



Exogenous melatonin enhances drought tolerance and secondary-metabolite accumulation in *Paeonia ostii* ‘Fengdan’ in association with improved PSI–PSII photochemical coordination

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Abstract

Drought limits the growth of *Paeonia ostii* ‘Fengdan’. While melatonin (MT) is known to enhance general drought tolerance, its association with Photosystem I–Photosystem II (PSI–PSII) photochemical coordination and secondary-metabolite accumulation in *P. ostii* remains unclear. We foliar-sprayed potted plants under simulated drought with 0, 50, 100, 200, and 400 $\mu\text{mol(MT)} \text{ L}^{-1}$. Drought induced severe oxidative damage, impaired photosynthesis, and disrupted photochemical coordination between PSI and PSII. Among all treatments, 100 $\mu\text{mol(MT)} \text{ L}^{-1}$ proved the most effective. It coincided with reduced reactive oxygen species and membrane damage, alongside increased antioxidants, osmolytes, and PSI/PSII performance. Furthermore, compared to drought alone, 100 $\mu\text{mol(MT)} \text{ L}^{-1}$ treatment increased the root-bark contents of total flavonoids, total phenolics, gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid, and paeonol by 48.1, 36.1, 17.7, 69.4, 69.9, 24.1, 7.7, and 22.7%, respectively. Thus, 100 $\mu\text{mol(MT)} \text{ L}^{-1}$ alleviated drought-induced physiological damage and increased selected secondary-metabolite contents in *P. ostii*.

Keywords: antioxidant system; drought stress; melatonin; *Paeonia ostii* ‘Fengdan’; photosynthesis.

Highlights

- Melatonin reduced drought-associated oxidative injury in *Paeonia ostii*
- Melatonin improved PSI- and PSII-related photochemical performance under drought
- Melatonin enhanced the accumulation of secondary metabolites in drought-stressed *P. ostii*

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Abbreviations: ABS/CS_m – absorption flux per excited cross-section; ABS/RC – the absorption flux per reaction center; Car – carotenoid; Chl – chlorophyll; C_i – intercellular carbon dioxide concentration; DI₀/CS_m – the dissipated energy flux per cross section; DI₀/RC – the dissipated energy flux per reaction center; *E* – transpiration rate; ET₀/CS_m – the electron transport flux per cross section; ET₀/RC – the electron transport flux per reaction center; F_v/F_m – the maximum photochemical efficiency of PSII under dark adaptation; g_s – stomatal conductance; M₀ – initial slope of relative variable fluorescence, reflecting the relative rate of the primary quinone electron acceptor of PSII (Q_A) reduction; MDA – malondialdehyde; MT – melatonin; PI_{ABS} – the performance index on absorption basis; P_N – the net photosynthetic rate; RC/CS_m – the density of RCs per excited cross section; REL – relative electrolyte leakage; SOD – superoxide dismutase; TR₀/CS_m – the trapped energy flux per cross section; TR₀/RC – the trapped energy flux per reaction center; V_J – the relative variable fluorescence intensity at the J step; W_K – the normalized relative variable fluorescence; ΔI/I₀ – the maximum redox activity of PSI; φD₀ – quantum yield of energy dissipation; φE₀ – the quantum yield for electron transport; Φ_{PSI/PSII} – the coordination between PSII and PSI.

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Introduction

Global warming has intensified extreme and persistent droughts (Hartmann *et al.* 2022), causing severe soil water deficits that trigger widespread agricultural drought stress (Xue *et al.* 2024). In China, the proportion of arable land affected by drought is projected to rise continuously (Qi *et al.* 2022), with the risk of drought stress being particularly prominent in the North China Plain (Yang *et al.* 2022). Drought stress impairs root function, nutrient transport, and leaf expansion, thereby disturbing organic compound metabolism, suppressing growth, reducing biomass, and ultimately decreasing yield, with severe cases even causing massive plant death (Guo *et al.* 2018, Zhu *et al.* 2024). For medicinal plants, drought not only hinders the yield but also affects the quality of medicinal materials by interfering with secondary metabolism (Cheng *et al.* 2018, Pant *et al.* 2021). Therefore, implementing effective approaches to strengthen drought adaptability of medicinal plants possesses important research significance.

Paeonia ostii ‘Fengdan’ is a perennial woody shrub classified into section Moutan within the genus *Paeonia* and the family *Paeoniaceae*, and it is widely recognized in China for both ornamental value and medicinal use (Peng *et al.* 2019). Its processed root bark, known as Cortex Moutan, is an official medicinal material recorded in the Chinese Pharmacopoeia (Wang *et al.* 2017). Cortex Moutan contains a variety of medicinal active ingredients, with high contents of terpenoids and their glycosides. Among them, paeonol can be used for tumor prevention and treatment, as well as antibacterial and anti-inflammatory purposes, while paeoniflorin exhibits anticoagulant and antithrombotic activities (Zhang *et al.* 2022, Wang *et al.* 2023). As a key medicinal material, Cortex Moutan has found extensive applications in numerous classic traditional Chinese medicine formulas such as Liuwei Dihuang Wan (Liu *et al.* 2023). Currently, *P. ostii* is extensively farmed across several Chinese provinces (Peng *et al.* 2019). Because these major production regions frequently experience water scarcity and drought, the sustainable yield and quality of Cortex Moutan are severely threatened (Li *et al.* 2022). Tree peonies exhibit significant physiological disorders under water deficit conditions (Guo *et al.* 2018), manifested as macroscopic symptoms such as leaf wilting, decreased pigment concentration, and inhibited photosynthetic capacity (Wei *et al.* 2023). Furthermore, water deficit not only compromises chloroplast architecture and suppresses the transcription of photosynthesis-associated genes, but also forces the plant to restructure its secondary metabolic networks as an adaptive survival strategy (Zhao *et al.* 2019). Despite extensive physiological research, how drought conditions affect the accumulation of active medicinal components in *P. ostii* still requires further exploration.

