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Exogenous melatonin enhances drought tolerance and germination in common buckwheat seeds through the coordinated effects of antioxidant and osmotic regulation

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Abstract

Background Drought stress is a major constraint on seed germination and crop productivity, particularly for drought-sensitive crops like common buckwheat (*Fagopyrum esculentum*). Exogenous melatonin has emerged as a promising strategy to mitigate drought stress by enhancing plant physiological and biochemical responses. However, its specific roles in regulating antioxidant defenses, osmotic adjustment, and plant compounds biosynthesis during buckwheat seed germination under drought stress remain poorly understood.

Results This study investigated the effects of 200 µM exogenous melatonin on common buckwheat germination under polyethylene glycol (PEG-6000)-induced drought stress. Melatonin significantly improved germination rates and radicle growth, reduced membrane damage, and enhanced osmotic regulation by increasing proline, soluble sugars, and proteins. Antioxidant enzyme activities (catalase, peroxidase, and superoxide dismutase) and associated gene expression (*FtCAT*, *FtPOD*, *FtSOD*) were markedly upregulated. Molecular docking and dynamics simulations revealed a stable interaction between rutin, a secondary metabolite, and catalase, suggesting enhanced enzyme stabilization. Additionally, melatonin increased rutin and methyl jasmonate synthesis, which contributed to antioxidant defenses and reduced oxidative damage. The coordinated effects of melatonin improved drought tolerance in buckwheat seeds by optimizing osmotic balance, strengthening antioxidant capacity, and stabilizing cellular structures.

Conclusions Exogenous melatonin enhances drought tolerance in common buckwheat seeds through the coordinated regulation of antioxidant defenses, osmotic adjustment, and plant compounds production, including methyl jasmonate and rutin, during germination. These findings offer valuable insights for developing practical strategies to improve drought resilience and crop establishment in sensitive agricultural species under water-limited conditions.

Keywords Antioxidant enzymes, Buckwheat germination, Drought stress, Melatonin, Osmotic regulation, Rutin

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Introduction

Drought stress (DS) is one of the most critical environmental stressors affecting plant growth and agricultural productivity, with its impact becoming increasingly significant in the context of global climate change. DS disrupts key physiological processes such as water uptake, osmotic regulation, and antioxidant defense systems, leading to impaired seed germination and plant growth, ultimately reducing crop yields [1, 2]. For crops like buckwheat (*Fagopyrum esculentum*), which are particularly sensitive to environmental stressors, DS often results in asynchronous seed germination, reduced seedling development, and lower yields [3, 4]. The increasing frequency of extreme weather events, especially reduced precipitation in many regions, exacerbates these challenges for buckwheat cultivation [5, 6].

Seed germination is a crucial stage in crop development, directly affecting both yield and economic outcomes. Under DS, seed germination is severely impaired due to disruptions in physiological and biochemical processes, such as decreased osmotic regulation and compromised antioxidant defense systems [7]. Insufficient soil moisture during sowing often leads to poor germination of buckwheat seeds, causing irregular seedling emergence and affecting subsequent crop growth [4]. Moreover, DS reduces seed water potential, hampers water uptake, and inhibits seed germination and seedling growth [8, 9]. To study the drought tolerance of seeds, polyethylene glycol (PEG)-induced DS is a commonly used technique in laboratory settings. Using PEG-6000, researchers have observed significant reductions in germination vigor, germination rate, and drought tolerance in various crops, along with increased cellular damage, membrane deterioration, and oxidative stress [10–12].

Melatonin has emerged in recent years as a key bioactive molecule with significant potential to enhance plant stress tolerance, particularly under DS [13]. Studies have demonstrated that melatonin promotes seed germination, root development, and overall plant growth while acting as a protective agent against environmental stressors like DS [14, 15]. It achieves this by activating antioxidant enzyme systems (such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) and inducing the biosynthesis of flavonoids, such as rutin, which help alleviate oxidative stress [16–19]. Additionally, melatonin reduces malondialdehyde (MDA) levels, protecting cellular membranes from oxidative damage, and enhances osmotic regulation by increasing the accumulation of osmotic regulators like proline (Pro) and soluble sugars (Ss), which mitigate DS-induced cellular damage [20, 21].

Despite the growing body of research on melatonin's role in improving plant stress tolerance, there is still limited understanding of how melatonin coordinates antioxidant enzyme activity, osmotic regulation, and flavonoid biosynthesis during the germination phase of buckwheat seeds under DS. Most existing research on buckwheat DS has focused on seedling growth, photosynthesis, antioxidant enzyme activities, and drought-responsive gene expression, leaving a significant gap in our understanding of how DS impacts seed germination in this crop [3, 22–24].

To address this gap, this study investigates the effects of melatonin on the DS tolerance of buckwheat seeds (Kanbaotianqiao, KBTQ) during germination under PEG-6000-induced DS. Specifically, we will examine melatonin's influence on key germination parameters such as germination rate, germination energy, and radicle growth. Furthermore, we will explore its role in regulating osmotic regulators, antioxidant enzyme activities, and the synthesis of flavonoid compounds such as rutin. By providing a comprehensive analysis of melatonin's regulatory effects on buckwheat seed germination under DS, this study aims to offer valuable insights into improving crop establishment and yield under increasingly frequent DS scenarios. Additionally, the findings could provide important theoretical and practical guidance for enhancing agricultural resilience to DS across a range of crops.

Materials and methods

Material and reagents

The experiment was conducted in the laboratory of the College of Landscape and Tourism, Hebei Agricultural University. The primary test material used was the buckwheat variety Kanbaotianqiao (KBTQ). Buckwheat (*Fagopyrum esculentum*) seeds were obtained from the Buckwheat Gene Resources Innovation Research Group at the Institute of Crop Science, Chinese Academy of Sciences. Melatonin (purity 99%) was procured from Sigma-Aldrich® (USA).

Determination of DS resistance

Buckwheat seeds were surface-sterilized by soaking in 75% ethanol for 30 min, followed by five rinsed with distilled water [25]. Sterilized seeds (30 per Petri dish) were placed on three layers of filter paper moistened with distilled water. Five replicates were prepared for each treatment. The seeds were then incubated under 4000 lx light intensity with a photoperiod of 8–10 h, exposed to various concentrations of polyethylene glycol (PEG-6000) solutions (5%, 10%, 15%, 20%, and 25%). Germination rate and germination potential were measured to determine the optimal PEG-6000 concentration for DS simulation.

Determination of melatonin concentration

For melatonin treatment (MT treatment), 1500 uniformly sized and healthy seeds were selected. These seeds were surface sterilized by soaking in 75% ethanol for 30 min, followed by rinsing with distilled water and air-drying. The seeds were then soaked in darkness for 16 h in melatonin solutions at concentrations of 0, 50, 100, 200, and 500 μM. For each treatment, 30 seeds were placed in Petri dishes, with five replicates per concentration. Germination was monitored daily, and germination potential and germination rate were calculated for each melatonin concentration.

Experimental setup

In subsequent tests, seeds were soaked in 200 μM melatonin at 22 °C for 16 h, with distilled water used as a control. Germination was carried out under the same conditions as previously described. Embryos and radicles (approximately 10 g) were collected from each treatment at 2, 4, and 6 days post-germination and stored at − 80 °C for further analyses, including assessments of SOD, POD, and CAT activities, as well as osmotic regulation. The four treatment groups were as follows: water immersion (CK), 200 μM melatonin immersion (MT), water immersion with 20% PEG-6000 (DS), and 200 μM melatonin immersion with 20% PEG-6000 (DS + MT).

Measurement of seed germination parameters

Germination was defined as the radicle reaching half the seed length. Germination counts were recorded daily from days 1 to 7, and germination potential and germination rate were calculated on the 3rd and 7 th days, respectively [26]. The calculations were as follows:

$$\text{Germination potential} = \frac{\text{Number of seeds germinated on the } 3^{\text{rd}} \text{ day}}{\text{Number of seeds tested}} \times 100\%$$

$$\text{Germination rate} = \frac{\text{Number of seeds germinated on the } 7^{\text{th}} \text{ day}}{\text{Number of seeds tested}} \times 100\%$$

Measurement of growth indicators

After 7 days of germination, 30 seeds from each treatment group were selected for analysis. Radicle length was measured using a vernier caliper, and this procedure was repeated three times for each treatment group [26, 27]. The fresh weight of the seeds was also determined using an electronic balance (Sartorius, BS124S, China) with an accuracy of 0.1 mg.

Measurement of physiological and biochemical indicators

The Ss content was measured using a modified version of the method by Borna et al. [28]. Briefly, 0.3 g of seed sample was immersed in 9 ml of distilled water and incubated in a boiling water bath for 30 min. Afterward, 1 ml of the supernatant was mixed with 5 ml of sulfuric acid-anthrone reagent, heated for 10 min, cooled, and the absorbance was recorded at 620 nm using a spectrophotometer.

