can effectively mitigate photoinhibition caused by high temperatures.

The Vj values of leaves from seedlings of the five buckwheat kinds were significantly higher under the W + H treatment compared to the W treatment (Fig. 3B). Furthermore, as stress duration grew, the rise in Vj values became more noticeable, peaking at 72 h, indicating increasing leaf damage due to high temperatures. However, the MT50+H, MT100+H, and MT200+H treatments successfully reduced Vj levels under high temperature stress, with the MT100+H treatment having the greatest impact. Except for the 'Fhth' variety, it significantly lowered Vj values for all other types across all time points.

3.4. Effects of exogenous melatonin on antioxidant enzyme activities in buckwheat leaves under high temperature stress

This study found that following the W + H treatment, the SOD activity of seedlings from five buckwheat types increased initially, but then decreased as the stress duration increased. At 24 and 48 h, SOD activity in seedlings of the five buckwheat cultivars increased significantly compared to the W treatment. Furthermore, the MT50+H, MT100+H,

and MT200+H treatments showed higher SOD activity than the W + H treatment. Notably, the MT100+H treatment had the largest effect on seedlings from the five buckwheat kinds at different time periods (12 h, 24 h, 48 h, and 72 h), particularly on 'Kbtq', which increased by 22.11%, 29.36%, 55.53%, and 76.96%, respectively (Fig. 4A).

Under the W + H treatment, the POD and CAT activities of seedlings from the five buckwheat types increased with longer stress duration, in contrast to the pattern found for SOD activity. However, the MT50+H, MT100+H, and MT200+H treatments significantly increased both POD and CAT activities as compared to the W + H therapy. Among these treatments, the MT100+H treatment had the greatest significant influence on seedlings from the five buckwheat types at different time points (12 h, 24 h, 48 h, and 72 h), however the effects varied between varieties. Specifically, at the 24th hour, the POD activity of 'Cq8h' increased the greatest, by 87.5%, while the CAT activity of 'Zk3h' increased by 28.78%. At 72 h, the POD activity of 'Kbtq' increased the greatest, by 85.71%, while the CAT activity of 'Fhth' increased by 39.82% (Fig. 4B, C).

3.5. Effects of exogenous melatonin on membrane damage in buckwheat leaves under high temperature stress

In comparison to the W treatment, the W + H treatment significantly raised MDA concentration in seedlings of the five buckwheat types. In particular, 'Kbtq' had the highest influence, while 'Cq8h' had the least impact, rising by 116.29% and 113.03% after 72 h, respectively (Fig. 5). However, with the MT50+H, MT100+H, and MT200+H treatments, all five types showed significant decreases when compared to the W + H treatment. The MDA concentration dropped dramatically with the MT100+H therapy. Among these therapies, 'Kbtq' had the greatest impact, while 'Zk3h' had the smallest, with decreases of 32.56% and 13.91%, respectively, at 72 h (Fig. 5).

The results showed that under the W + H treatment, the REC of the leaves of the five buckwheat seedlings rose considerably over time when compared to the W treatment. Notably, 'Kbtq' had the most influence, increasing by 59.09%, and 'Zk3h' increased by just 19.51%. However, with the MT50+H, MT100+H, and MT200+H treatments, all five kinds had lower relative conductivity than the W + H treatment. Among these therapies, the MT100+H therapy had the greatest benefit, reducing

Table 3

Effect of different concentrations of melatonin and high temperature on the Relative electrical conductivity of five varieties of buckwheat. Data (means $\pm$ SD, $n = 3$) followed by different letters among treatments within the same varieties indicate significant differences at the 5% level. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

Varieties	Relative electrical conductivity(%)				
	W	W + H	MT ₅₀ +H	MT ₁₀₀ +H	MT ₂₀₀ +H
'Fhth'	55.1 $\pm$ 3.04 c	76.19 $\pm$ 1.83 a	68.65 $\pm$ 1.26 b	60.59 $\pm$ 4.57 c	70.74 $\pm$ 3.56 ab
'Kbtq'	49.54 $\pm$ 1.42 c	78.82 $\pm$ 1.09 a	72.38 $\pm$ 2.61 b	66.65 $\pm$ 2.84 b	69.52 $\pm$ 4.98 b
'Zk3h'	60.84 $\pm$ 4.27 c	72.71 $\pm$ 5.21 a	69.78 $\pm$ 2.86 ab	63.85 $\pm$ 0.87 bc	66.03 $\pm$ 4.22 abc
'Cq8h'	56.02 $\pm$ 2.32 c	71.65 $\pm$ 1.48 a	68.42 $\pm$ 1.14 a	62.44 $\pm$ 2.66 b	69.34 $\pm$ 2.23 a
'Xn9978'	55.74 $\pm$ 3.31 d	71.87 $\pm$ 3.43 a	64.19 $\pm$ 4.39 bc	59.1 $\pm$ 2.89 cd	66.13 $\pm$ 3.3 ab

'Fhth' by 20.5%. 'Zk3h' had the least influence, with a decrease of 12.19% (Table 3).

3.6. Effects of exogenous melatonin on osmotic adjustment substance content in buckwheat leaves under high temperature stress

The study found that the W + H treatment significantly increased the concentrations of proline, soluble sugars, and soluble proteins in seedlings from five different buckwheat kinds over time when compared to the W treatment (Fig. 6). Furthermore, seedlings treated with MT50+H, MT100+H, and MT200+H had significantly higher levels of proline, soluble carbohydrates, and soluble proteins than those treated with W + H alone. The 'Xn9978' variety was especially interesting, with the MT100+H treatment showing the greatest increase in proline and soluble protein levels. At the 12th, 24th, 48th, and 72 nd h, proline rose by 39.68%, 37.16%, 42.32%, and 24.88%, respectively, whereas soluble protein increased by 20.49%, 22.3%, 22.58%, and 35.09%. Furthermore, among the 'Kbtq' types, the MT100+H treatment had the greatest impact on increasing the content of soluble sugars, with increases of 33.81%, 46.52%, 31.63%, and 35.85% at 12, 24, 48, and 72 h.

3.7. Comprehensive analysis of exogenous melatonin on physiological changes of buckwheat seedlings under high temperature stress

A correlation value of 0.99 was found between CAT and POD. POD showed a substantial positive connection with SOD, Cc, Ca, SFW, and SDW ($p < 0.01$). The weakest linkages were found between SOD and MDA and CAT and SDW. MDA and PSII photochemistry's highest quantum yield (Fv/Fm) showed a substantial negative correlation ($p < 0.01$) (Fig. 7).

Principal Component Analysis (PCA) was conducted on growth and physiological indicators across all treatments to assess the impact of exogenous melatonin under high temperature stress. The PCA extracted two main components for both growth and physiological indicators: PC1 and PC2, with contribution rates of 78.16% and 17.25% for growth indicators, and 46.00% and 25.31% for physiological indicators, respectively. In the growth indicators, stem fresh weight (SFW) had the highest loading on PC1 with a value of 0.176 (Fig. 7), indicating it made the most significant contribution to PC1's composition. On PC2, all indicators had lower loadings, suggesting they contributed less significantly.

Examine the effect of exogenous melatonin under high temperature stress using principal component analysis on all growth and physiological indications of the therapy. The contributions of PC1 and PC2 to the growth and physiological indices recovered by principal component analysis were 78.16% and 17.25%, 46.00% and 25.31%, respectively. In the seedling growth indicators, the characteristic of stem fresh weight (SFW) has the largest load on PC1, with a load value of 0.176 (Fig. 8A), suggesting that it makes the most important contribution to the composition of PC1. On PC2, all indicators indicate a low workload, implying a low contribution rate. In the physiological indicators of seedlings, the characteristic of SOD (superoxide dismutase activity) has

the highest load on PC1, with a load value of -36.62 (Fig. 8B), suggesting that it makes the most important contribution to the composition of PC1 and has a negative load. The highest load for REC on PC2 is 6.88, suggesting that it makes the most substantial contribution to PC2 and is both positive and negative.

Grey correlation degree can be used to analyze the relationship between exogenous melatonin concentration and physiological indicators, allowing for an assessment of their degree of correlation. Grey relational analysis revealed that the activities of SOD, CAT, and Pro have the strongest correlation with exogenous melatonin concentration. This implies that these indicators are effective in measuring the alleviating effects of exogenous melatonin on buckwheat seedling growth under high-temperature stress. Consequently, SOD activity, CAT activity, and Pro content are the most reliable indicators for evaluating exogenous melatonin's effectiveness in mitigating high-temperature stress. Additionally, soluble sugar, POD activity, MDA concentration, soluble protein, and the Fv/Fm ratio also indicate that exogenous melatonin positively influences buckwheat seedling development under high-temperature conditions (Fig. 9).