Photosynthesis is the core driver of plant productivity, and its efficiency relies on the coordinated operation of photosynthetic pigments and the two key photosynthetic complexes, PSI and PSII (Zhang *et al.* 2024). Drought stress impairs the photosynthetic apparatus by deactivating

PSII reaction centers and obstructing electron transport, thereby reducing light-use efficiency and inducing severe photoinhibition across diverse plant species (Guo *et al.* 2020, Hu *et al.* 2023, Pereira *et al.* 2026). As a sensitive probe of this process, chlorophyll fluorescence parameters are indirect indicators of PSII photochemical performance and electron transport (Strasser *et al.* 2010), providing a rapid, noninvasive diagnostic tool. Beyond PSII, PSI catalyzes the terminal stage of photosynthetic electron transport and supplies reducing power for nicotinamide adenine dinucleotide phosphate (NADPH) formation, making it indispensable for sustained photosynthetic activity. Under water-deficit conditions, dissociation of the photosystem I–light-harvesting complex I (PSI–LHCI) supercomplex can diminish the light-harvesting antenna size and compromise the structural integrity of PSI (Pandey *et al.* 2023). The subsequent accumulation of nonfunctional complexes further suppresses PSI photochemical efficiency (Wada *et al.* 2019), rendering PSI another pivotal regulatory site for photosynthetic downregulation during drought (Sapeta *et al.* 2023). In the traditional medicinal tree peony, *Paeonia ostii*, soil water deficit has been shown to decrease PSII maximum quantum efficiency (F_v/F_m) and effective quantum yield $Y_{(II)}$, while concurrently increasing nonphotochemical quenching (NPQ) and the coefficient of nonphotochemical quenching (q_N) (Zhao *et al.* 2019). However, existing studies have largely emphasized PSII, whereas the physiological regulation of PSI in drought-stressed *P. ostii* leaves remains insufficiently understood.

As a pleiotropic signaling molecule with potent endogenous antioxidant activity, melatonin (MT) is involved in diverse aspects of plant development and stress acclimation (Tan *et al.* 2010, Arnao and Hernández-Ruiz 2014). Exogenous MT confers drought tolerance in crops by promoting osmotic adjustment and antioxidant capacity, thereby preventing reactive oxygen species (ROS) bursts and subsequent lipid peroxidation (Altaf *et al.* 2022). Furthermore, MT has been reported to protect the photosynthetic apparatus. Exogenous application restricts chlorophyll degradation by modulating specific metabolic enzymes and restores photochemical efficiency and electron transport in PSII (Du *et al.* 2024). In maize, MT further ameliorates drought-induced structural damage and re-establishes the coordination of electron transport between PSII and PSI (Guo *et al.* 2020). However, whether MT regulates the coordinated operation of the two photosystems in tree peony remains unknown.

In addition to its protective effects on photosynthesis, MT can modulate secondary metabolism under water deficit. Previous studies found that exogenous MT activated phenylpropanoid metabolism in drought-stressed pecan and promoted the accumulation of flavonoids, flavanones, and phenolic acids associated with stress tolerance (Sharma *et al.* 2020). Preliminary hydroponic experiments indicate that MT enhances drought acclimation in *P. ostii* plants by elevating antioxidant capacity and PSII efficiency (Fan *et al.* 2020). Although melatonin-mediated drought responses have been reported in several plant species, it remains unclear how exogenous melatonin affects

the coordination of PSI- and PSII-related photochemical processes in *P. ostii* under drought. It is also unknown whether changes in photosynthetic performance are accompanied by altered accumulation of medicinally relevant metabolites in the root bark, and how these responses vary with melatonin concentration. Given that seasonal water deficits in major *P. ostii* cultivation regions frequently coincide with the critical period of root bark metabolite accumulation, elucidating these processes holds practical significance for optimizing MT application in drought-resilient cultivation. We hypothesized that exogenous melatonin would alleviate drought-induced physiological impairment in *P. ostii*, and that this protection would be associated with improved coordination of PSI- and PSII-related photochemistry and increased accumulation of selected secondary metabolites. To test this hypothesis, this study examined the effects of exogenous melatonin on antioxidant defense, osmotic adjustment, gas exchange, PSI–PSII-related photochemical parameters, and root-bark secondary metabolites in drought-stressed four-year-old potted *P. ostii* ‘Fengdan’ plants.

Materials and methods

Materials and experimental design: In October 2024, sixty uniform four-year-old *P. ostii* ‘Fengdan’ plants were individually cultivated in plastic pots (28 × 23 × 25 cm; top diameter × basal diameter × height), each containing 11 kg of air-dried soil. Melatonin was dissolved in 95% (v/v) ethanol, and the solution was brought to a final volume of 50 mL to obtain a 100 mmol L⁻¹ stock solution. Appropriate aliquots of the stock solution were diluted with deionized water to obtain the required MT concentrations. The final ethanol concentration was adjusted to 0.5% (v/v) in all MT treatment solutions and the corresponding solvent controls. *Tween-80* was added to all final solutions at 0.1% (v/v). Foliar sprays were applied on 10, 20, and 30 April 2025. To prevent potential photodegradation, all spraying procedures were performed at dusk under dim light, ensuring both the adaxial and abaxial leaf surfaces were thoroughly wetted until dripping.