The Pro content was determined following the method by Subramanyam et al. [29]. A 0.3 g sample was homogenized in 3 ml of 3% aqueous sulfosalicylic acid and centrifuged. Then, 2 ml of the supernatant was mixed with equal volumes of glacial acetic acid and ninhydrin reagent. After heating in boiling water for 30 min, the mixture was cooled and centrifuged at 10,000 rpm for 5 min. The absorbance was measured at 520 nm using a spectrophotometer.

The Sp content was assessed using the Coomassie Brilliant Blue method described by Yasmeen et al. [30]. The Coomassie Brilliant Blue G-250 solution was prepared by dissolving 0.1 g of the dye in 50 ml of 90% ethanol, followed by the addition of 100 ml of 85% phosphoric acid. The volume was adjusted to 1,000 ml with distilled water and stored in an amber glass bottle for up to one month. For the assay, 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 homogenized and left to stand for 2 min before measuring absorbance at 595 nm.

The REC was determined following the method by Lim et al. [31]. Fresh samples of ornamental buckwheat were rinsed with deionized water, dried, and weighed to 0.1 g. The samples were soaked in deionized water for 24 h, and initial conductivity readings (R1) were taken. After boiling the samples for 20 min, a second reading (R2) was taken.

The CAT activity was measured using the method by Baureder et al. [32]. Two 50 ml Erlenmeyer flasks were used for each test—one as the control and one for the test. The test flask contained 2.5 ml of enzyme extract, while the control flask had 2.5 ml of boiled enzyme extract. Both flasks received 2.5 ml of H_2O_2 and were incubated at 30 °C for 10 min. The reaction was stopped

by adding 2.5 ml of 10% H_2SO_4, and the mixture was titrated with 0.1 mol/L $KMnO_4$ until a pink color persisted for 30 s.

The MDA content was measured using the acid ninhydrin method described by Landi [33]. A 0.1 g tissue sample was homogenized with 2 ml of 10% trichloroacetic acid and a small amount of quartz sand. The homogenate was mixed with an additional 8 ml of 10% trichloroacetic acid, transferred to a 10 ml centrifuge tube, and centrifuged at 2000 rpm for 10 min. The supernatant was used as the sample extract. In a new centrifuge tube, 3 ml of 0.5% thiobarbituric acid was combined with 1 ml of enzyme extract. The mixture was incubated in boiling water for 30 min, rapidly cooled, and centrifuged at 9,000 rpm for 15 min. Absorbance was measured at 450 nm, 532 nm, and 600 nm with thiobarbituric acid as the blank.

The SOD activity was assessed using the nitroblue tetrazolium reduction method [34]. The assay included two sets of test tubes, one for treatment and one for sample analysis. The reaction mixture comprised 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, and 0.3 ml of riboflavin. One test tube contained pH 7.8 phosphate buffer instead of enzyme solution and was placed in the dark, while the other was exposed to 4000 LX sunlight (25 °C, level 6 light) for 20 min. After the reaction, the tubes were covered with black plastic bags to halt the reaction. Absorbance was measured at 560 nm, using the unilluminated tube as the blank.

The POD activity was measured using the guaiacol method [34]. The reaction mixture included 2.9 ml of phosphate buffer, 1 ml of 2% H_2O_2, 1 ml of guaiacol solution, and 0.1 ml of enzyme solution (added last). The mixture was incubated in a 37 °C water bath for 15 min and then rapidly cooled in an ice bath. The reaction was stopped by adding 2 ml of trichloroacetic acid, and absorbance was measured at 470 nm, with a control comprising 2.5 ml of heated and boiling enzyme solutions.

The contents of rutin (No. NT20230820) and MeJA (No. NT20230825) were determined using ELISA kits from Shanghai Jingkang Biological Engineering Co., LTD (www.gelatins.com.cn, Shanghai, China).

RNA extraction and qRT-PCR: the RNA was extracted from the seeds using the RNeasy® Mini Kit (Qiagen), following the manufacturer's protocol, the RNA was subsequently treated with the TURBO DNAfreeTM Kit (Ambion) to eliminate possible DNA contamination. Reverse transcription to generate single-stranded cDNA was carried out using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas GmbH, St. Leon-Rot, Germany) with 1 μL random primer. All primer names and sequences used for semi-quantitative, and qPCR are listed in Table S1. The reaction comprised the following steps: incubation at 65 °C for 10 min, 55 °C for 20 min, and 85 °C for 5 min. The final volume of the cDNA was 20 μL, which was diluted to 100 μL. The qRT-PCR was conducted using the LightCycler® 96 Real-Time PCR System (Roche). The reaction mixture consisted of 2.5 μL cDNA, 5 μL 2× FastStart Essential DNA Green Master (Roche), and 0.5 μL of each primer (5 μM), in a total reaction volume of 10 μL. The PCR was performed according to the following program: initial denaturation at 95 °C for 600 s, followed by 45 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s. The run ended with a melting curve analysis to avoid nonspecific PCR products. The Ct values and baseline range were determined using the LightCycler® 96 software. The relative quantification was evaluated using the $2^{-\Delta\Delta CT}$ method [35], taking *FtH3* serving as the housekeeping gene. The transcript levels were normalized against the expression value in the water immersion treatment.

Statistical analysis

Data were recorded using Microsoft Excel, and statistical analyses were performed with SPSS 26.0. Duncan's multiple range test was used to compare average values and conduct the least significant difference (LSD) test. In addition, correlation, path, and principal component analyses (PCA) were conducted. Graphs were generated using Prism 9.0 statistical software, while PCA was performed using the Chiplot online platform (https://www.chiplot.online/).

Correlation analysis

Pearson correlation analysis was employed to assess the strength and direction of linear relationships between variables, with correlation coefficients ranging from −1 to 1. A value of 1 indicates a perfect positive correlation, −1 indicates a perfect negative correlation, and 0 indicates no linear association [36]. The formula used for calculating the Pearson correlation coefficient is as follows:

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

where X_i, Y_i represent the different values of the measurement index, and X_{av}, Y_{av} represent the corresponding average values of the measured indicators.

Grey relational analysis method

Grey relational analysis was conducted to assess the correlations among physiological and molecular indicators under melatonin treatment and drought stress, following the methodology of Javed et al. [37]. This method quantifies

the relational degree between variables based on their data sequences, enabling comprehensive evaluation of their interactions. Data were analyzed using SPSSAU software (https://spssau.com). The indicators included two categories: physiological parameters (Pro, Ss, Sp, SOD, POD, MDA, CAT, REC, Rutin, and MeJA) and gene expression levels (*FtCAT*, *FtSOD*, *FtPOD*, *FtSOS1*, and *FtAVP1*).

Path analysis

In the path analysis [38], the independent variables included SOD, POD, CAT, Sp, Ss, Pro, MDA, rutin, MeJA, *FtSOD*, *FtPOD*, *FtCAT*, *FtSOS1*, and *FtAVP1*, with REC serving as the dependent variable. Initially, a normality test was conducted on the dependent variable. A linear regression equation was generated through stepwise regression analysis. The indirect path coefficient (n) of each variable was calculated using the direct path coefficient and the regression equation, based on the following formula:

Independent variables in this study included SOD, POD, CAT, Sp, Ss, Pro, MDA, rutin, MeJA, *FtSOD*, *FtPOD*, *FtCAT*, *FtSOS1*, and *FtAVP1*, with REC as the dependent variable. Initially, a normalcy test for the dependent variable was performed. The linear regression equation was obtained through stepwise regression analysis. The indirect path coefficient (n) of each variable to the dependent variable is calculated using the direct path coefficient and the linear regression equation. The calculation formula is:

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

where X_i and X_j represent any two independent variables; 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.

Coupling coordination analysis

Coupling coordination analysis was performed using the method proposed by Dong et al. [39]. This analysis calculates three indices: the coupling coordination index (T value), the coupling coordination degree (C value), and the coupling coordination (D value). The formulas are as follows:

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

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

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

In these formulas, C represents the coupling degree of the antioxidant enzyme system and osmotic regulating substances, T represents the comprehensive level of these systems, and D represents their coupling coordination. U and A are the comprehensive scores of the antioxidant enzyme system and osmotic regulating substances, respectively, and α and β are their contribution coefficients. Generally, both systems are considered equally important, i.e., $\alpha = \beta = 0.5$. The classification standard of coupling coordination levels is shown in Table S2.

In silico binding studies of antioxidant enzymes and plant compounds

The in silico binding studies were conducted following the protocol outlined by Bag et al. [40]. Three plant compounds, melatonin (PubChem CID: 896), MeJA, PubChem CID: 5,319,693), and rutin (PubChem CID: 280,805), and five antioxidant enzymes, AVP1, CAT, POD, SOD, and SOS1, were investigated. The three-dimensional (3D) structures of the plant compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF file format and subsequently converted to PDB format using PyMOL 3.0 [41]. The 3D structures of the antioxidant enzymes were modeled using the SWISS-MODEL tool (https://swissmodel.expasy.org/) based on their respective FASTA sequences (Table S1). Molecular docking studies were performed using AutoDock 4.2, and binding affinities between rutin and the antioxidant enzymes were calculated in terms of kcal/mol [42].