The size of the path coefficients reflects the extent to which physiological markers influence membrane permeability in buckwheat seedlings, indicating the potential for membrane damage. The study found that various physiological variables impact REC to different extents. Among these variables, SFW had the greatest influence on REC, with a path coefficient of 1.55, while Cb had the smallest impact, with a path coefficient of 0.266 (Fig. 10).

3.8. Analysis of heat tolerance in buckwheat seedlings under different treatments using the membership function method

The membership function approach is an effective method for quantitatively assessing plant stress resistance. In a study on buckwheat's heat tolerance, this approach was applied under various treatments. The findings revealed that during high-temperature treatment, the cultivar 'Cq8h' exhibited significantly higher membership function values than other types, indicating superior heat tolerance (Fig. 11A1, A2). Conversely, 'Kbtq' had the lowest membership function values, suggesting it was the least heat-tolerant (Fig. 11A1, A2). Additionally, exogenous melatonin doses of 50 μ M and 200 μ M did not influence the heat tolerance of the different buckwheat cultivars under high-temperature conditions (Fig. 11B1, B2, D1, D2). However, at a concentration of 100 μ M, the cultivar 'Zk3h' showed higher membership function values than 'Xn9978', indicating better heat tolerance (Fig. 11C1, C2).

3.9. The coordination of the antioxidant enzyme system with osmotic regulating chemicals in five buckwheat types

Coupling coordination analysis is a method used to evaluate and analyze the degree of interaction and coordination between the various components of a system (Fig. 12). In the examination of coupling coordination, the coupling coordination D value are predicted to lie between

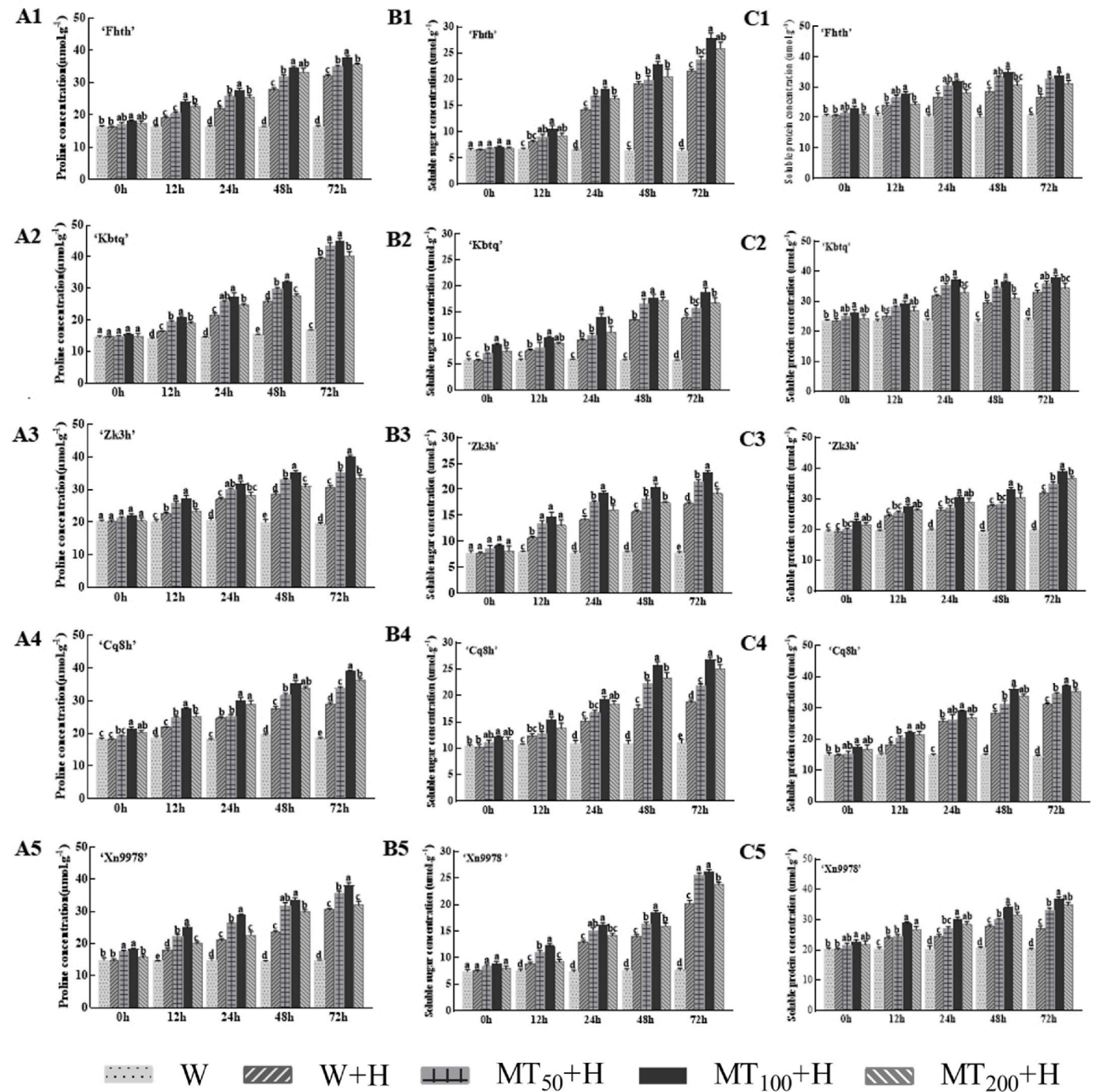


Fig. 6. Effect of different concentrations of melatonin and high temperature on the concentrations of proline (A1-A5), soluble sugars (B1-B5) and soluble proteins (C1-C5) in leaves of five varieties of buckwheat at 0, 12, 24, 48 and 72 h post-treatment. Data (means $\pm$ SD, $n = 3$) are significantly different ($p < 0.05$) if followed by different letters above the bars within the same varieties. W: leaf spraying with water at 25 °C; W + H: leaf spraying with water at 40 °C; MT50+H: leaf spraying with 50 μ M melatonin at 40 °C; MT100+H: leaf spraying with 100 μ M melatonin at 40 °C; MT200+H: leaf spraying with 200 μ M melatonin at 40 °C.

0 and 1, with higher values suggesting a greater degree of system co-ordination. These parameters are then used to classify the amount and degree of coordination.

The coupling coordination index of the antioxidant enzyme system and osmotic regulating chemicals in five buckwheat varieties gradually increased from 0 to 72 h. Specifically, under normal settings, these components in the five buckwheat kinds consistently exhibit an imbalance in coordination. However, with high-temperature stress, the alterations were more significant than in other five treatments. Notably, 'Cq8h' progressed through three stages, from strong coordination at 0h

to main coordination at 12h and intermediate coordination at later time periods. Similarly, after 48 h of exposure to high temperatures, 'Xn9978' transitioned from mild imbalance to intermediate coordination (Fig. 12).

However, treatment with varied amounts of exogenous MT had a substantial effect on the coupling coordination index of the buckwheat's antioxidant enzyme system and osmotic regulating chemicals when compared to their coordination index under high-temperature stress, which occurred in two stages. The coupling coordination indexes of 'Fhth', 'Kbtq', and 'Zk3h' tended to stabilize at various time intervals.