Before the first foliar application, 60 potted plants were randomly assigned to six treatment groups, with 10 plants per group. Plants assigned to the MT50, MT100, MT200, and MT400 groups were sprayed with 50, 100, 200, and 400 µmol(MT) L⁻¹, respectively, on 10, 20, and 30 April 2025. Plants assigned to the Control (CK) and Drought (DR) groups were sprayed with the solvent control solution on the same dates. The solvent control contained the same final concentrations of ethanol and *Tween-80* as the MT solutions but no melatonin. All plants were maintained under well-watered conditions until the drought treatment was initiated in May 2025. Thereafter, CK plants were maintained at 75 ± 5% of soil field capacity (FC), whereas DR and all MT-pretreated plants were subjected to drought stress at 50 ± 5% FC for 8 d. Soil moisture was maintained gravimetrically by weighing each pot daily at 18:00 h and replenishing the water lost. After 8 d of drought treatment, gas exchange traits, chlorophyll fluorescence parameters, and 820-nm

modulated-reflectance parameters were measured. Leaves were then collected for physiological and biochemical analyses, and roots were harvested for quantification of secondary metabolites in the root bark.

Antioxidant enzyme activity: Superoxide dismutase (SOD, EC 1.15.1.1) activity was quantified using the nitroblue tetrazolium (NBT) method (Li *et al.* 2018). Fresh foliage (~0.30 g) was cryo-ground and homogenized in phosphate buffer. The supernatant, recovered after centrifugation (3,000 rpm, 10 min, 4°C), served as the crude extract. Following absorbance readings at 560 nm, one unit of SOD activity was defined as the amount of enzyme required to inhibit NBT photoreduction by 50%. Activity was expressed as U g⁻¹ fresh mass (FM).

Osmotic adjustment substances: Soluble sugars were evaluated following the protocol of Dong *et al.* (2019). Aqueous extraction was performed by boiling 0.1 g of fresh foliage for 30 min. The filtered and diluted supernatant was subsequently reacted with an ethyl acetate–anthrone solution, water, and concentrated sulfuric acid. After heating in a boiling water bath and subsequent cooling, sample absorbance was quantified at 630 nm.

Following the protocol of Zhang *et al.* (2021), soluble proteins were extracted by homogenizing fresh leaves (0.2 g) in 8 mL of 1% polyvinylpyrrolidone (PVP). After centrifugation (10,000 rpm, 10 min, 4°C), the supernatant was combined with *Coomassie Brilliant Blue G-250* reagent. Absorbance was recorded at 595 nm after a 2-min room-temperature incubation.

The quantification of proline followed the procedure of Bates *et al.* (1973). Leaf material (0.2 g) was homogenized in sulfosalicylic acid and subjected to a hot water bath. Aliquots of the supernatant were mixed with glacial acetic acid and acidic ninhydrin, followed by incubation in boiling water. After rapid cooling, the mixture was vigorously extracted with toluene. The absorbance of the upper organic layer was then recorded at 520 nm against a toluene blank. The content of proline was expressed as µg g⁻¹ on a fresh-mass basis.

Relative electrolyte leakage: Relative electrolyte leakage (REL) was quantified following Johal *et al.* (2020). Washed and surface-dried leaf segments (0.05 g, ~0.5 cm in length) were incubated in 20 mL of deionized water (24 h, dark, room temperature) before recording the baseline conductivity (R₁). The same samples were then boiled for 30 min and cooled to measure the maximum conductivity (R₂). Membrane permeability was expressed as the percentage of initial to total leakage (REL = R₁/R₂ × 100%).

Malondialdehyde content: Following the protocol of Cai *et al.* (2019), lipid peroxidation was assessed by quantifying MDA content. Leaves (0.25 g) were extracted in 5% trichloroacetic acid (TCA), and the resulting supernatant was reacted with 0.67% thiobarbituric acid (TBA). After a 30-min heating period at 100°C and rapid cooling, the mixture was subjected to a second

centrifugation to clarify the solution. Absorbance values were measured spectrophotometrically at 450, 532, and 600 nm against a reagent blank. The content of MDA was expressed as nmol g^{-1} on a fresh-mass basis.

Hydrogen peroxide content: Based on the colorimetric assay by Velikova *et al.* (2000), leaf samples (0.3 g) were subjected to cold extraction in 3 mL of 0.1% TCA. The homogenate was centrifuged, and 700 μL of the resulting supernatant was reacted with KI in the presence of a phosphate buffer. The optical density was subsequently measured at 390 nm against a 0.1% TCA background to determine H_2O_2 content. H_2O_2 content was expressed in $\mu\text{mol g}^{-1}$.

Superoxide anion ($\text{O}_2^{\cdot-}$) content: The $\text{O}_2^{\cdot-}$ production was determined according to Liu *et al.* (2016). Briefly, 0.5 g of tissue was extracted in a 65 mM phosphate buffer (pH 7.8) and centrifuged. The cleared supernatant (2 mL) was incubated with hydroxylamine hydrochloride (25°C, 20 min). Subsequently, color development was induced by adding equivolumetric amounts of 17 mM sulfanilic acid and 7 mM α -naphthylamine. Following a 30-min incubation at 30°C, the optical density was measured spectrophotometrically at 530 nm. The content of $\text{O}_2^{\cdot-}$ was expressed as $\mu\text{g g}^{-1}$.

Photosynthetic pigment content: Leaf pigments were extracted and determined based on the method of Zhang *et al.* (2023). Midrib-free leaf segments (0.05 g) were macerated in 80% acetone and kept in total darkness until complete tissue bleaching was achieved. Spectrophotometric absorbances of the clarified extracts were recorded at 663 nm (chlorophyll *a*), 646 nm (chlorophyll *b*), and 470 nm (carotenoids) against an 80% acetone background. Photosynthetic pigment content was expressed in mg g^{-1} .

Gas-exchange parameters: Photosynthetic traits, encompassing net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration rate (E), were quantified using a LI-6400 analyzer (LI-COR, Lincoln, NE, USA) mid-morning (09:00–11:00 h) on sunny days. Measurements were performed under strictly controlled leaf chamber conditions: 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD), 380 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, 250 $\mu\text{mol s}^{-1}$ flow rate, and a 25°C leaf temperature. Five biological replicates per treatment were assessed.