Molecular dynamics simulation study

In this study, all-atom molecular dynamics simulations of protein–ligand complexes derived from molecular docking were conducted using GROMACS 2022.4 software [43, 44]. The protein was parameterized using the Amber14SB force field, while topology files for the small molecule ligands were generated via ACPYPE and Antechamber. The system was solvated in a cubic solvent box, maintaining a minimum distance of 1 nm between the complex and the system boundary. The TIP3P water model was employed, and appropriate quantities of sodium and chloride ions were introduced to neutralize the system's overall charge. Energy minimization was performed using the steepest descent algorithm to resolve steric clashes and reduce potential energy. Subsequently, the system underwent equilibration in two stages: first using the NVT ensemble to stabilize the temperature at 300 K, followed by the NPT ensemble to maintain pressure at 101.325 kPa. Following equilibration, a 100-ns MD simulation was carried out at 300 K, resulting in the generation of 10,000 simulation frames.

Key parameters were extracted from the resulting trajectory files, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius

of gyration (Rg), and the number of hydrogen bonds between the protein and the ligand. In addition, solvent-accessible surface area (SASA) was calculated to evaluate the solvent-exposed surface of the protein in different conformational states. RMSD was utilized to assess the stability of the complex throughout the simulation, relative to its initial structure. RMSF was used to quantify fluctuations in atomic positions, thereby indicating the local flexibility of protein regions. The Rg provided a measure of the overall compactness of the protein. SASA quantified the area of the protein surface accessible to the solvent.

Results

Effect of exogenous melatonin on the growth of buckwheat seeds under DS

As shown in Fig. S1 and Table 1, DS significantly reduced the germination rate and germination potential of buckwheat seeds in the absence of melatonin by 18.48% and 32.95%, respectively. However, when seeds were immersed in melatonin, no significant reductions in these parameters were observed under DS. In addition to its impact on germination, DS also significantly decreased the fresh weight, dry weight, and radicle length of buckwheat seeds, irrespective of the MT treatment. Without melatonin immersion, the reductions in fresh weight, dry weight, and radicle length were 7.96%, 37.26%, and 49.95%, respectively. For seeds treated with melatonin, these reductions were slightly different at 13.91%, 40.49%, and 26.57%.

Under normal (non-stressed) conditions, melatonin immersion had no significant effect on the germination rate, germination potential, fresh weight, dry weight, or radicle length of buckwheat seeds. However, under DS conditions, the MT treatment notably improved all these growth parameters. Specifically, melatonin-treated seeds showed increases of 24.42% in germination rate, 36.71% in germination potential, 3.85% in fresh weight, 6.04% in dry weight, and a substantial 61.99% increase in radicle length compared to untreated seeds under DS.

Effect of exogenous melatonin on the osmotic regulation and membrane stability of buckwheat seeds under DS

As shown in Fig. 1A, DS significantly increased the Pro content of buckwheat seeds on the 2nd, 4th, and 6th days, irrespective of the MT treatment. In addition, the MT treatment significantly elevated the Pro content on the 4th and 6th days under both normal and DS conditions. However, on the 2nd day, melatonin did not significantly affect Pro levels under normal conditions, though it markedly increased Pro content in seeds exposed to DS.

At all time points, DS substantially increased the Ss content of buckwheat seeds, regardless of the MT treatment (Fig. 1B). On the 4th and 6th days, melatonin immersion further increased Ss content under DS conditions but had no significant impact under normal conditions. Notably, on the 2nd day, melatonin significantly enhanced Ss content in seeds, regardless of the DS treatment.

As illustrated in Fig. 1C, DS also significantly elevated the Sp content of buckwheat seeds on the 2nd, 4th, and 6th days, independent of the MT treatment. Melatonin immersion further increased Sp content in buckwheat seeds under both normal and DS conditions across all time points.

The patterns of MDA content (Fig. 1D) and REC (Fig. 1E) were similar. DS significantly increased both MDA content and REC on the 2nd, 4th, and 6th days, regardless of the MT treatment. Melatonin immersion, however, significantly reduced MDA content and REC on the 4th and 6th days under both normal and DS conditions. On the 2nd day, melatonin did not significantly affect MDA content or REC under normal conditions, but it significantly reduced both parameters in seeds under DS.

Effect of exogenous melatonin on the antioxidant enzyme activity and compounds content of buckwheat seeds under DS

As shown in Figs. 2 A-C, significantly increased the activities of CAT, POD, and SOD in buckwheat seeds on the 2nd, 4th, and 6th days, irrespective of the MT treatment. The MT treatment further enhanced CAT

Table 1 Impact of exogenous melatonin on the germination and growth indicators of buckwheat under drought stress. Data (means ± SD, $n = 3$) are significantly different ($P < 0.05$) if followed by different letters within the same column. CK: water immersion; MT: 200 μM melatonin immersion; DS: 20% PEG-6000 + water immersion; DS + MT: 20% PEG-6000 + 200 μM melatonin immersion

Treatments	Germination rate (%)	Germination potential (%)	Fresh weight (g)	Dry weight (g)	Radicle length (cm)
CK	89.33 ± 14a	80.00 ± 17a	1.017 ± 0.09a	0.950 ± 0.09a	10.67 ± 0.35a
MT	95.67 ± 11a	86.67 ± 28a	1.129 ± 0.28a	1.062 ± 0.29a	11.78 ± 0.17a
DS	72.82 ± 12b	53.64 ± 22b	0.936 ± 0.19c	0.596 ± 0.16c	5.34 ± 0.08c
DS + MT	91.33 ± 21a	73.33 ± 18a	0.972 ± 0.18b	0.632 ± 0.26b	8.65 ± 0.23b

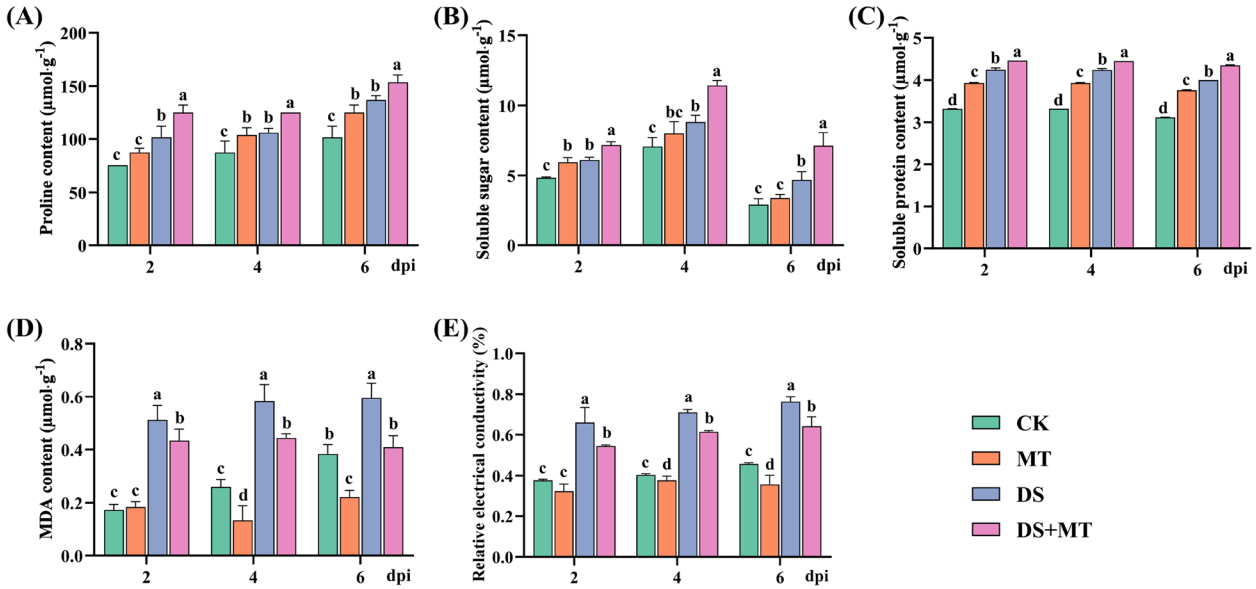

Fig. 1 Effects of exogenous melatonin on the on the (**A**) proline, (**B**) soluble sugars, (**C**) soluble protein, (**D**) malonaldehyde contents and (**E**) relative electrical conductivity in buckwheat seeds under normal and drought stress conditions. Data (means ± SD, *n* = 3) followed by different letters above the bars indicated significant differences at the 5% level. CK: water immersion; MT: 200 μM melatonin immersion; DS: 20% PEG-6000 + water immersion; DS + MT: 20% PEG-6000 + 200 μM melatonin immersion