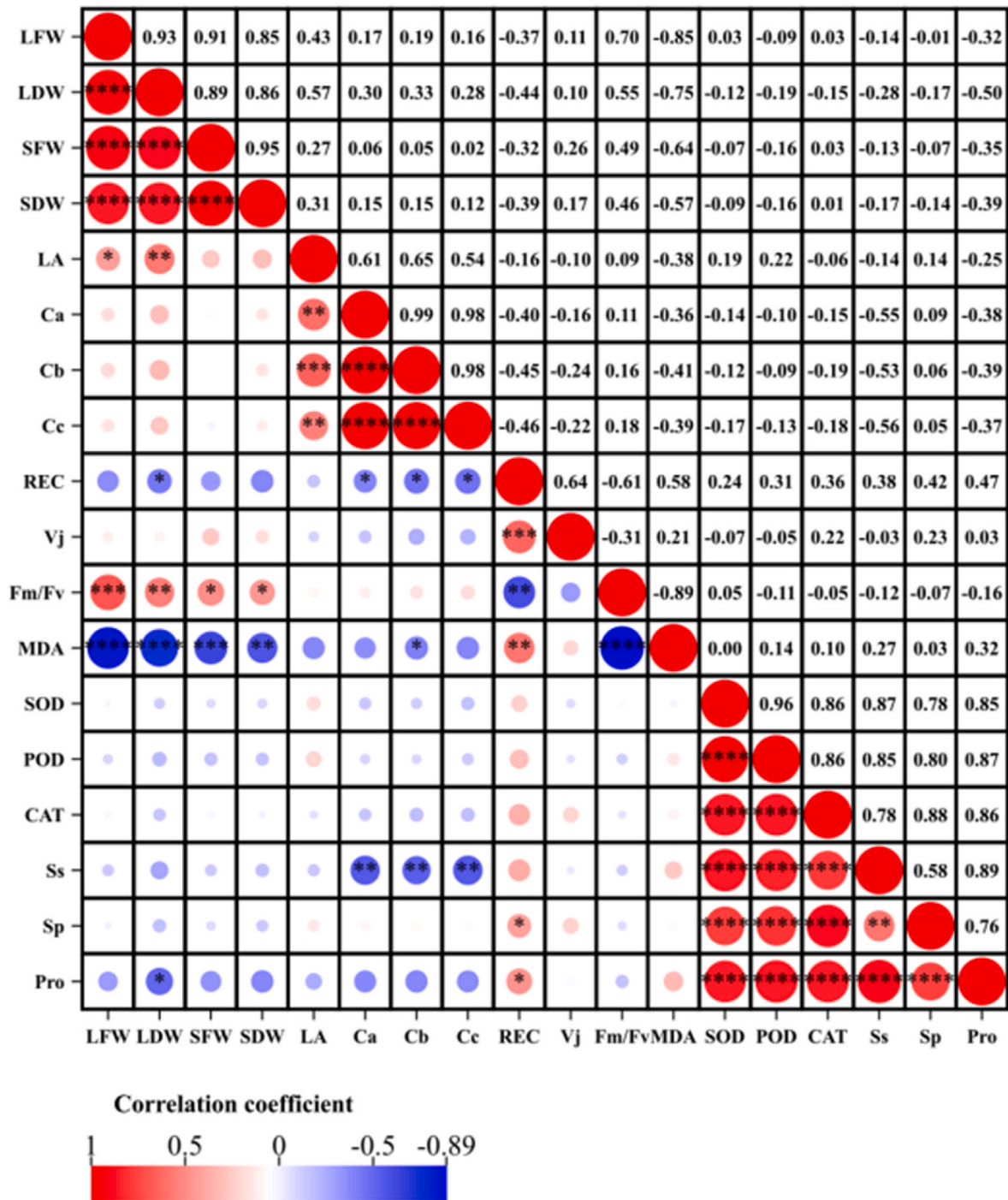


Fig. 7. Correlation analysis of the correlation between physiological indicators. LFW: leaf fresh weight; LDW: leaf dry weight; SFW: stem fresh weight; SDW: stem dry weight; Ca: chlorophyll α concentration; Cb: chlorophyll β concentration; Cc: carotenoid concentration; REC: relative electrical conductivity; Vj: Relatively variable fluorescence; Fv/Fm: the maximal PSII efficiency of dark-adapted leaves; MDA: malonaldehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; Ss: soluble sugars; Sp: soluble proteins; Pro: proline.

Notably, the coupling coordination index of ‘Cq8h’ and ‘Xn9978’ continuously showed outstanding coordination, with ‘Xn9978’ obtaining exceptional coordination after 12 h of 100uM MT treatment (Fig. 12).

This demonstrates that only at the ideal MT concentration can the coupling coordination between the antioxidant system and osmotic regulating chemicals be maximized, resulting in the greatest coordination impact. This coordinated reaction seeks to alleviate the damage caused by high temperatures to buckwheat seedlings, reduce cell membrane permeability, and lower MDA levels.

4. Discussion

4.1. Differential responses of buckwheat varieties to high-temperature stress and the role of exogenous melatonin in enhancing photosynthesis

When crops encounter high-temperature stress during their growth stage, they initiate a cascade of physiological and biochemical responses that help modify the growth process, enabling plants to acclimatize to elevated temperatures and sustain normal survival. However, prolonged exposure to high temperatures can cause irreparable harm, ultimately

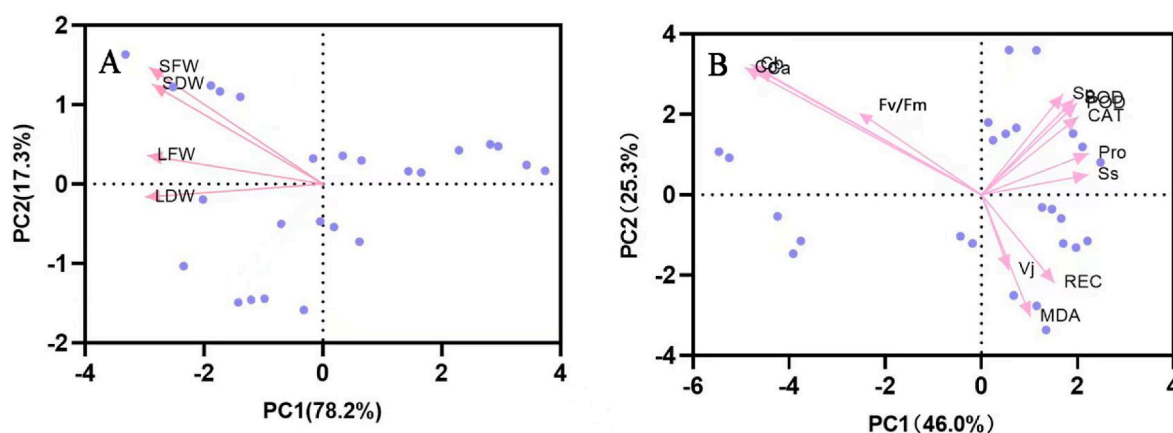


Fig. 8. Principal component analysis of morphology and physiological correlation. LFW: leaf fresh weight; LDW: leaf dry weight; SFW: stem fresh weight; SDW: stem dry weight; Ca: chlorophyll α concentration; Cb: chlorophyll β concentration; Cc: carotenoid concentration; REC: relative electrical conductivity; Vj: Relatively variable fluorescence; Fv/Fm: the maximal PSII efficiency of dark-adapted leaves; MDA: malonaldehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; Ss: soluble sugars; Sp: soluble proteins; Pro: proline. (A: Principal Component Analysis of Growth Indicators in Buckwheat; B: Principal Component Analysis of Physiological Indicators in Buckwheat.)

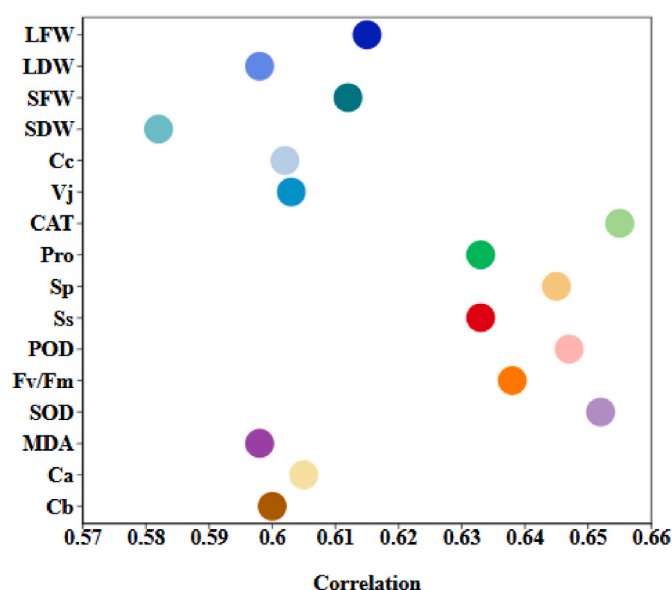


Fig. 9. Analysis of the grey relational grade of exogenous melatonin and high temperature stress. LFW: leaf fresh weight; LDW: leaf dry weight; SFW: stem fresh weight; SDW: stem dry weight; Ca: chlorophyll α concentration; Cb: chlorophyll β concentration; Cc: carotenoid concentration; REC: relative electrical conductivity; Vj: Relatively variable fluorescence; Fv/Fm: the maximal PSII efficiency of dark-adapted leaves; MDA: malonaldehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; Ss: soluble sugars; Sp: soluble proteins; Pro: proline.