Chlorophyll fluorescence parameters and 820-nm modulated reflectance: The *M-PEA* plant performance analyzer (Hansatech, United Kingdom) was used to obtain the fast chlorophyll *a* fluorescence transient (O–J–I–P) and modulated 820-nm reflectance data. All tested leaves underwent a 30-min dark-adaptation period before illumination with a 3,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ saturating pulse. Parameters including F_v/F_m , DI_0/RC , TR_0/RC , ET_0/RC , ABS/RC , $\Delta I/I_0$, and $\Phi_{\text{PSI/PSII}}$, along with additional yields and kinetic parameters (PI_{abs} , ϕE_0 , W_K , V_J , M_0 , *etc.*), were calculated according to Strasser *et al.* (2010). The relative variable fluorescence was determined using

the following equation: $V_t = (F_t - F_0)/(F_m - F_0)$ (Chang *et al.* 2023). Concurrently, the MR signal (MR/MR_0) driven by PSI oxidation was recorded under far-red illumination [$250 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$], where MR_0 represents the initial reflectance value at 0.7 ms. All assessments were performed with 10 independent biological replicates per treatment.

Root-bark secondary-metabolite contents: Total flavonoids were extracted and quantified according to Zhang *et al.* (2024). Fresh roots were washed, the woody medulla removed, and the remaining bark was oven-dried (105°C for 30 min, then 50°C to constant mass). The resulting powder was filtered using a 60-mesh screen. Flavonoids were extracted from the fine powder using hot ethanol (70°C). The clarified extract underwent sequential complexation with NaNO_2 , $\text{Al}(\text{NO}_3)_3$, and 4% NaOH. The absorbance of the chromogenic complex was recorded at 510 nm, and the content was calculated as mg g^{-1} dry mass (DM).

Total phenolics were determined via the Folin–Ciocalteu method (Feng *et al.* 2014). Briefly, the sample powder was subjected to ultrasonic extraction with 80% methanol. The resulting supernatant was reacted with Folin–Ciocalteu reagent, neutralized with Na_2CO_3 , and vigorously vortexed. Following color development, the spectrophotometric absorbance was recorded at 765 nm. Total phenolics content was expressed in $\text{mg g}^{-1}(\text{DM})$.

Six specific secondary metabolites (paeonol, paeoniflorin, gallic acid, benzoic acid, albiflorin, and catechin) were quantified using an Agilent 1260 High-Performance Liquid Chromatography (HPLC) system equipped with a Waters C18 column (4.6 mm $\times$ 250 mm, 5 μm) (Zhang *et al.* 2024). The mobile phase consisted of acetonitrile (solvent A) and 0.05% aqueous phosphoric acid adjusted to pH 2.7 (solvent B). The gradient elution program was configured as described below: 8–12% A within 0–5 min, 12–20% A from 5 to 20 min, maintained at 20% A during 20–25 min, 20–45% A in 25–35 min, kept 45% A for 35–40 min, and 45–8% A at 40–50 min. Following a 10 μL sample injection, compounds were resolved at a column temperature of 30°C (1.0 mL min^{-1}) and detected at 230 nm. The metabolite contents were ultimately calculated as $\text{mg g}^{-1}(\text{DM})$.

Data processing and analysis: All experimental data are expressed as the mean $\pm$ standard deviation (SD) of at least three independent biological replicates. Statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). To evaluate the statistical significance among different treatments, a one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test for post hoc comparisons. Differences were considered statistically significant at $P < 0.05$.

Results

Impact of MT on biochemical indices: Compared with unstressed conditions, controlled soil water deficit triggered noticeable surges in foliar SOD capacity and