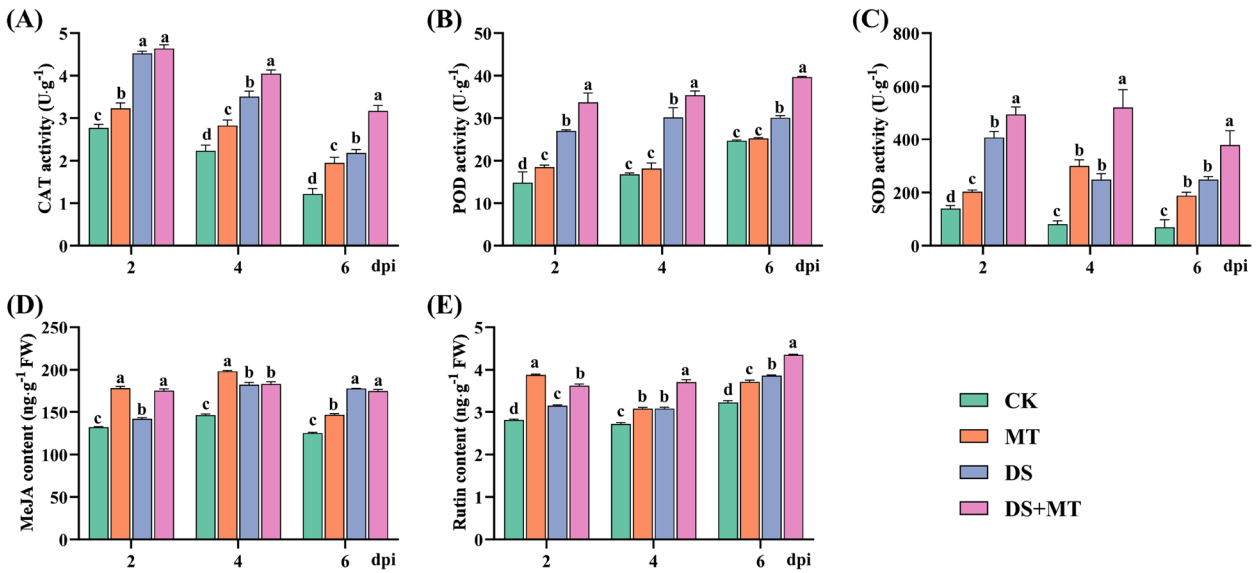

Fig. 2 Effects of exogenous melatonin on the on the (**A**) catalase, (**B**) peroxidase, (**C**) superoxide dismutase activities and (**D**) methyl jasmonate, and (**E**) rutin contents in buckwheat seeds under normal and drought stress conditions. Data (means ± SD, n = 3) followed by different letters above the bars indicated significant differences at the 5% level. CK: water immersion; MT: 200 μM melatonin immersion; DS: 20% PEG-6000 + water immersion; DS + MT: 20% PEG-6000 + 200 μM melatonin immersion

activity under both normal and DS conditions at all time points, except for the 2nd day under DS (Fig. 2A). Under normal conditions, melatonin significantly increased POD activity only on the 2nd day, with no significant effects observed on the 4th and 6th days (Fig. 2B). However, under DS, melatonin significantly elevated POD activity across all time points (Fig. 2B). For SOD, the MT treatment led to a significant increase

in activity at all measured time points, regardless of whether the seeds were under normal or DS conditions (Fig. 2C).

Regarding plant compounds, DS significantly increased the MeJA content in buckwheat seeds at all time points without the MT treatment (Fig. 2D). When seeds were treated with melatonin, DS significantly elevated MeJA content on the 2nd day, but there were no significant changes on the 4th and 6th days (Fig. 2D).

At all time points, melatonin immersion significantly increased rutin content in buckwheat seeds under both normal and DS conditions (Fig. 2E). While DS reduced rutin content on the 2nd day under the MT treatment, it significantly elevated rutin content on the 4th and 6th days, irrespective of the MT treatment(Fig. 2E).

Effect of exogenous melatonin on the relative expression of antioxidant enzyme genes in buckwheat seeds under DS

In the absence of melatonin, DS had varying effects on the expression of antioxidant enzyme genes in buckwheat seeds. On day 2, DS did not significantly affect the expression levels of *FtPOD* and *FtSOD*, but it significantly reduced the expression of *FtAVP1*, *FtCAT*, and *FtSOS1*. By day 4, DS led to a significant increase in the expression of *FtAVP1* and *FtSOD*, while it had no effect on *FtPOD* and *FtSOS1*, and significantly reduced *FtCAT* levels. On day 6, DS markedly elevated the expression levels of *FtAVP1*, *FtCAT*, *FtPOD*, and *FtSOD*, but it had no significant effect on *FtSOS1* (Fig. 3). In the MT treatment, DS significantly enhanced the expression of *FtAVP1*, *FtCAT*, *FtPOD*, *FtSOD*, and *FtSOS1* at all time points, with a few exceptions. These exceptions include *FtCAT*, *FtSOD*, and *FtSOS1* on day 2, and *FtCAT* and *FtPOD* on day 4, where no significant changes were observed (Fig. 3).

Under normal hydration conditions, melatonin immersion did not significantly alter the relative expression of *FtCAT* and *FtSOD* on day 2. However, it significantly reduced the expression of FtAVP1 and FtSOS1, while increasing *FtPOD* expression. By day 4, the MT treatment had no significant impact on *FtPOD* and *FtSOD*, but it markedly increased the expression of *FtAVP1*, *FtCAT*, and *FtSOS1*. On day 6, melatonin immersion had no significant effect on *FtAVP1*, *FtCAT*, *FtSOD*, and *FtSOS1*, though it significantly enhanced *FtPOD* expression (Fig. 3). Under DS conditions, melatonin consistently elevated the expression of *FtAVP1*, *FtCAT*, *FtPOD*, *FtSOD*, and *FtSOS1* at all time points, except for *FtSOD* and *FtSOS1* on day 2, *FtPOD* on day 4, and *FtCAT* on day 6, where no significant changes were observed (Fig. 3).

Correlation analysis and principal component analysis

Correlation analysis was conducted to clarify the relationships between the physiological indices of buckwheat under DS treated with exogenous melatonin. Among all parameters, the correlation between REC and rutin was the most notable, with a correlation coefficient of 1. Other significant correlations included MDA with rutin and REC, SOD with Sp, POD with Pro, CAT with

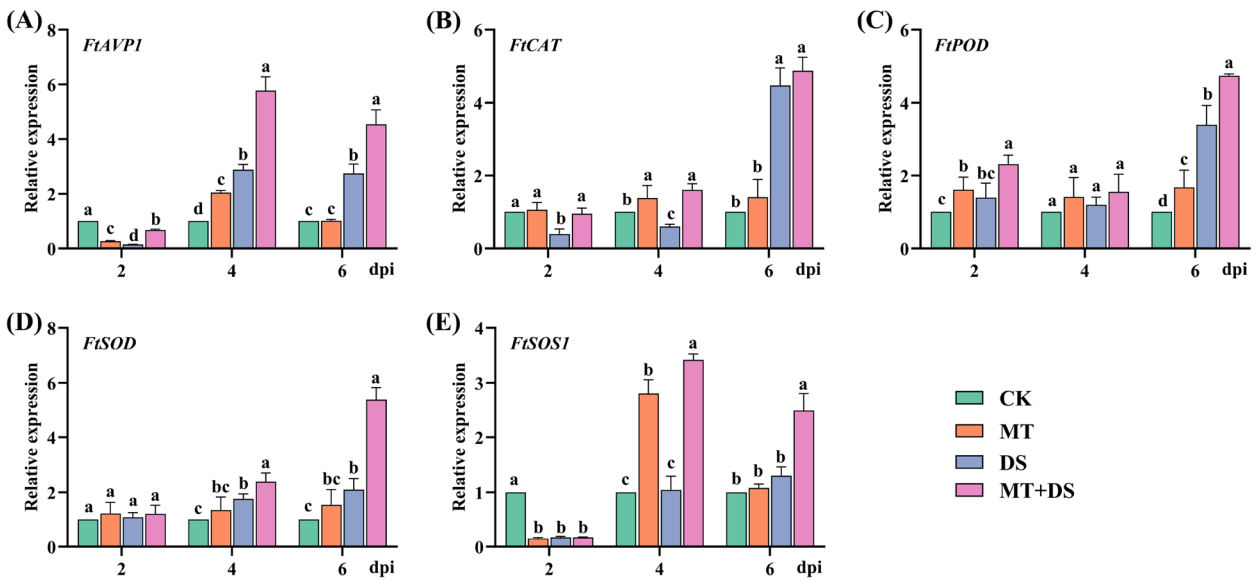

Fig. 3 Effects of exogenous melatonin on the on relative expression levels of (**A**) *FtAVP1*, (**B**) *FtCAT*, (**C**) *FtPOD*, (**D**) *FtSOD*, and (**E**) *FtSOS1* in buckwheat seeds under normal and drought stress conditions. Data (means ± SD, $n = 3$) followed by different letters above the bars indicated significant differences at the 5% level. CK: water immersion; MT: 200 μM melatonin immersion; DS: 20% PEG-6000 + water immersion; DS + MT: 20% PEG-6000 + 200 μM melatonin immersion

Sp, CAT with MeJA, POD with REC, and Pro with REC. The weakest correlation was observed between MeJA and MDA (Fig. 4A). Regarding gene parameters, the strongest correlation was found between *FtPOD* and *FtAVP1*, also with a correlation coefficient of 1. Additional significant correlations were observed between *FtSOD* and *FtCAT*, as well as between *FtSOS1* and *FtCAT* (Fig. 4B).