leading to plant mortality (Krasensky and Jonak, 2012; Raza, 2021; Raza et al., 2019; Szymańska et al., 2017). In this study, significant differences were observed in the regulatory response mechanisms of five buckwheat varieties to high-temperature stress. Notably, the 'Zk3h' variety exhibited the most pronounced effects on fresh weight, dry weight, and leaf photosynthesis under high-temperature stress, including a marked reduction in the fluorescence parameter Fv/Fm. Although the leaf and stem weights of the other three varieties ('Fhth', 'Cq8h', and 'Xn9978') also showed significant differences compared to normal conditions, the extent of variation was smaller than that of 'Zk3h' and 'Kbtq'. Previous studies have shown that high-temperature stress adversely affects plant photosynthesis, reducing the accumulation and distribution of dry matter (Mathur et al., 2014; Wang et al.,

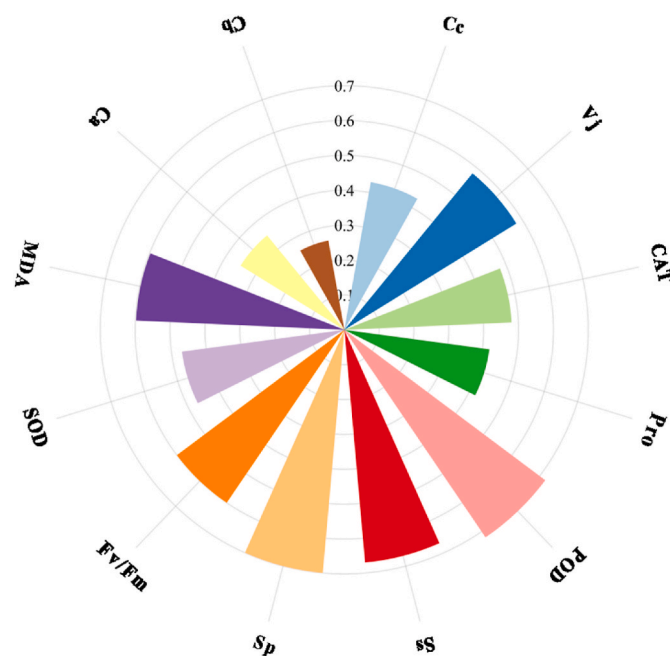
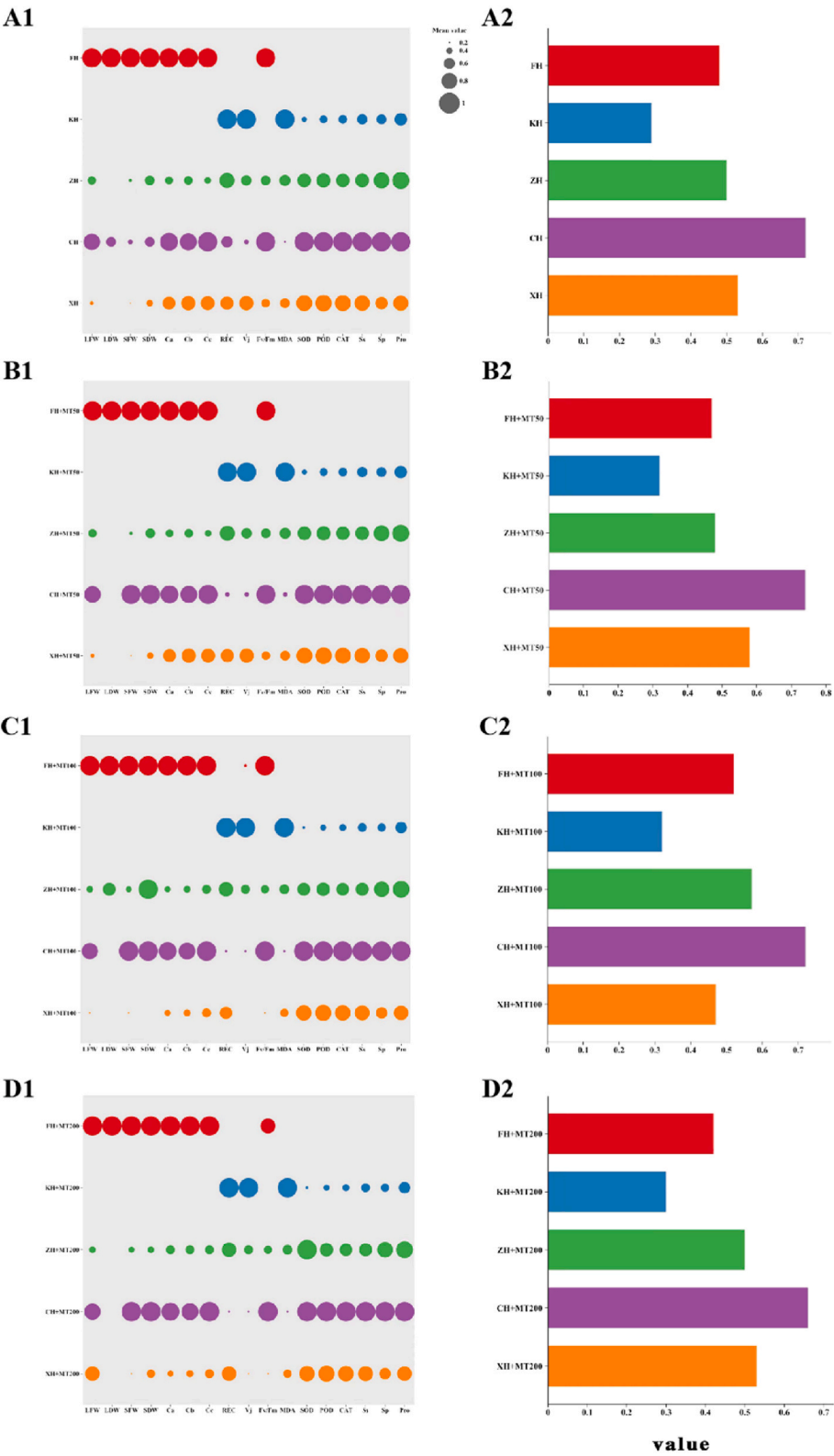


Fig. 10. Path analysis of relative conductivity and physiological indices. LFW: leaf fresh weight; LDW: leaf dry weight; SFW: stem fresh weight; SDW: stem dry weight; Ca: chlorophyll α concentration; Cb: chlorophyll β concentration; Cc: carotenoid concentration; REC: relative electrical conductivity; Vj: Relatively variable fluorescence; Fv/Fm: the maximal PSII efficiency of dark-adapted leaves; MDA: malonaldehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; Ss: soluble sugars; Sp: soluble proteins; Pro: proline.

2020). This suggests that high temperatures damage the photosynthetic tissue, hinder photosynthetic capacity, disrupt the photosynthetic electron transport chain, and consequently reduce the synthesis and accumulation of photosynthetic products (Djanaguiraman et al., 2020; Yan et al., 2013). Additionally, high-temperature stress may interfere with the distribution of dry matter to stems by disrupting the balance of various hormones and metabolic pathways in plants (Abdelrahman et al., 2020; Impa et al., 2019). For example, in this study, a decrease in dry matter allocation to buds was observed in 'Zk3h' leaves, indicating that 'Zk3h' primarily copes with high-temperature stress by adjusting its growth strategy and structural model.



(caption on next page)

Fig. 11. Comprehensive evaluation of exogenous melatonin and high temperature stress on plant growth indexes. H: leaf spraying with water at 40 °C; H + MT50: leaf spraying with 50 µM melatonin at 40 °C; H + MT100: leaf spraying with 100 µM melatonin at 40 °C; H + MT200: leaf spraying with 200 µM melatonin at 40 °C. F: 'Fhth'; K: 'Kbtq'; Z: 'Zk3h'; C: 'Cq8h'; X: 'Xn9978'. (A1: The membership values of various physiological indicators in the five buckwheat varieties under the 40 °C treatment condition; A2: The comprehensive evaluation of heat tolerance based on the membership values of physiological indicators in the five buckwheat varieties under the 40 °C treatment condition; B1: The membership values of various physiological indicators in the five buckwheat varieties under the 40 °C and MT50 treatment condition; B2: The comprehensive evaluation of heat tolerance based on the membership values of physiological indicators in the five buckwheat varieties under the 40 °C and MT50 treatment condition; C1: The membership values of various physiological indicators in the five buckwheat varieties under the 40 °C and MT100 treatment condition; C2: The comprehensive evaluation of heat tolerance based on the membership values of physiological indicators in the five buckwheat varieties under the 40 °C and MT100 treatment condition; D1: The membership values of various physiological indicators in the five buckwheat varieties under the 40 °C and MT200 treatment condition; D2: The comprehensive evaluation of heat tolerance based on the membership values of physiological indicators in the five buckwheat varieties under the 40 °C and MT200 treatment condition.)