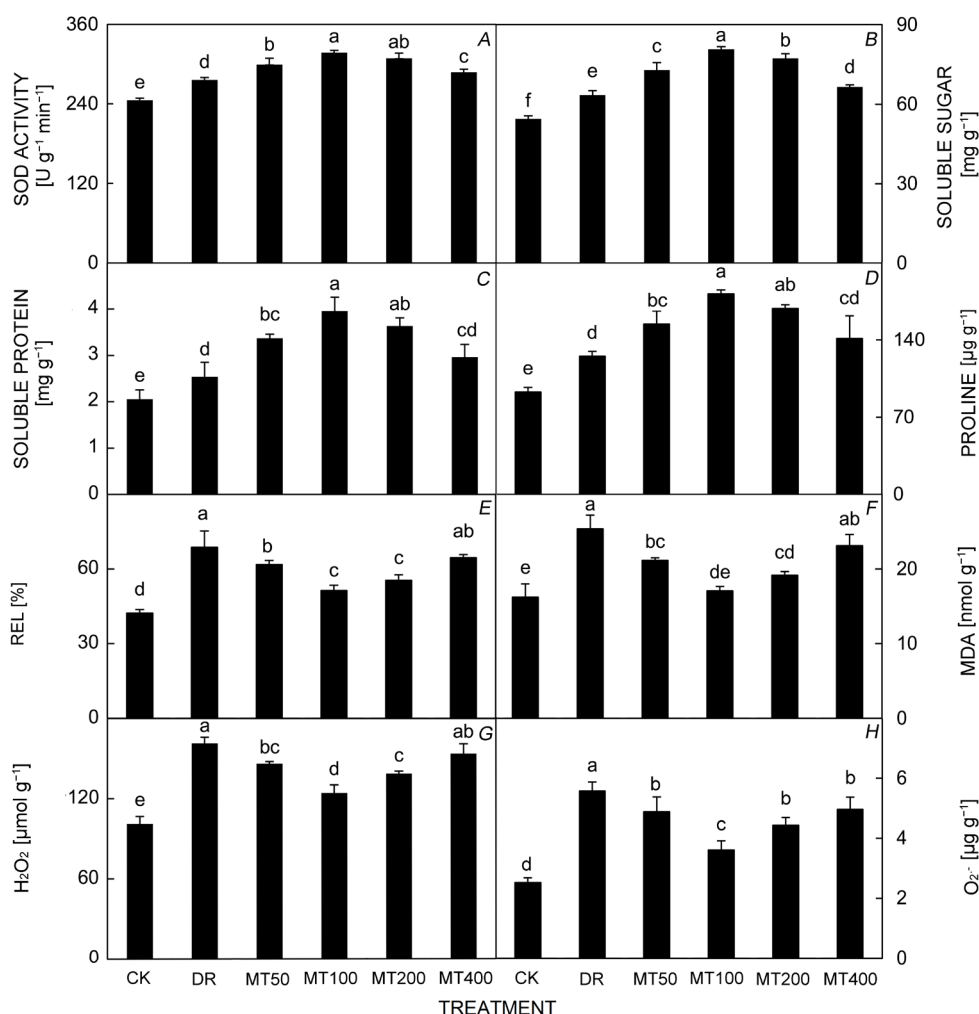


Fig. 1. Changes in SOD activity (A) and soluble sugar (B), soluble protein (C), proline (D), REL (E), MDA (F), H₂O₂ (G), and O₂⁻ (H) contents in *Paeonia ostii* leaves following different melatonin (MT) applications. Each value represents the mean $\pm$ SD ($n = 3$). SOD – superoxide dismutase; MDA – malondialdehyde; REL – relative electrolyte leakage. Distinct lowercase letters above the bars denote significant differences between treatments based on Duncan's multiple range test at $P < 0.05$. CK – Control; DR – Drought; MT50 – 50 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT100 – 100 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT200 – 200 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT400 – 400 $\mu\text{mol(MT)} \text{ L}^{-1}$.

osmoprotectant contents (Fig. 1). MT treatment further altered these traits relative to DR, with the largest responses generally observed at 100 $\mu\text{mol L}^{-1}$. Specifically, relative to the drought-only group, the 100 $\mu\text{mol(MT)} \text{ L}^{-1}$ treatment not only elevated SOD activity (+14.8%) but also drove dramatic accumulations of soluble sugars (+27.3%), soluble proteins (+55.9%), and proline (+45.2%).

Drought stress increased REL, MDA, O₂⁻, and H₂O₂ contents, indicating enhanced membrane injury and oxidative stress (Fig. 1). Exogenous application of melatonin robustly alleviated this drought-induced cellular damage. Specifically, peaking at a dosage of 100 $\mu\text{mol L}^{-1}$, the treatment markedly ameliorated the negative impacts of stress, with REL decreased by 25.4%, O₂⁻ by 23.1%, H₂O₂ by 35.3%, and MDA by 32.7%.

Pigment contents and photosynthetic performance indicators: The contents of chlorophyll *a* and chlorophyll *b* in *P. ostii* leaves were significantly reduced under drought

conditions in comparison with the control treatment, with carotenoids and total chlorophyll presenting the same trend (Fig. 2). After exogenous melatonin application, pigment concentrations went up at first and then decreased gradually. The peak values were recorded at 100 $\mu\text{mol L}^{-1}$ melatonin, with increases of 97.2, 115.8, and 39.2% for chlorophyll *a*, chlorophyll *b*, and carotenoids, respectively, compared with the DR treatment.

Compared with the controls, drought stress severely impaired leaf gas exchange, evidenced by marked declines in P_N , E , and g_s , together with an increase in intercellular CO₂ concentration (C_i) (Fig. 2). However, foliar application of melatonin effectively reversed these drought-induced physiological disruptions. Consistent with prior observations, the 100 $\mu\text{mol L}^{-1}$ dosage yielded the optimal recovery. Relative to the untreated drought-stressed plants, this specific treatment dramatically boosted P_N , E , and g_s . Simultaneously, the C_i level was significantly suppressed.