PCA was performed to assess the impact of exogenous melatonin on the physiological indices and antioxidant enzyme genes of buckwheat under DS. The results showed that the first two principal components (PC1 and PC2) accounted for 77.08% and 22.92% of the total variance, respectively, allowing clear differentiation among the various indices. Among these, CAT and POD had the highest eigenvalues, exerting significant influence on both PC1 and PC2, while Ss contributed primarily to PC2 (Fig. 4C). Similarly, the PCA of antioxidant enzyme genes revealed that PC1 and PC2 explained 78.57% and 22.43% of the total variance, respectively, enabling clear differentiation of the genes. *FtCAT* and *FtSOD* had the highest eigenvalues, significantly affecting both PC1 and PC2, while *FtAVP1* mainly influenced PC2 (Fig. 4D).

Grey correlation analysis, path coefficient analysis, and coordination of metabolites in buckwheat seeds under DS

Grey correlation analysis was conducted to identify the physiological indicators and peroxidase genes most significantly influenced by exogenous melatonin under DS conditions. The results showed that among the physiological indicators, CAT activity had the strongest correlation with melatonin, while MDA content exhibited the

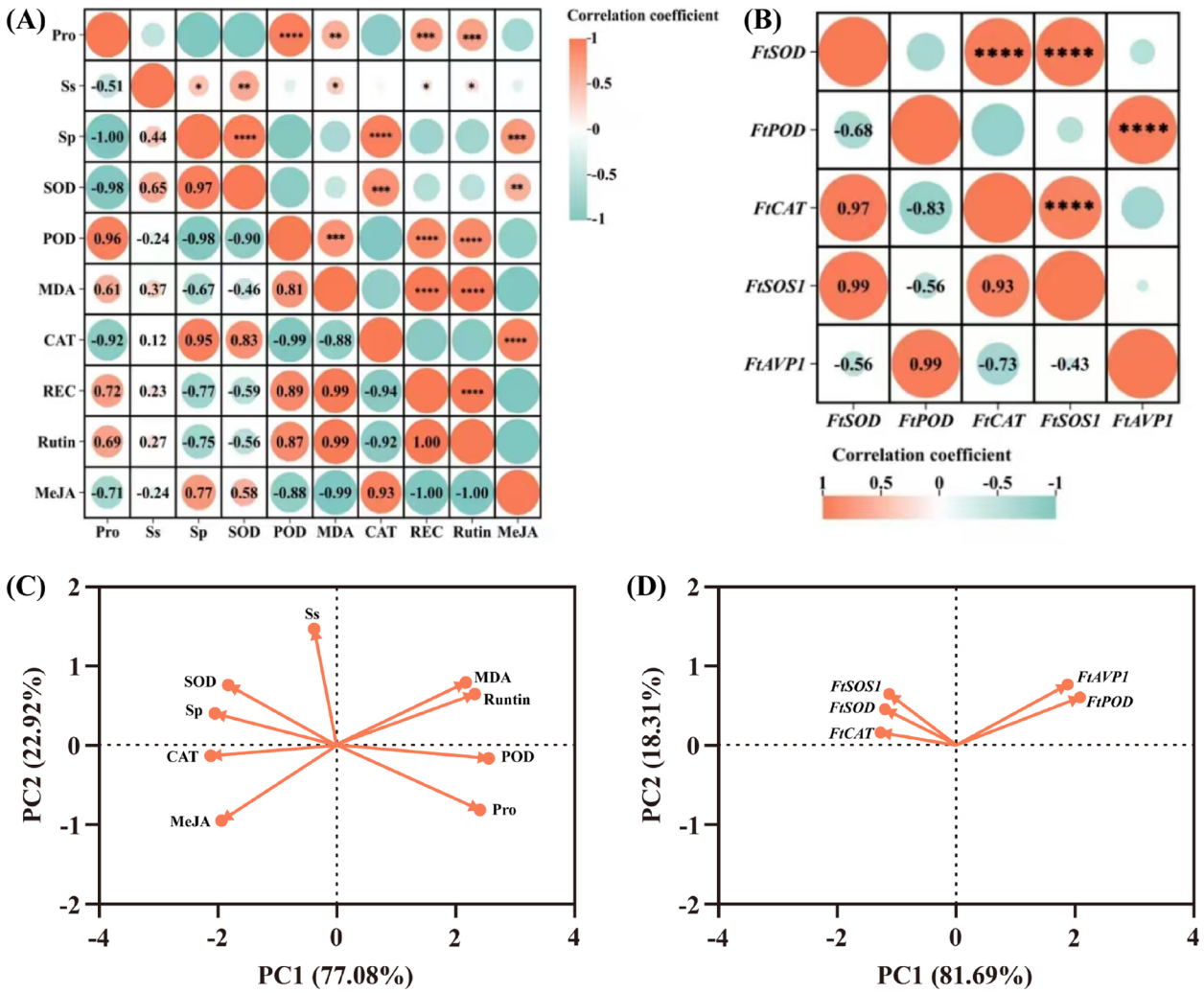

Fig. 4 Correlation analysis and principal component analysis of (**A**, **C**) physiological indicators and (**B**, **D**) antioxidant enzyme gene expression levels. Pro: proline; Ss: soluble sugars; Sp: soluble proteins; SOD: superoxide dismutase; POD: peroxidase; MDA: malonaldehyde; CAT: catalase; REC: relative electrical conductivity; MeJA: methyl jasmonate

weakest correlation (Fig. 5A). Similarly, among the gene parameters, *FtPOD* had the strongest correlation with melatonin, whereas *FtSOD* showed the weakest correlation (Fig. 5B).

Path coefficient analysis was then employed to evaluate the impact of these physiological indicators and antioxidant enzyme genes on membrane permeability, which was reflected by REC. Under DS, CAT had the most significant effect on REC, with a path coefficient of 2.446, while Ss had the least effect, with a path coefficient of 0.011 (Fig. 5C). Regarding gene expression, *FtCAT* had the largest impact on REC, with a path coefficient of 1, whereas *FtSOD* had the smallest impact, with a path coefficient of 0.427 (Fig. 5D).

Further analysis examined the coordination between plant compounds, osmotic regulators, and antioxidant enzymes. The coupling coordination model, represented by the D-value, was used to assess the interactions between physiological indicators or genes, helping to clarify whether these factors synergistically mitigate membrane damage. Path analysis revealed that CAT, MeJA, and Pro significantly influenced REC, as did *FtCAT* and *FtPOD*. The D-values across various treatments ranged from 0 to 1, indicating different coordination levels (Table S2).

On the 2nd day of the MT treatment, the D-value for the coordination between CAT and MeJA increased by 14.21%, raising the coordination level from moderate (Level 8) to high-quality (Level 10). By the 4th day, the D-values stabilized at a good coordination level (Level 9). By the 6th day, the D-value increased ninefold, shifting from severe discoordination (Level 2) to high-quality coordination (Level 10), demonstrating the significant positive effect of melatonin on CAT-MeJA interaction. However, under the DS + MT treatment, after an initial improvement, the D-value markedly decreased by the 6th day, suggesting potential negative long-term effects (Fig. 5E).

For CAT and Pro, melatonin consistently exerted positive effects at all time points, improving coordination from mild discoordination to higher levels, indicating a beneficial influence on CAT-Pro coordination in both the short and long term. In contrast, the DS + MT treatment showed fluctuations in coordination, reflecting variability over time (Fig. 5F).

Regarding CAT and POD, melatonin initially showed a strong positive impact on coordination, though this effect diminished over the long term. Under the DS + MT treatment, significant improvement was observed in the mid-term, but as with the CAT-MeJA interaction, long-term coordination showed variability, with both positive and negative impacts (Fig. 5G).

For *FtCAT* and *FtPOD*, melatonin treatment led to a significant reduction in the D-value on days 2 and 4, decreasing coordination from high quality (Level 10) to moderate discoordination (Level 3). However, by the 6th day, the D-value returned to high-quality coordination (Level 10) (Fig. 5H). Under the DS + MT treatment, the D-values on days 2 and 4 were 1.24 and 1.31 times higher than those under the DS treatment alone, improving coordination from severe (Level 2) to moderate discoordination (Level 3). By the 6th day, both treatments achieved high-quality coordination (Level 10), suggesting that while melatonin initially impaired *FtCAT-FtPOD* coordination under DS, it ultimately promoted optimal long-term coordination.

In summary, melatonin treatment generally enhanced the coordination between antioxidant enzymes and osmotic regulators in buckwheat seeds under DS, though the effects varied depending on specific factors and the duration of DS. These findings provide valuable insights into the protective mechanisms facilitated by melatonin in response to DS.