Chlorophyll, an essential pigment in green plants, is the primary material for photosynthesis in higher crops and a crucial indicator of plant vitality (Gräf et al., 2022). Recent studies have found that exogenous melatonin can enhance the photosynthetic cycle, increase photosynthetic efficiency, protect chlorophyll, promote fruit ripening, and delay aging, with functions similar to auxin (Jiang et al., 2021). In this study, high-temperature stress in buckwheat led to a decrease in photosynthetic pigments (chlorophyll- α , chlorophyll- β , carotenoids, and total chlorophyll), along with reductions in photosynthetic gas exchange parameters (*Pn*, *Gs*, *Tr*, and *CI*) and the fluorescence parameter *Fv/Fm*, while *Vj* increased. Various concentrations of exogenous melatonin were found to increase photosynthetic pigment content, improve photosynthetic gas exchange parameters, and enhance fluorescence parameters in leaves. Consistent with previous studies, these findings suggest that exogenous melatonin can mitigate the damage to chloroplasts and thylakoids caused by high-temperature stress, regulate the synthesis of photosynthetic pigments, improve photosynthetic efficiency, protect PSI and PSII reaction centers, reduce photoinhibition, and promote biomass accumulation in plants (Jahan et al., 2021).

4.2. Exogenous melatonin activates antioxidant defense in buckwheat to mitigate heat stress-induced membrane damage

Plant cells suffer damage from heat stress, and the first impact is on the cell membrane. High temperature stress can rupture the membrane of leaves, and changes in membrane permeability lead to an increase in relative conductivity (Khan et al., 2017). Qi et al. (2021) showed that under high temperature stress intensity, spraying a certain concentration of MT would reduce the relative electrical conductivity of leaves to a certain extent, which was consistent with the results of this study. Melatonin can reduce MDA content by increasing the activity of plant antioxidant enzymes (Muhammad et al., 2022). Under high temperature stress, peroxidation of plant cell membrane and polymerization of membrane proteins will accelerate, resulting in damage to membrane system, which is characterized by an increase in intracellular MDA content. Three enzymes, SOD, POD and CAT, work together in the plant antioxidant oxidase system to clear ROS and maintain its homeostase in plants (Yang et al., 2023). In this experiment, five buckwheat leaves treated with exogenous melatonin had higher antioxidant enzyme activity, which could prevent the accumulation of hydrogen peroxide. At different times after spraying exogenous MT on the leaves of five buckwheat varieties, the relative electrical conductivity and MDA content of leaves were significantly lower than CK, while the activities of SOD, POD and CAT in leaves were higher than CK, the most significant in 'Kbtq', indicating that MT can activate the antioxidant system of plants. Reducing or preventing ROS damage in plants effectively alleviates cell membrane damage in buckwheat leaves, and the results show that 'Kbtq' mainly counteracts the influence of heat stress by activating antioxidant defense mechanisms.

4.3. Exogenous melatonin enhances osmoregulation in buckwheat to alleviate heat stress-induced cellular damage

Plants usually accumulate osmoregulatory substances under high

temperature conditions, thereby reducing the water potential in cells, promoting the absorption of water by plant cells, and maintaining the osmotic balance of cells to maintain normal physiological functions. Studies have shown that intracellular soluble protein content is significantly positively correlated with stress resistance, that is, exogenous MT can increase soluble protein content in leaves under high temperature stress, thereby improving plant stress resistance (Wu et al., 2021). The results showed that the soluble protein content of buckwheat leaves treated with 100 µmol/L MT was significantly higher than that of CK. Under high temperature stress, plants can accumulate a large amount of soluble sugar, increase the concentration of cell fluid, and delay the high temperature damage of cells (Wang et al., 2015). The activities of antioxidant enzymes (SOD, POD and CAT) and osmoregulatory substances (proline, soluble sugar and soluble protein) of 'Fhth', 'Cq8h' and 'Xn9978' were significantly increased. This increase helps to stabilize membrane permeability and reduce the damage to photosynthetic tissues, thereby ultimately reducing the degree of change in leaf and stem dry and fresh weight. This observation further suggests that 'Fhth', 'Cq8h' and 'Xn9978' respond to high temperature stress mainly by regulating antioxidant defense mechanisms and osmoregulatory substances.

4.4. Comprehensive analysis of exogenous melatonin's impact on heat stress resistance in different buckwheat varieties

The combined analysis of principal component analysis and grey correlation analysis found that exogenous melatonin had the greatest effect on SOD activity in the antioxidant oxidase system, indicating that melatonin enhanced the overall antioxidant defense mechanism of plants mainly by regulating SOD activity. Pathway analysis showed that POD activity had the greatest effect on cell membrane permeability under exogenous melatonin treatment, indicating that POD activity played a key role in regulating cell membrane permeability under exogenous melatonin treatment (Ding et al., 2018). Grey correlation analysis showed that exogenous melatonin had the strongest correlation with the concentration of proline in osmoregulatory substances, indicating that melatonin enhanced the overall osmoregulatory mechanism of plants mainly by regulating the content of proline. This further proves that the application of exogenous melatonin can maintain ROS balance, reduce membrane lipid peroxidation, and alleviate oxidative damage caused by abiotic stress (Chen et al., 2018; Li et al., 2019; Shi et al., 2015). The application of exogenous melatonin can also enhance osmoregulation and maintain intracellular osmotic pressure, thereby maintaining membrane stability (Hassan et al., 2022; Imran et al., 2021). Therefore, the application of exogenous melatonin plays an important role in enhancing cell membrane stability, improving photosynthetic efficiency and promoting biomass accumulation under high temperature stress.

When the concentration of exogenous melatonin was 100 µM, the heat resistance of some buckwheat varieties was improved. This suggests that exogenous melatonin can enhance the response of certain species to high temperatures by improving their processes and strategies, thereby improving their own heat resistance. Additional coupled coordination studies show that the coordination index of 'Cq8h' is