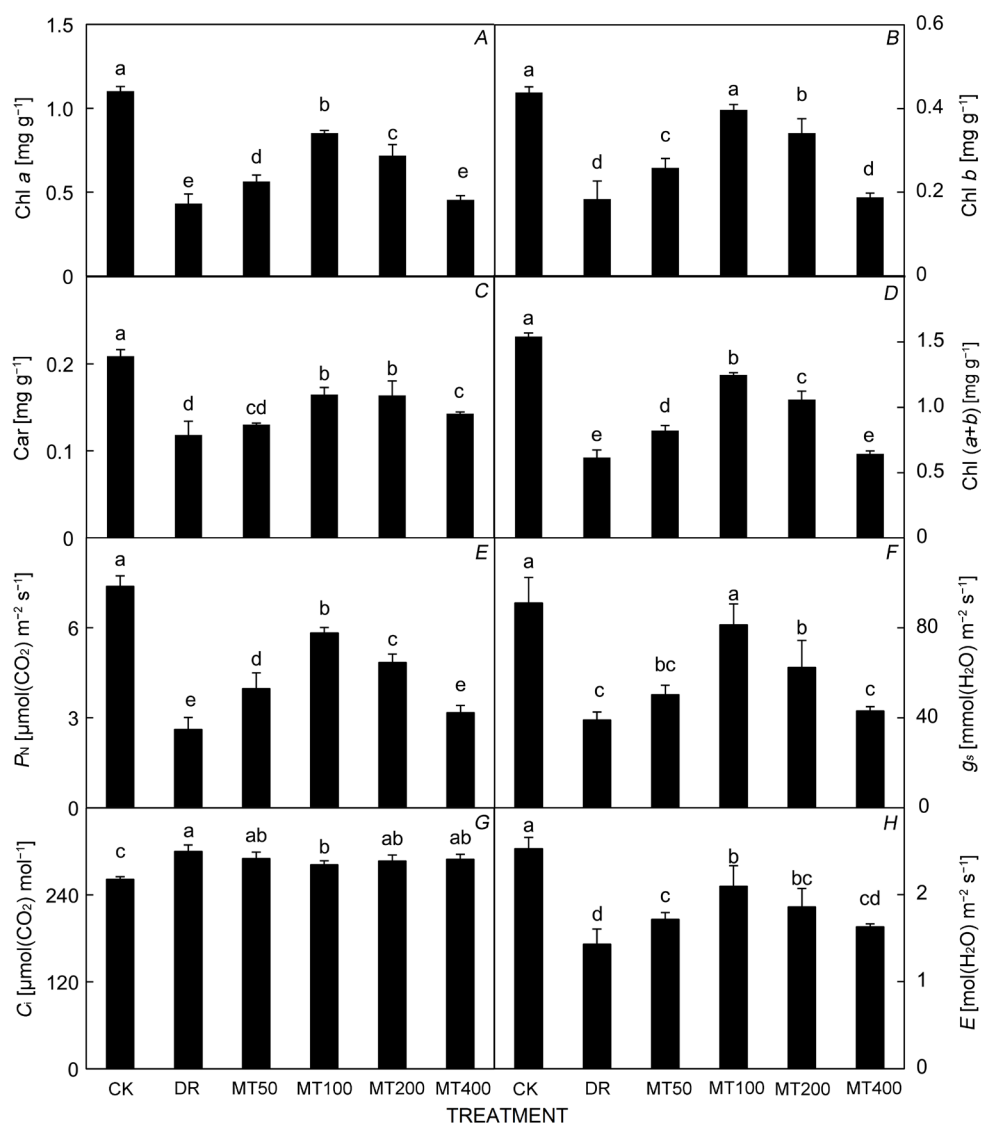


Fig. 2. Changes in Chl *a* (A), Chl *b* (B), Car (C), and total Chl (*a*+*b*) (D) contents, as well as P_N (E), g_s (F), C_i (G), and E (H), in leaves of *Paeonia ostii* under varying melatonin (MT) treatments. Chl – chlorophyll; Car – carotenoid; P_N – net photosynthetic rate; g_s – stomatal conductance; C_i – intercellular CO₂ concentration; E – transpiration rate. Values are shown as mean $\pm$ SD; $n = 3$ for photosynthetic pigment concentrations and $n = 5$ for photosynthetic parameters. Lowercase letters above the bars denote significant differences between treatments according to Duncan's multiple range test at $P < 0.05$. CK – Control; DR – Drought; MT50 – 50 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT100 – 100 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT200 – 200 $\mu\text{mol(MT)} \text{ L}^{-1}$; MT400 – 400 $\mu\text{mol(MT)} \text{ L}^{-1}$.

Chlorophyll fluorescence OJIP curves and 820-nm modulated reflectance curves: Kinetic analysis revealed drought markedly altered the shape of the OJIP transients, specifically manifested as amplified fluorescence signals at the K-step (0.3 ms) and J-step (2 ms) (Fig. 3). Parallely, the 820-nm modulated reflectance kinetics displayed a distinct elevation in the minimal MR/MR₀ ratio. Consistent with the quantitative JIP parameters, melatonin treatment effectively reversed these stress-induced kinetic variations. Among the concentrations tested, 100 $\mu\text{mol(MT)} \text{ L}^{-1}$ generally produced the greatest recovery in these transient signals.

Rapid Chl fluorescence kinetics, functional variation, and PSII-PSI cooperativity: Chlorophyll fluorescence

analysis indicated that water deficit severely compromised PSII electron transport, significantly increasing W_K , V_J , and M_0 while suppressing ϕE_0 compared to the unstressed group (Fig. 4). Foliar melatonin spraying dose-dependently reversed these adverse trends. Among the MT treatments, MT100 showed the lowest W_K , V_J , and M_0 values and the highest ϕE_0 value.

As depicted in Fig. 4, prolonged drought exposure induced a drastic reduction in F_v/F_m , PI_{abs} , $\Delta I/I_0$, and $\Phi_{PSI/PSII}$ relative to the unstressed group. These changes were consistent with impaired PSI- and PSII-related photochemical performance and altered coordination between the two photosystems. Foliar melatonin supplementation successfully alleviated this photochemical inhibition. The restorative impact was