In silico binding studies and molecular dynamics simulations of antioxidant enzymes and plant compounds

In silico molecular docking studies were conducted to investigate the interactions between four antioxidant enzymes and three plant-derived compounds: melatonin, rutin, and MeJA (Fig. 6A). Among these, rutin displayed the strongest binding affinity with the antioxidant enzymes, suggesting stable and well-matched interactions. CAT showed the highest binding affinity with rutin, with a binding free energy of −9.714 kcal/mol. Rutin formed seven hydrogen bonds with specific amino acids in CAT (Fig. 6B; Fig. S2 A), including three with THR551, and also engaged in Pi-Pi stacking with PHE452 and Pi-alkyl interactions with TYR462 and ILE460. Similarly, rutin formed seven hydrogen bonds with SOD (Fig. S2B), including the longest bond at 5.65 Å with ARG126, along with alkyl interactions with VAL14 and LEU162. However, unfavorable receptor-receptor interactions were noted with SER122 and ILE124. Rutin's interaction with POD involved six hydrogen bonds (Fig. S2 C), Pi-Pi stacking with PHE452, and Pi-alkyl interactions with TYR462 and ILE460, though it also showed an unfavorable interaction with LYS361. In SOS1 (Fig. S2D), rutin formed seven hydrogen bonds, including two with ARG888, as well as Pi-alkyl interactions with VAL971 and PRO903. Additionally, carbon-hydrogen bonding with ASP973, PRO904, and PRO941 was observed. Rutin also interacted with AVP1 (Fig. S2E), forming two hydrogen bonds and engaging in Pi-alkyl and Pi-anion interactions.

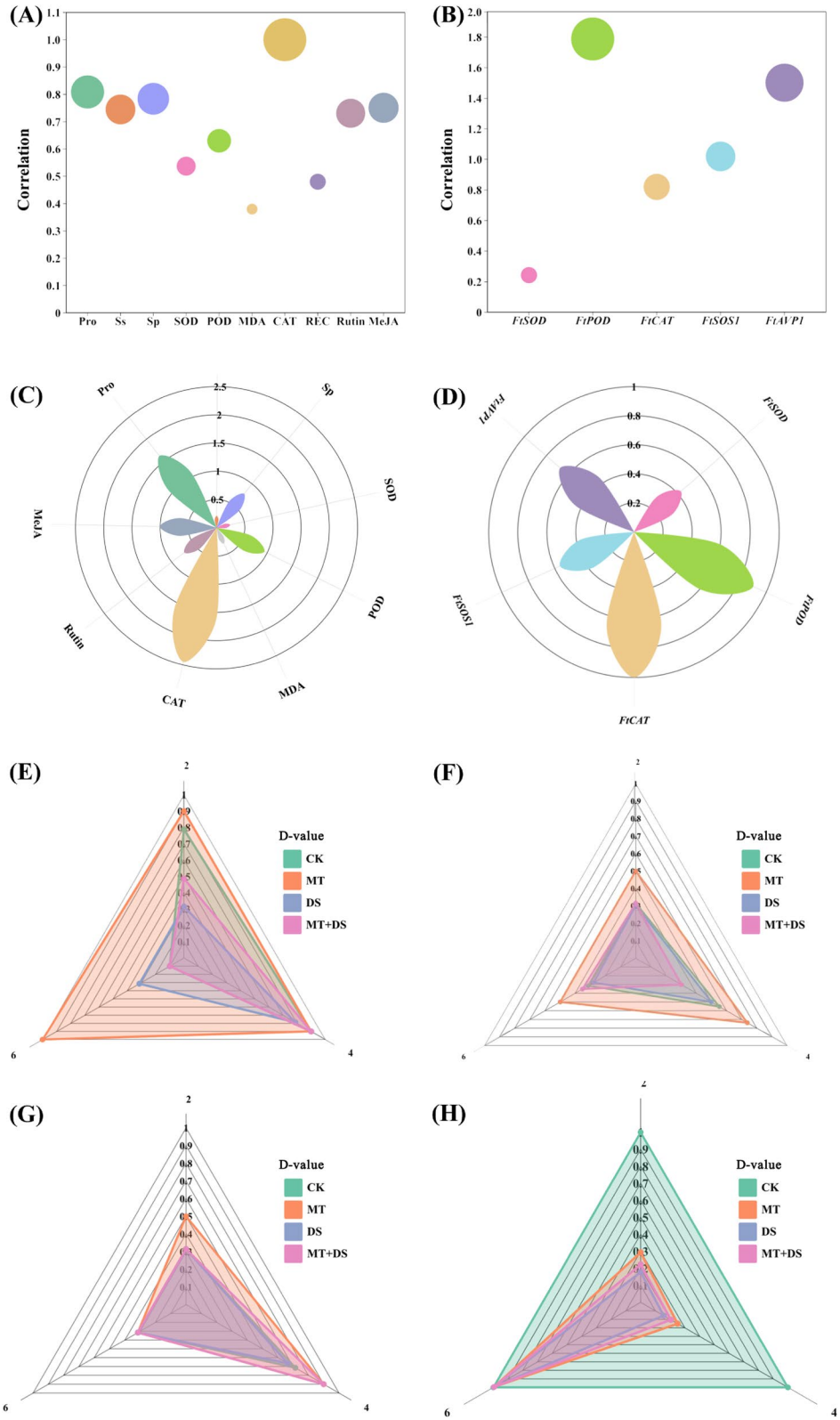

Fig. 5 Grey relational grade, path, and coordination analyses of (**A**, **C**) physiological indicators and (**B**, **D**) antioxidant enzyme gene expression levels, and (**E**–**H**) plant compounds under stress with exogenous melatonin. Pro: proline; Ss: soluble sugars; Sp: soluble proteins; SOD: superoxide dismutase; POD: peroxidase; MDA: malonaldehyde; CAT: catalase; REC: relative electrical conductivity; MeJA: methyl jasmonate. CK: water immersion; MT: 200 μM melatonin immersion; DS: 20% PEG-6000 + water immersion; DS + MT: 20% PEG-6000 + 200 μM melatonin immersion

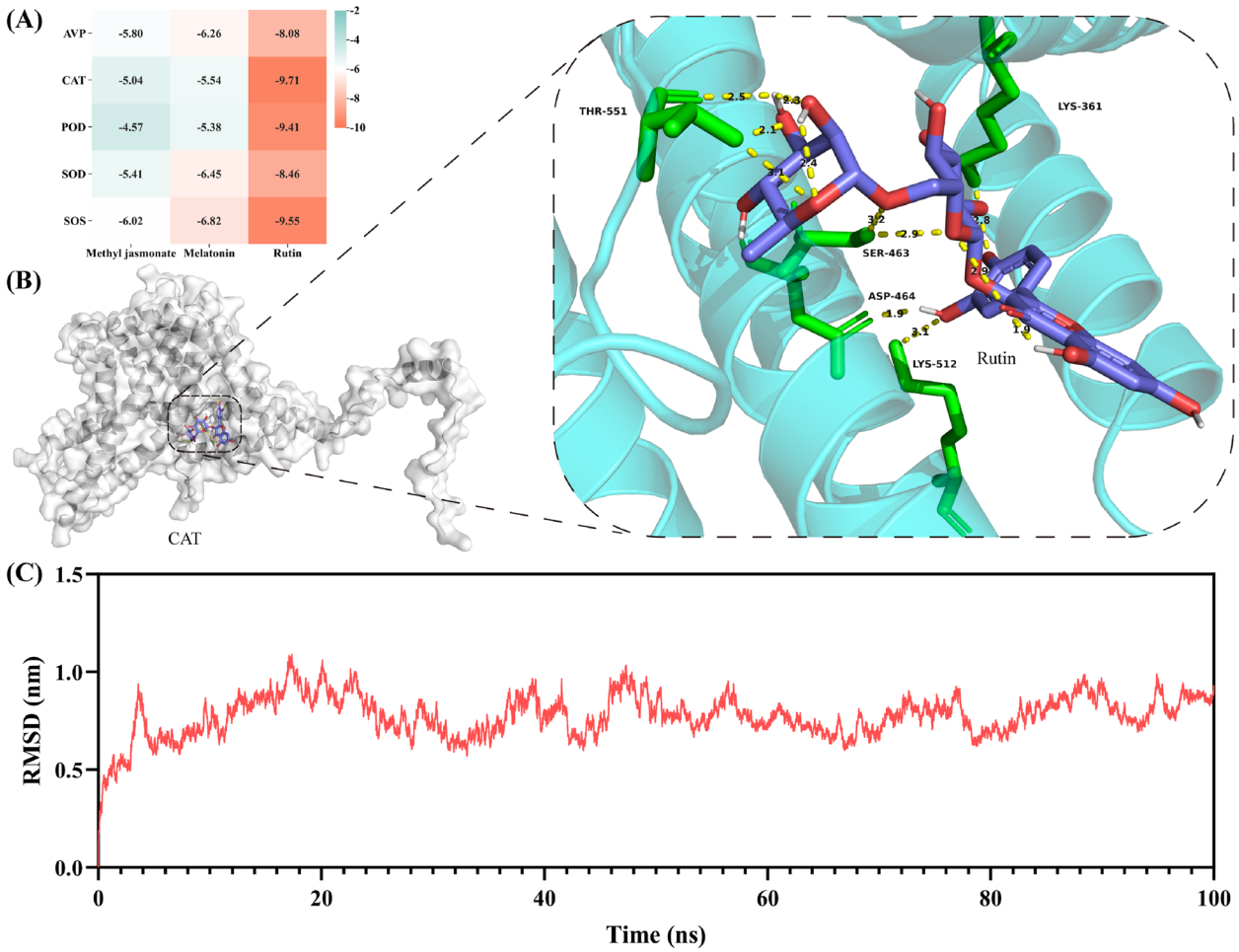

Fig. 6 Illustrates the in silico binding interactions between antioxidant enzymes and plant compounds. **A** Heatmap of the binding energies for these interactions. **B** 3D model highlighting the amino acid residues involved in the interaction between catalase (CAT) and rutin. **C** Root mean square deviation (RMSD) of the CAT and rutin complex over 100 ns