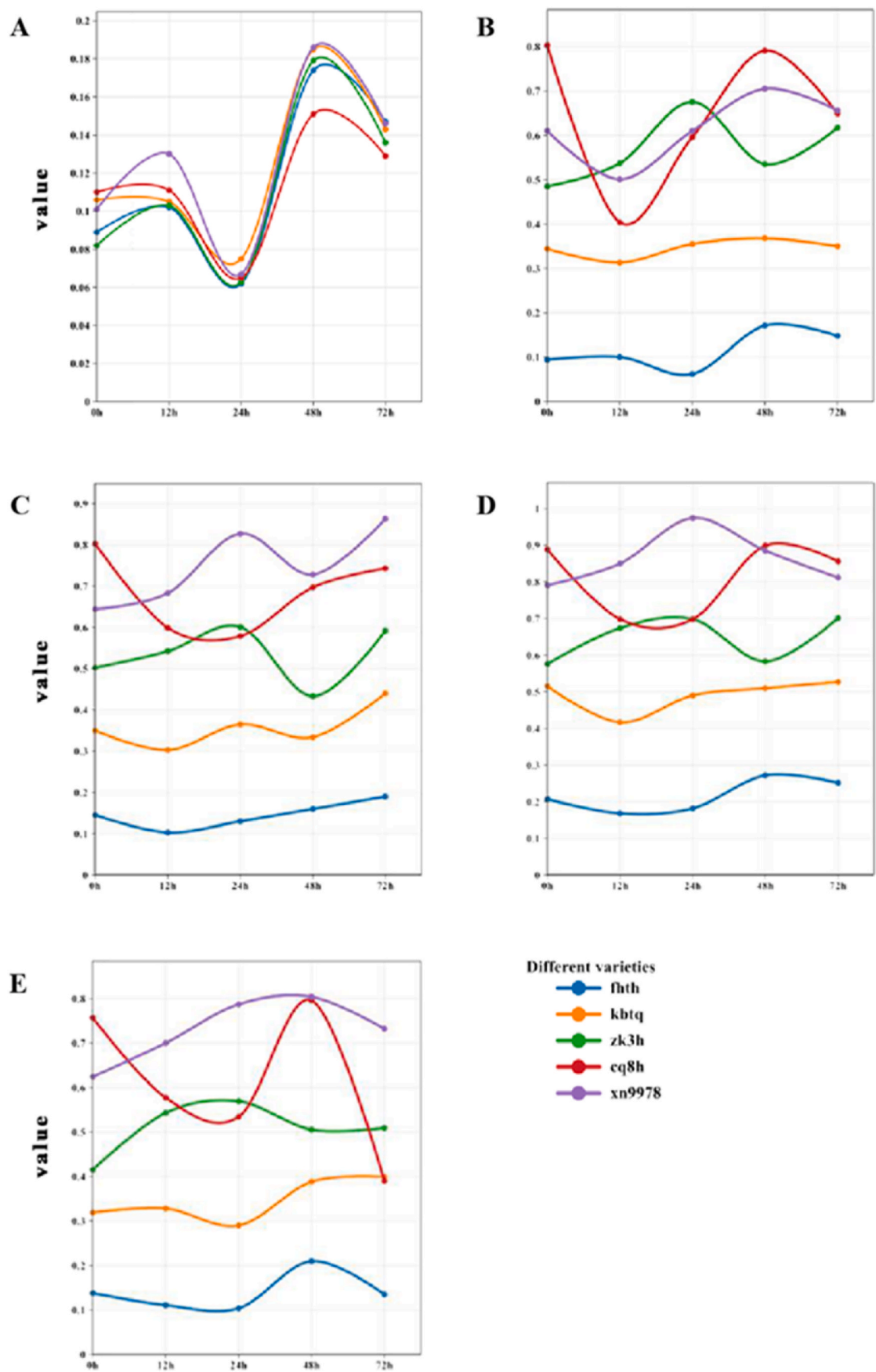


Fig. 12. Coupling coordination analysis of the antioxidant enzyme system and osmotic regulatory substances (A: normal treatment; B: high-temperature stress treatment; C: 50uM MT treatment; D: 100uM MT treatment; E: 200uM MT treatment).

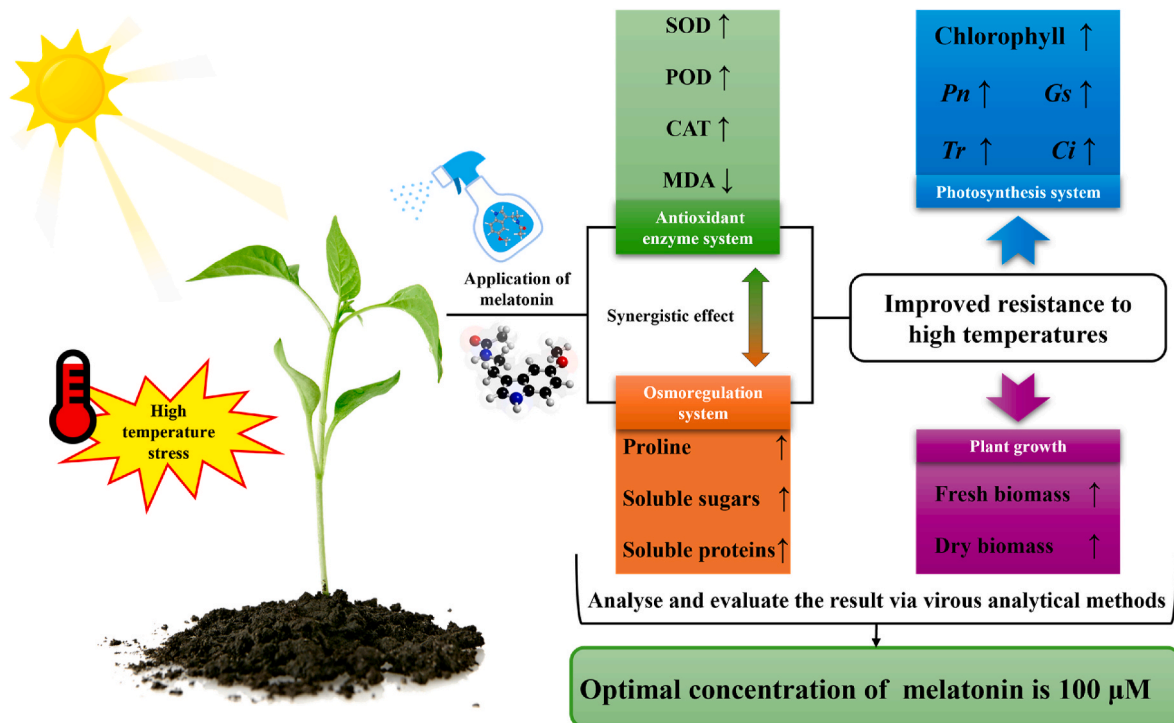


Fig. 13. When exogenous melatonin is applied to buckwheat seedlings under high-temperature stress: 1. Melatonin activates antioxidant enzymes and enhances osmoregulatory substances, 2. Seedlings exhibit modifications such as: Improved cell membrane stability, enhanced photosynthetic efficiency, increased chlorophyll content, and reduced biomass loss to overcome heat stress.

significantly different between the two stages. Its resistance to high temperature stress was enhanced with the enhancement of coordination, indicating that exogenous melatonin had a greater effect on 'Cq8h'. However, when exogenous melatonin was administered, the 'Fthh' coordination index did not change much; This may be attributed to the unique qualities of the breed. The effects of melatonin on plant signaling pathways, gene expression regulation and stress-related protein activity under high temperature stress need to be further studied in order to fully understand the molecular processes behind this effect.

Different buckwheat varieties have different response mechanisms to high temperature stress, which may be related to variety-specific response and genetic background differences among varieties. In this study, the heat resistance of 5 different buckwheat varieties was compared by membership function method and coupling coordination analysis. The results show that the heat resistance of 'Cq8h' is the highest and that of 'Kbtq' is the lowest. This highlights the importance and effectiveness of the synergistic action of antioxidant defense systems and osmoregulatory chemicals in improving buckwheat's resistance to high temperature stress.

5. Conclusion

High-temperature stress severely limits buckwheat seedling growth, but exogenous MT effectively mitigates these effects by regulating the antioxidant and osmoregulation systems. MT enhances the activities of antioxidant enzymes such as SOD, POD, and CAT, reducing ROS accumulation and MDA content, which protects cell membranes and decreases REC to maintain integrity. Simultaneously, MT promotes the accumulation of osmoregulatory substances like proline, soluble sugars, and proteins, helping cells maintain water balance and osmotic pressure under heat stress. The coordinated regulation of these systems enhances the heat tolerance of buckwheat seedlings, leading to a stronger photosynthetic system with increased PSII activity and higher levels of chlorophyll and carotenoids, ensuring sustained photosynthesis. Consequently, MT-treated seedlings show improved growth with

reduced biomass loss. Comprehensive analysis indicates that 100 μ M MT provides the most effective enhancement of heat resistance, highlighting its potential in improving crop resilience to high temperatures (Fig. 13). Meanwhile, exogenous MT enhances the heat tolerance of all five buckwheat varieties, though the effects vary. Among them, the improvement in the 'Zk3h' variety is the most pronounced.

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Declaration of competing interest

All 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.

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Data availability

Data will be made available on request.

References

- Abdelrahman, M., Burritt, D.J., Gupta, A., Tsujimoto, H., Tran, L.-S.P., 2020. Heat stress effects on source-sink relationships and metabolome dynamics in wheat. *J. Exp. Bot.* 71, 543–554. <https://doi.org/10.1093/jxb/erz296>.