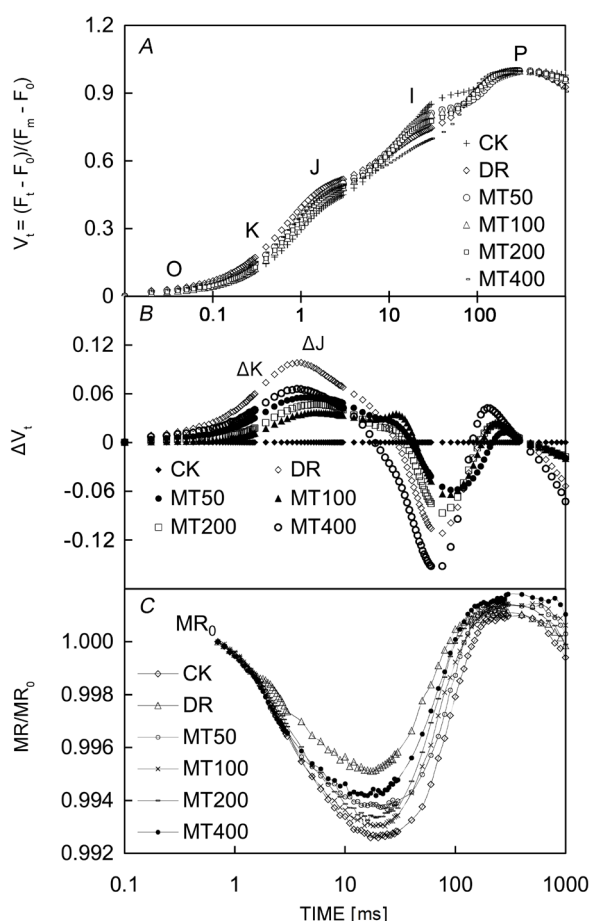


Fig. 3. V_t curves (A), ΔV_t curves (B), and 820-nm modulated reflectance (C) obtained from *Paeonia ostii* leaves under varied melatonin (MT) supplementation. V_t – the relative variable fluorescence at arbitrary time t , which is computed via the equation $V_t = (F_t - F_0)/(F_m - F_0)$; ΔV_t – the difference in V_t between a treatment and CK, calculated as $\Delta V_t = V_{t(\text{treat})} - V_{t(\text{CK})}$; MR/MR_0 – the normalized 820-nm modulated reflectance signal, where MR is the reflectance at a given time point, and MR_0 is the initial value under far-red light at 0.7 ms. CK – Control; DR – Drought; MT50 – 50 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT100 – 100 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT200 – 200 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT400 – 400 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$.

maximized at 100 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$, which significantly elevated all four parameters to their highest levels among the MT treatments.

As detailed in Table 1, drought and MT treatment altered several specific- and cross-sectional energy-flux parameters. The responses varied with MT concentration. MT100 showed lower ABS/RC , TR_0/RC , ϕD_0 , DI_0/RC , and DI_0/CS_m and higher RC/CS_m , ABS/CS_m , TR_0/CS_m , and ET_0/CS_m than DR, whereas ET_0/RC did not differ significantly among treatments.

Contents of total flavonoids, total phenolics, and secondary metabolites: As shown in Fig. 5, while water deficit alone stimulated the root accumulation of major phytochemicals (including phenolics, flavonoids, and specific monoterpene glycosides), foliar melatonin supplementation further robustly fortified this metabolic

accumulation. Compared with the drought treatment, the 100 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$ treatment increased total flavonoid and total phenolic contents by 48.1 and 36.1%, respectively. The contents of gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid, and paeonol increased by 17.7, 69.4, 69.9, 24.1, 7.7, and 22.7%, respectively. Conversely, the 50 and 400 $\mu\text{mol} \text{ L}^{-1}$ treatments elicited comparable, nonsignificant fluctuations for most compounds.

Discussion

Effects of melatonin on SOD activity and osmotic adjustment substance accumulation under drought conditions: In *P. ostii*, drought increased $\text{O}_2^{\cdot-}$, H_2O_2 , and MDA contents and elevated electrolyte leakage, indicating enhanced oxidative stress and membrane injury. However, exogenous melatonin robustly counteracted this drought-induced cellular toxicity. It was associated with two groups of responses: increased SOD activity and enhanced accumulation of osmolytes. First, melatonin fortified the enzymatic antioxidant machinery, particularly by upregulating SOD activity to detoxify superoxide (Ahmad *et al.* 2021). Second, it stimulated a massive accumulation of osmoprotectants, including soluble sugars, proteins, and proline (Altaf *et al.* 2022). Our findings perfectly align with previous results suggesting that these osmolytes, especially proline, function beyond simple osmotic adjustment; they act synergistically to scavenge hydroxyl radicals, stabilize membrane integrity, and ultimately safeguard PSII functionality against drought stress (Haider *et al.* 2017, Zhao *et al.* 2019, Moustafa-Farag *et al.* 2020).

Melatonin, a potent ROS scavenger with both lipophilic and hydrophilic properties, can penetrate membranes and organelles to eliminate ROS directly (Zhang *et al.* 2015). Notably, melatonin and its metabolic derivatives can trigger sequential cascade responses after neutralizing free radicals, sustaining the efficient scavenging of newly produced ROS over time (Galano *et al.* 2013). Exogenous melatonin application markedly enhanced SOD activity in *P. ostii* plants, accompanied by a substantial accumulation of key osmoprotectants (proline, soluble sugars, and soluble proteins). Additionally, melatonin intervention significantly suppressed intracellular ROS overproduction and MDA accumulation, while preventing electrolyte leakage. Collectively, these findings indicate that exogenous melatonin optimizes the antioxidant enzyme defense system and maintains cellular osmotic homeostasis in *P. ostii* leaves exposed to drought stress. The enhanced antioxidant and osmotic responses observed after MT application were associated with improved physiological performance under drought stress. Previous studies have reported that melatonin may affect drought-responsive, antioxidant-related, and secondary-metabolism-related genes through regulatory components such as dehydration-responsive element-binding (DREB) transcription factors, WRKY transcription factors, MYB transcription factors, and mitogen-activated protein kinase (MAPK) signaling in other plant species (Sharma *et al.* 2020, Muhammad *et al.* 2022). However, these molecular components were not examined in the present study, and their involvement