To assess the stability of these protein–ligand interactions, 100 ns molecular dynamics simulations were performed, analyzing RMSD, RMSF, Rg, SASA, and the number of hydrogen bonds over time. The RMSD analysis showed significant fluctuations in the first 20 ns, reflecting a transition from the initial conformation to a more stable one, after which the system stabilized between 0.5 and 1.0 nm (Fig. 6C). RMSF analysis revealed higher flexibility in the N- and C-terminal regions (residues 220 and 580), while the core region (residues 300 to 500) remained rigid, suggesting its importance in ligand interaction (Fig. S3 A). The Rg analysis indicated that the protein became more compact over time, with Rg values decreasing from 3.0 nm to 2.6 nm, reflecting a more stable conformation, likely induced by ligand binding (Fig. S3B). The SASA analysis showed a reduction in solvent-exposed surface area from 240 nm^2 to 200 nm^2, suggesting internal structural rearrangements as the protein compacted during the simulation (Fig. S3 C). The number of hydrogen bonds fluctuated between 2 and 6, indicating dynamic protein–ligand interactions, but critical bonds were maintained, contributing to the stability of the complex (Fig. S3D). These results suggest that rutin significantly enhances the antioxidant activity of these enzymes by stabilizing their structures and modulating their interactions, potentially playing a critical role in regulating oxidative stress responses in plants. The detailed interaction and stability analyses offer insights into how plant compounds like rutin contribute to stress resistance by interacting with key antioxidant proteins.

Discussion

Exogenous melatonin enhances seed germination and alleviates DS

The application of natural plant growth regulators is an ecologically sustainable strategy to enhance seed

germination under adverse environmental conditions and activate plant defense mechanisms during early development [45, 46]. Melatonin, a small indoleamine crucial for plant life, plays a pivotal role in regulating growth, development, and stress responses, even at low concentrations [47]. Thus, melatonin can function as an effective natural plant growth regulator. Previous studies have shown that exogenous melatonin improves seed germination, reduces stress-induced damage, and supports seedling growth, particularly under stressful conditions [48–50]. Our findings align with these reports.

In this study, treating common buckwheat seeds with exogenous melatonin significantly increased germination rates, seed vigor, and radicle length, while alleviating the inhibitory effects of DS on seed germination. These results confirm the efficacy of exogenous melatonin as a stress mitigator, particularly in drought conditions. The observed benefits are likely due to melatonin's regulation of key physiological and biochemical processes that help seeds overcome drought-induced inhibition of germination. This suggests that melatonin could serve as a valuable tool for enhancing seed germination and early seedling establishment in crops under DS, thereby improving agricultural resilience to climate change.

Exogenous melatonin enhances antioxidant enzyme activity in buckwheat seeds to combat DS

DS often triggers the overproduction of reactive oxygen species (ROS) in plant cells, including hydroxyl radicals and singlet oxygen. The excessive accumulation of ROS accelerates membrane lipid peroxidation, compromises the structural integrity and function of cellular membranes, disrupts the cell cycle, and can ultimately lead to cell death or the failure of the entire plant organism [51, 52]. To mitigate the oxidative damage caused by ROS, plants have developed a sophisticated antioxidant defense system. Key components of this system include SOD, POD, and CAT, which neutralize excess ROS and protect cellular membranes from oxidative damage [53]. Previous studies have shown that exogenous melatonin can enhance the expression of antioxidant enzyme genes, such as Cu/Zn-SOD, Fe/Zn-SOD, POD, and CAT, thereby boosting enzyme activities and preserving cellular membrane integrity under oxidative stress [53, 54].

Our findings are in line with these studies. Treatment with 200 μM melatonin significantly upregulated the expression of *FtSOD*, *FtPOD*, and *FtCAT*, leading to increased enzymatic activities by day six. This rise in antioxidant enzyme activity was accompanied by a marked reduction in MDA content and decreased cell membrane permeability, both indicators of reduced lipid peroxidation and oxidative damage. Further analysis revealed a strong correlation between POD activity, MDA levels, and relative conductivity, indicating that exogenous melatonin significantly affects CAT activity and *FtPOD* expression under DS. Notably, CAT activity and *FtCAT* expression had the most pronounced impact on cell conductivity changes, underscoring their crucial roles in maintaining cellular membrane stability.

These results suggest that POD plays a key role in mitigating oxidative damage to cellular membranes, while CAT is essential for preserving membrane integrity under DS. Overall, exogenous melatonin enhanced the antioxidant defense capacity of common buckwheat seeds, effectively scavenging ROS and stabilizing cellular structures. However, it is important to recognize that the optimal concentration of melatonin may vary between plant species. For instance, Xiao et al. [55] found that a 20 μM melatonin solution was most effective in reducing MDA accumulation and enhancing SOD and POD activities in cotton seeds under DS. This suggests that different plant species may exhibit varying sensitivities to melatonin, necessitating species-specific optimization of the MT treatment concentrations to achieve maximum protective effects.

Exogenous melatonin regulates osmotic adjustment substances in buckwheat seeds to combat DS

Osmotic regulation is a key mechanism that enables plants to tolerate DS by maintaining water balance and cellular integrity [56]. Under drought conditions, plants accumulate osmotic adjustment substances, such as Pro, Ss, and Sp, through biosynthetic pathways. These compounds play a vital role in regulating cellular water potential, thereby preventing dehydration and preserving cellular structure [57]. In our study, treatment with 200 μM melatonin significantly increased the accumulation of Pro, soluble sugars, and soluble proteins in common buckwheat seeds, consistent with the findings of [58]. Notably, Pro accumulation was strongly correlated with reduced MDA content and lower relative conductivity, indicating that exogenous melatonin alleviates drought-induced membrane damage by enhancing osmolyte accumulation and maintaining intracellular water potential.

Pro is widely recognized as a key marker of plant drought tolerance [49], functioning as an osmoprotectant that shields cellular membranes and proteins from dehydration stress. Melatonin-induced Pro accumulation has also been observed in maize under drought conditions, further underscoring its crucial role in enhancing drought resistance [18]. Besides increasing Pro content, melatonin also elevated the levels of soluble sugars and proteins, which are essential for osmotic adjustment and maintaining metabolic activity under stress conditions.

Additionally, our study showed that the MT treatment upregulated the expression of *FtSOS1* and *FtAVP1* at days

2, 4, and 6 of DS. These genes are integral to maintaining ion homeostasis and osmotic pressure. SOS1 is particularly important for Na^+ homeostasis, as it regulates Na^+ extrusion to ensure optimal intracellular ion concentrations, which is crucial for sustaining osmotic balance during DS [59, 60]. Furthermore, overexpression of AVP1 enhances vacuolar proton gradients, facilitating ion sequestration within vacuoles and improving water retention, which significantly boosts drought tolerance [61, 62]. By upregulating *FtSOS1* and *FtAVP1* expression, melatonin promotes ion regulation, thereby maintaining osmotic pressure and enhancing drought resistance.

In summary, these findings indicate that exogenous melatonin not only increases the accumulation of key osmotic adjustment substances, such as Pro, soluble sugars, and proteins, but also upregulates the expression of genes critical for maintaining cellular ion homeostasis. Together, these processes enhance the drought tolerance of common buckwheat seeds by regulating osmotic pressure and preserving cellular stability.

Exogenous melatonin regulates meja and rutin synthesis to enhance buckwheat seed drought tolerance

MeJA is a crucial signaling molecule in plants, playing a central role in both stress responses and the regulation of secondary metabolite synthesis [63–65]. We measured MeJA to investigate its role in melatonin-mediated drought tolerance, as it is known to mediate stress signaling and induce secondary metabolite accumulation under abiotic stress [63, 64]. The jasmonic acid signaling pathway has been shown to activate genes associated with secondary metabolism, including those involved in rutin biosynthesis [66]. In this study, the MT treatment significantly elevated MeJA levels during the early stages of DS, which later stabilized. This early MeJA accumulation likely enhances drought tolerance by triggering the jasmonic acid pathway, which, in coordination with melatonin, promotes rutin synthesis and strengthens antioxidant defenses. However, rutin content continued to increase throughout the stress period, suggesting that melatonin promotes rutin synthesis by stimulating early MeJA accumulation and activating the jasmonic acid pathway.