- Adler, J., Parmryd, I., 2010. Quantifying colocalization by correlation: the Pearson correlation coefficient is superior to the Mander's overlap coefficient. *Cytometry* 77A, 733–742. <https://doi.org/10.1002/cyto.a.20896>.
- Arnao, M.B., 2014. Phyto-melatonin: discovery, content, and role in plants. *Adv. Bot.* 2014, 1–11. <https://doi.org/10.1155/2014/815769>.
- Arnao, M.B., Hernández-Ruiz, J., 2006. The physiological function of melatonin in plants. *Plant Signal. Behav.* 1, 89–95. <https://doi.org/10.4161/psb.1.3.2640>.
- Aubert, L., Konrádová, D., Kebbas, S., Barris, S., Quinet, M., 2020. Comparison of high temperature resistance in two buckwheat species *Fagopyrum esculentum* and *Fagopyrum tataricum*. *J. Plant Physiol.* 251, 153222. <https://doi.org/10.1016/j.jplph.2020.153222>.
- Baureder, M., Barane, E., Hederstedt, L., 2014. *In vitro* assembly of catalase. *J. Biol. Chem.* 289, 28411–28420. <https://doi.org/10.1074/jbc.M114.596148>.
- Borna, R.S., Hoque, M., Sarker, R., 2019. *In vitro* microtubule induction and regeneration of plantlets from microtubule discs of cultivated potato (*Solanum tuberosum* L.). *Plant Tissue Cult. Biotechnol.* 29, 63–72. <https://doi.org/10.3329/ptcb.v29i1.41979>.
- Bose, S.K., Howlader, P., 2020. Melatonin plays multifunctional role in horticultural crops against environmental stresses: a review. *Environ. Exp. Bot.* 176, 104063. <https://doi.org/10.1016/j.envexpbot.2020.104063>.
- Chen, Y., Mao, J., Sun, L., Huang, B., Ding, C., Gu, Y., Liao, J., Hu, C., Zhang, Z., Yuan, S., Yuan, M., 2018. Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. *Physiol. Plantarum* 164, 349–363. <https://doi.org/10.1111/ppi.12737>.
- Christa, K., Soral-Smietana, M., 2008. Buckwheat grains and buckwheat products - nutritional and prophylactic value of their components - a review. *Czech J. Food Sci.* 26, 153–162. <https://doi.org/10.17221/1602-CJFS>.
- Ding, F., Wang, G., Zhang, S., 2018. Exogenous melatonin mitigates methyl viologen-triggered oxidative stress in poplar leaf. *Molecules* 23, 2852. <https://doi.org/10.3390/molecules23112852>.
- Djanaguiraman, M., Narayanan, S., Erdayani, E., Prasad, P.V.V., 2020. Effects of high temperature stress during anthesis and grain filling periods on photosynthesis, lipids and grain yield in wheat. *BMC Plant Biol.* 20, 268. <https://doi.org/10.1186/s12870-020-02479-0>.
- Dong, G., Ge, Y., Liu, J., Kong, X., Zhai, R., 2023. Evaluation of coupling relationship between urbanization and air quality based on improved coupling coordination degree model in Shandong province, China. *Ecol. Indic.* 154, 110578. <https://doi.org/10.1016/j.ecolind.2023.110578>.
- Gräf, M., Pucher, B., Hietz, P., Hofbauer, K., Allabashi, R., Pitha, U., Hood-Nowotny, R., Stangl, R., 2022. Application of leaf analysis in addition to growth assessment to evaluate the suitability of greywater for irrigation of *Tilia cordata* and *Acer pseudoplatanus*. *Sci. Total Environ.* 836, 155745. <https://doi.org/10.1016/j.scitotenv.2022.155745>.
- Halbrecht, B., Romedenne, P., Ledent, J.F., 2005. Evolution of flowering, ripening and seed set in buckwheat (*Fagopyrum esculentum* Moench): quantitative analysis. *Eur. J. Agron.* 23, 209–224. <https://doi.org/10.1016/j.eja.2004.11.006>.
- Hassan, M.U., Ghareeb, R.Y., Nawaz, M., Mahmood, A., Shah, A.N., Abdel-Megeed, A., Abdelsalam, N.R., Hashem, M., Alami, S., Thabit, M.A., Qari, S.H., 2022. Melatonin: a vital pro-protectant for crops against heat stress: mechanisms and prospects. *Agronomy* 12, 1116. <https://doi.org/10.3390/agronomy12051116>.
- He, J.-D., Zou, Y.-N., Wu, Q.-S., Kuca, K., 2020. Mycorrhizas enhance drought tolerance of trifoliate orange by enhancing activities and gene expression of antioxidant enzymes. *Sci. Hortic.* 262, 108745. <https://doi.org/10.1016/j.scienta.2019.108745>.
- Hornýák, M., Pláček, A., Kopeć, P., Dziurka, M., Pastuszak, J., Szczerba, A., Hura, T., 2020. Photosynthetic activity of common buckwheat (*Fagopyrum esculentum* Moench) exposed to thermal stress. *Photosynthetica* 58, 45–53. <https://doi.org/10.32615/ps.2019.140>.
- Hu, D., Zhang, X., Xue, P., Nie, Y., Liu, J., Li, Y., Wang, C., Wan, X., 2023. Exogenous melatonin ameliorates heat damages by regulating growth, photosynthetic efficiency and leaf ultrastructure of carnation. *Plant Physiol. Biochem.* 198, 107698. <https://doi.org/10.1016/j.plaphy.2023.107698>.
- Impa, S.M., Sunoj, V.S.J., Krassovskaya, I., Bheemanahalli, R., Obata, T., Jagadish, S.V.K., 2019. Carbon balance and source-sink metabolic changes in winter wheat exposed to high night-time temperature. *Plant Cell Environ.* 42, 1233–1246. <https://doi.org/10.1111/pce.13488>.
- Imran, M., Latif Khan, A., Shahzad, R., Aaqil Khan, M., Bilal, S., Khan, A., Kang, S.-M., Lee, I.-J., 2021. Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. *AoB PLANTS* 13, plab026. <https://doi.org/10.1093/aobpla/plab026>.
- Jahan, M.S., Guo, S., Sun, J., Shu, S., Wang, Y., El-Yazied, A.A., Alabdallah, N.M., Hikil, M., Mohamed, M.H.M., Ibrahim, M.F.M., Hasan, MdM., 2021. Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. *Plant Physiol. Biochem.* 167, 309–320. <https://doi.org/10.1016/j.plaphy.2021.08.002>.
- Jiang, D., Lu, B., Liu, L., Duan, W., Meng, Y., Li, J., Zhang, K., Sun, H., Zhang, Y., Dong, H., Bai, Z., Li, C., 2021. Exogenous melatonin improves the salt tolerance of cotton by removing active oxygen and protecting photosynthetic organs. *BMC Plant Biol.* 21, 331. <https://doi.org/10.1186/s12870-021-03082-7>.
- Khan, A., Numan, M., Khan, A.L., Lee, I.-J., Imran, M., Asaf, S., Al-Harassi, A., 2020. Melatonin: awakening the defense mechanisms during plant oxidative stress. *Plants* 9, 407. <https://doi.org/10.3390/plants9040407>.
- Khan, N., Ahmad, I., Azhar, M.T., 2017. Genetic variation in relative cell injury for breeding upland cotton under high temperature stress. *Turk. J. Field Crops* 22, 152–159. <https://doi.org/10.17557/jtfc.315544>.
- Kopeć, P., Hornýák, M., Pastuszak, J., Szczerba, A., Rapacz, M., Waga, J., Pláček, A., 2021. Changes in the flower and leaf proteome of common buckwheat (*Fagopyrum esculentum* Moench) under high temperature. *Int. J. Mol. Sci.* 22, 2678. <https://doi.org/10.3390/ijms22052678>.
- Krasensky, J., Jonak, C., 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* 63, 1593–1608. <https://doi.org/10.1093/jxb/err460>.
- Kumari, P., Rastogi, A., Yadav, S., 2020. Effects of Heat stress and molecular mitigation approaches in orphan legume. *Chickpea. Mol. Biol. Rep.* 47, 4659–4670. <https://doi.org/10.1007/s11033-020-05358-x>.
- Landi, M., 2017. Commentary to: “improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds” by hodge et al., *planta* (1999) 207:604–611. *Planta* 245. <https://doi.org/10.1007/s00425-017-2699-3>, 1067–1067.
- Lee, K.-A., Kim, Y.-N., Kantharaj, V., Lee, Y.B., Woo, S.Y., 2023. Seedling growth and photosynthetic response of *Pterocarpus indicus* L. to shading stress. *Plant Signal. Behav.* 18, 2245625. <https://doi.org/10.1080/15592324.2023.2245625>.
- Li, J., Yang, Y., Sun, K., Chen, Y., Chen, X., Li, X., 2019. Exogenous melatonin enhances cold, salt and drought stress tolerance by improving antioxidant defense in tea plant (*Camellia sinensis* (L.) O. Kuntze). *Molecules* 24, 1826. <https://doi.org/10.3390/molecules24091826>.
- Lim, J.H., Hou, J., Chun, J., Lee, R.D., Yun, J., Jung, J., Lee, C.H., 2022. Importance of hydroxide ion conductivity measurement for alkaline water electrolysis membranes. *Membranes* 12, 556. <https://doi.org/10.3390/membranes12060556>.
- Lu, Q., Yu, X., Wang, H., Yu, Z., Zhang, X., Zhao, Y., 2022. Quantitative trait locus mapping for important yield traits of a sorghum-sudangrass hybrid using a high-density single nucleotide polymorphism map. *Front. Plant Sci.* 13, 1098605. <https://doi.org/10.3389/fpls.2022.1098605>.
- Mao, L., Zhang, L., Zhao, X., Liu, S., van der Werf, W., Zhang, S., Spiertz, H., Li, Z., 2014. Crop growth, light utilization and yield of relay intercropped cotton as affected by plant density and a plant growth regulator. *Field Crops Res.* 155, 67–76. <https://doi.org/10.1016/j.fcr.2013.09.021>.
- Mathur, S., Agrawal, D., Jajoo, A., 2014. Photosynthesis: response to high temperature stress. *J. Photochem. Photobiol., B* 137, 116–126. <https://doi.org/10.1016/j.jphotobiol.2014.01.010>.
- Muhammad, I., Yang, L., Ahmad, S., Mosaad, I.S.M., Al-Ghamdi, A.A., Abbasi, A.M., Zhou, X.-B., 2022. Melatonin application alleviates stress-induced photosynthetic inhibition and oxidative damage by regulating antioxidant defense system of maize: a meta-analysis. *Antioxidants* 11, 512. <https://doi.org/10.3390/antiox11030512>.
- Pláček, A., Slomka, A., Kopeć, P., Dziurka, M., Hornýák, M., Sychta, K., Pastuszak, J., Dubert, F., 2019. Effects of high temperature on embryological development and hormone profile in flowers and leaves of common buckwheat (*Fagopyrum esculentum* Moench). *Int. J. Mol. Sci.* 20, 1705. <https://doi.org/10.3390/ijms20071705>.
- Prasad, P.V.V., Gowsiga, S., Djanaguiraman, M., 2023. Influence of high temperature stress on grain crops. In: Harohalli Masthigowda, M., Gopalareddy, K., Khobra, R., Singh, G., Pratap Singh, G. (Eds.), *Translating Physiological Tools to Augment Crop Breeding*. Springer Nature, Singapore, pp. 371–389. https://doi.org/10.1007/978-981-19-7498-4_17.
- Qi, X.-Y., Wang, W.-L., Hu, S.-Q., Liu, M.-Y., Zheng, C.-S., Sun, X.-Z., 2021. Effects of exogenous melatonin on photosynthesis and physiological characteristics of chrysanthemum seedlings under high temperature stress. *Ying Yong Sheng Tai Xue Bao J. Appl. Ecol.* 32, 2496–2504. <https://doi.org/10.13287/j.1001-9332.202107.025>.
- Raza, A., 2021. Eco-physiological and biochemical responses of rapeseed (*Brassica napus* L.) to abiotic stresses: consequences and mitigation strategies. *J. Plant Growth Regul.* 40, 1368–1388. <https://doi.org/10.1007/s00344-020-10231-z>.
- Raza, A., Razzaq, A., Mehmood, S., Zou, X., Zhang, X., Lv, Y., Xu, J., 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. *Plants* 8, 34. <https://doi.org/10.3390/plants8020034>.
- Sajid, M., Kifayat, M., Salman, M., Usman, M., Rahman, S., Saddique, M.A.B., 2022. Effects of High Temperature on Morphological and Physiological Stages of Different Cultivated Crops.
- Schroeter-Zakrzewska, A., Pradita, F.A., 2021. Effect of colour of light on rooting cuttings and subsequent growth of chrysanthemum. *Chrysanthemum × grandiflorum* Ramat./Kitam.). *Agriculture* 11, 671. <https://doi.org/10.3390/agriculture11070671>.
- Sehar, Z., Fatma, M., Khan, S., Mir, I.R., Abdi, G., Khan, N.A., 2023. Melatonin influences methyl jasmonate-induced protection of photosynthetic activity in wheat plants against heat stress by regulating ethylene-synthesis genes and antioxidant metabolism. *Sci. Rep.* 13, 7468. <https://doi.org/10.1038/s41598-023-34682-y>.
- Shi, H., Jiang, C., Ye, T., Tan, D., Reiter, R.J., Zhang, H., Liu, R., Chan, Z., 2015. Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [cynodon dactylon (L.) Pers.] by exogenous melatonin. *J. Exp. Bot.* 66, 681–694. <https://doi.org/10.1093/jxb/eru373>.
- Stephenie, S., Chang, Y.P., Gnanasekaran, A., Esa, N.M., Gnanaraj, C., 2020. An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. *J. Funct. Foods* 68, 103917. <https://doi.org/10.1016/j.jff.2020.103917>.
- Subramanyam, K., Du Laing, G., Van Damme, E.J.M., 2019. Sodium selenate treatment using a combination of seed priming and foliar spray alleviates salinity stress in rice. *Front. Plant Sci.* 10, 407836. <https://doi.org/10.3389/fpls.2019.00116>.
- Szymańska, R., Ślesak, I., Orzechowska, A., Kruk, J., 2017. Physiological and biochemical responses to high light and temperature stress in plants. *Environ. Exp. Bot.* 139, 165–177. <https://doi.org/10.1016/j.envexpbot.2017.05.002>.
- Wan, Y., Zhang, Y., Zhang, M., Hong, A., Yang, H., Liu, Y., 2020. Shade effects on growth, photosynthesis and chlorophyll fluorescence parameters of three *Paonia* species. *PeerJ* 8, e9316. <https://doi.org/10.7717/peerj.9316>.
- Wang, B., Chen, J., Chen, L., Wang, X., Wang, R., Ma, L., Peng, S., Luo, J., Chen, Y., 2015. Combined drought and heat stress in camellia oleifera cultivars: leaf characteristics,