Rutin, a potent antioxidant, plays a protective role in buckwheat by mitigating oxidative damage under stress conditions [67, 68]. Additionally, rutin has been shown to modulate the catalytic activities of enzymes involved in oxidative and metabolic processes [69, 70]. Our molecular docking analysis revealed strong binding interactions between rutin and *FtCAT*, suggesting that rutin may stabilize or enhance *FtCAT* activity. The stable complex formed through hydrogen bonding, Pi-Pi interactions, and Pi-alkyl interactions indicates that rutin can activate or stabilize *FtCAT*, thus enhancing its enzymatic function.

To further investigate the interaction between the receptor protein and the small molecule during dynamic conditions, and to evaluate the stability of the binding site, we performed a 100 ns molecular dynamics simulation of the CAT-rutin complex. The molecular dynamics simulations provided additional insights into the stability of the CAT-rutin complex, showing stable RMSD values after 20 ns, along with consistent hydrogen bonding and decreased SASA. These findings suggest reduced exposure of the protein surface to solvents, indicative of a more compact and stable structure. Overall, these results confirm the crucial role of rutin in stabilizing CAT activity, thereby contributing to enhanced drought tolerance by mitigating oxidative damage.

Previous studies have demonstrated that rutin upregulates CAT gene expression and increases CAT enzymatic activity [71], further supporting its role in reducing oxidative stress by regulating CAT activity and lowering ROS levels. This reduction in ROS accumulation helps protect cellular structures from oxidative damage, thereby supporting normal physiological functions during DS.

These findings suggest that exogenous melatonin enhances drought tolerance in common buckwheat by modulating both MeJA and rutin synthesis. The early accumulation of MeJA likely activates the jasmonic acid signaling pathway, promoting rutin biosynthesis, which subsequently contributes to mitigating oxidative stress. Together, these mechanisms indicate that melatonin strengthens the plant's overall defense system by regulating key plant compounds and enzymes involved in oxidative stress protection.

Exogenous melatonin enhances buckwheat seed drought tolerance through synergistic effects on multiple physiological processes

The coupling coordination degree model is a valuable tool for quantifying the relationships between multiple physiological indicators, providing insights into the synergistic effects under specific conditions [72]. In plants, the coordinated regulation of physiological processes is critical for mitigating environmental stress, as various systems must function in harmony to effectively reduce stress-induced damage [73–75]. By applying this model, it becomes possible to comprehensively assess the interactions among different physiological processes, thus illuminating the complex mechanisms underlying plant stress tolerance.

Previous research has demonstrated that exogenous melatonin enhances drought tolerance by improving antioxidant enzyme activities, osmolyte accumulation,

secondary metabolite production, and related gene expression [76, 77]. However, these studies primarily focused on individual physiological parameters and did not systematically examine their coordination. In our study, we used the coupling coordination model to quantify the interactions between key physiological indicators, such as CAT, POD, MeJA, Pro, and the gene expression of *FtCAT* and *FtPOD*, at different time points during DS.

Results revealed that exogenous melatonin significantly enhanced the coordination between CAT, POD, MeJA, and Pro during the early and middle stages of DS. Notably, the coordination between *FtCAT* and *FtPOD* peaked on day six. These findings suggest that melatonin plays a pivotal role in mitigating early membrane damage by promoting the synergistic regulation of antioxidant enzymes and osmolytes. As DS progresses, melatonin further strengthens the plant's antioxidant defense system by coordinating gene expression related to stress responses, thereby reducing cellular membrane damage and enhancing drought tolerance.

The coordinated regulation of these multiple physiological processes highlights the complex, multilayered defense mechanisms activated by melatonin to protect common buckwheat from DS. Initially, melatonin alleviates oxidative stress and membrane damage through the combined action of antioxidant enzymes and osmolytes. As DS intensifies, melatonin continues to support drought tolerance by upregulating stress-related gene expression and reinforcing the antioxidant defense system. This study provides further evidence that exogenous melatonin enhances drought tolerance in common buckwheat by orchestrating a range of physiological responses, offering a comprehensive mechanism for plant adaptation to stress conditions.

Conclusions

This study demonstrated that exogenous melatonin significantly enhances drought tolerance in common buckwheat seeds under PEG-6000-induced DS (Fig. 7). The MT treatment improved key germination parameters, such as germination rate and radicle length, alleviating the negative effects of DS. Melatonin enhanced osmotic adjustment by increasing the accumulation of Pro, Ss, and Sp, thereby maintaining water potential

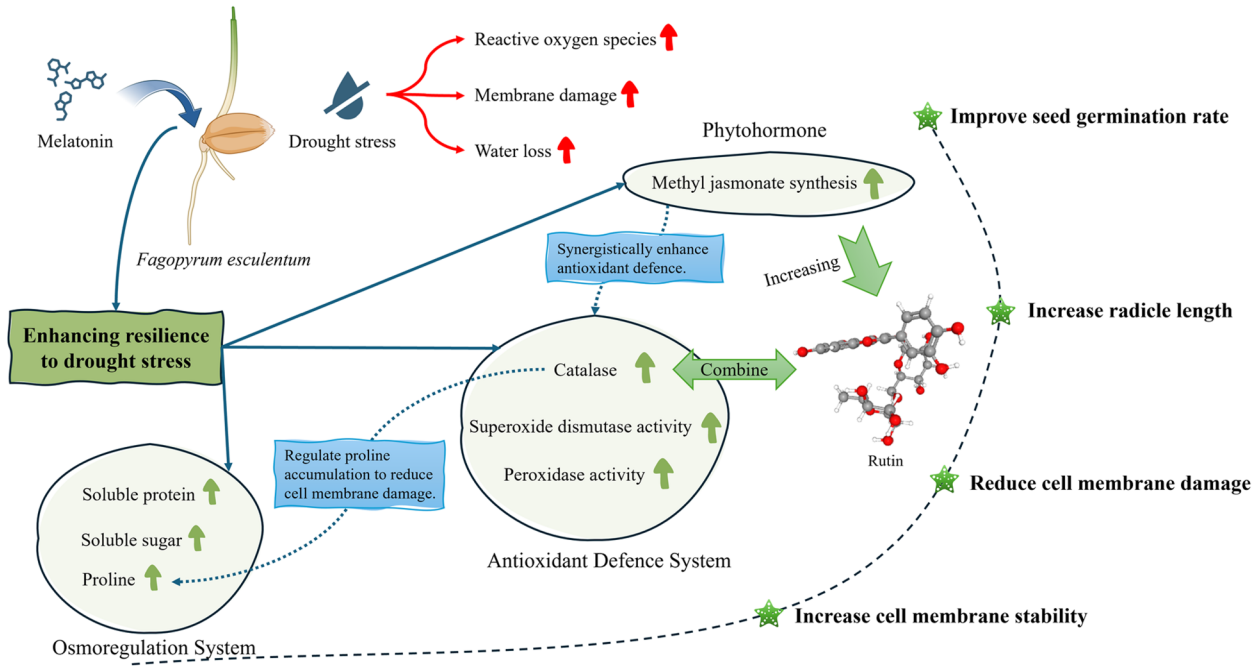

Fig. 7 Mechanism of exogenous melatonin enhancing seed germination characteristics of buckwheat under drought stress. Under drought stress, buckwheat seeds experience water loss, increased membrane damage, and elevated reactive oxygen species (ROS) levels. Exogenous melatonin counteracts these effects by promoting osmotic regulation, boosting antioxidant defenses, and stimulating plant compounds synthesis. It increases soluble sugars, proteins, and proline, maintaining osmotic balance, reducing water loss, and preserving metabolic functions. Melatonin also enhances superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, improving ROS scavenging and protecting cell membranes. Additionally, melatonin promotes methyl jasmonate (MeJA) synthesis, activating the jasmonic acid pathway and increasing rutin content, further enhancing antioxidant capacity. Proline and CAT synergistically stabilize membranes, while MeJA and CAT boost antioxidant defense. Combined action of POD, CAT, and rutin strengthens ROS elimination and mitigates oxidative damage. These mechanisms collectively improve drought tolerance, increasing germination rates, radicle length, and membrane stability, while reducing damage

and preventing dehydration. It also upregulated the expression of genes related to osmotic regulation (*FtAVP1* and *FtSOS1*), contributing to ion homeostasis. Additionally, melatonin boosted antioxidant enzyme activities (CAT, POD, SOD) and related gene expression, reducing oxidative damage and stabilizing cell membranes. Molecular docking and molecular dynamics simulations revealed a stable interaction between rutin and CAT, suggesting enhanced enzyme stability and a strengthened antioxidant defense. The increase in rutin content, along with its interaction with antioxidant enzymes, further supported stress tolerance. In summary, exogenous melatonin enhances drought tolerance by coordinating antioxidant defense, osmotic regulation, plant compounds synthesis, and stabilizing protein interactions, offering a practical strategy to improve crop establishment and resilience under drought conditions (Fig. 7).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06632-5.

Supplementary Material 1.

Authors' contributions

ZT, JH and YM conceived and designed the study. ZT performed the experiments. JH wrote the original manuscript. ZT and YM analyzed the data. ZT, JH, ZW, ZZ, MQ and YM contributed to manuscript development and revisions.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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