- soluble sugar and protein contents, and rubisco gene expression. *Trees (Berl.)* 29, 1483–1492. <https://doi.org/10.1007/s00468-015-1229-9>.
- Wang, W., Cai, C., He, J., Gu, J., Zhu, G., Zhang, W., Zhu, J., Liu, G., 2020. Yield, dry matter distribution and photosynthetic characteristics of rice under elevated CO₂ and increased temperature conditions. *Field Crops Res.* 248, 107605. <https://doi.org/10.1016/j.fcr.2019.107605>.
- Wu, S., Wang, Yao, Zhang, J., Gong, X., Zhang, Z., Sun, J., Chen, X., Wang, Yanling, 2021. Exogenous melatonin improves physiological characteristics and promotes growth of strawberry seedlings under cadmium stress. *Hortic. Plant J.* 7, 13–22. <https://doi.org/10.1016/j.hpj.2020.06.002>.
- Yan, K., Chen, P., Shao, H., Shao, C., Zhao, S., Brestic, M., 2013. Dissection of photosynthetic electron transport process in sweet sorghum under heat stress. *PLoS One* 8, e62100. <https://doi.org/10.1371/journal.pone.0062100>.
- Yang, J.-W., Park, S.-U., Lee, H.-U., Nam, K.J., Lee, K.-L., Lee, J.J., Kim, J.H., Kwak, S.-S., Kim, H.S., Kim, Y.-H., 2023. Differential responses of antioxidant enzymes and lignin metabolism in susceptible and resistant sweetpotato cultivars during root-knot nematode infection. *Antioxidants* 12, 1164. <https://doi.org/10.3390/antiox12061164>.
- Yasmeen, A., Basra, S.M.A., Farooq, M., Rehman, H. ur, Hussain, N., Athar, H. ur R., 2013. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regul.* 69, 225–233. <https://doi.org/10.1007/s10725-012-9764-5>.
- Zhang, H., Zhang, Y., 2014. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. *J. Pineal Res.* 57, 131–146. <https://doi.org/10.1111/jpi.12162>.
- Zhu, X., Liu, T., Xu, K., Chen, C., 2022. The impact of high temperature and drought stress on the yield of major staple crops in northern China. *J. Environ. Manag.* 314, 115092. <https://doi.org/10.1016/j.jenvman.2022.115092>.