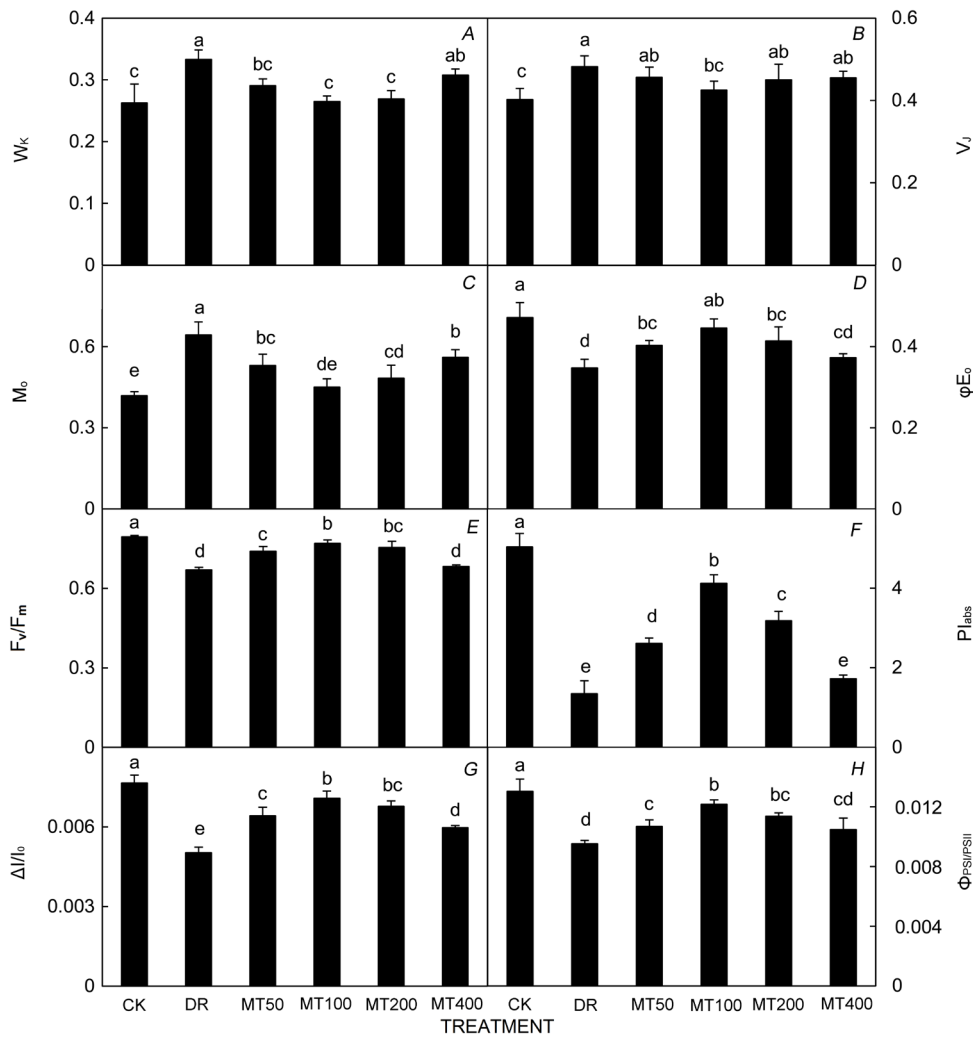


Fig. 4. Dynamic alterations of W_k (A), V_j (B), M_0 (C), ϕE_0 (D), F_v/F_m (E), PI_{abs} (F), $\Delta I/I_0$ (G), and $\Phi_{PSI/PSII}$ (H) in *Paeonia ostii* leaves after foliar melatonin (MT) application under drought conditions. W_k – the normalized relative variable fluorescence; V_j – the relative variable fluorescence intensity at the J step; M_0 – the initial slope of relative variable fluorescence, reflecting the relative rate of Q_A reduction; ϕE_0 – the quantum yield of electron transport; F_v/F_m – the maximum photochemical efficiency of PSII under dark adaptation; PI_{abs} – the performance index on an absorption basis; $\Delta I/I_0$ – the maximum redox activity of PSI; and $\Phi_{PSI/PSII}$ – the coordination between PSII and PSI. Values are shown as means $\pm$ SD ($n = 10$). Lowercase letters above the bars denote significant differences between treatments at $P < 0.05$, based on Duncan's multiple range test. CK – Control; DR – Drought; MT50 – 50 $\mu\text{mol(MT) L}^{-1}$; MT100 – 100 $\mu\text{mol(MT) L}^{-1}$; MT200 – 200 $\mu\text{mol(MT) L}^{-1}$; MT400 – 400 $\mu\text{mol(MT) L}^{-1}$.

Table 1. Alterations of energy fluxes in *Paeonia ostii* foliage via melatonin (MT) supplementation under drought. Values are shown as means $\pm$ SD. Duncan's test ($\alpha = 0.05$) was applied to evaluate treatment differences, and significant outcomes are denoted by different lowercase letters.

Parameter	CK	DR	MT50	MT100	MT200	MT400
ABS/RC	1.11 $\pm$ 0.14 ^c	1.66 $\pm$ 0.16 ^a	1.31 $\pm$ 0.02 ^b	1.14 $\pm$ 0.05 ^{bc}	1.19 $\pm$ 0.1 ^{bc}	1.50 $\pm$ 0.04 ^a
DI ₀ /RC	0.24 $\pm$ 0.03 ^c	0.55 $\pm$ 0.11 ^a	0.34 $\pm$ 0.02 ^b	0.26 $\pm$ 0.03 ^{bc}	0.29 $\pm$ 0.05 ^{bc}	0.47 $\pm$ 0.00 ^a
TR ₀ /RC	0.88 $\pm$ 0.10 ^c	1.11 $\pm$ 0.05 ^a	0.97 $\pm$ 0.04 ^{bc}	0.88 $\pm$ 0.03 ^c	0.90 $\pm$ 0.04 ^c	1.03 $\pm$ 0.03 ^{ab}
ET ₀ /RC	0.53 $\pm$ 0.11 ^a	0.57 $\pm$ 0.04 ^a	0.53 $\pm$ 0.02 ^a	0.51 $\pm$ 0.05 ^a	0.50 $\pm$ 0.08 ^a	0.56 $\pm$ 0.02 ^a
RC/CS _m	27,914.89 $\pm$ 5,592.17 ^a	14,240.67 $\pm$ 2,534.04 ^c	18,899.56 $\pm$ 444.44 ^{bc}	23,500.94 $\pm$ 1,700.65 ^{ab}	22,026.24 $\pm$ 505.52 ^b	15,212.47 $\pm$ 945.05 ^c
ϕD_0	0.21 $\pm$ 0.01 ^c	0.33 $\pm$ 0.03 ^a	0.26 $\pm$ 0.02 ^b	0.22 $\pm$ 0.01 ^c	0.25 $\pm$ 0.02 ^{bc}	0.32 $\pm$ 0.01 ^a
ABS/CS _m	30,556.67 $\pm$ 2,898.19 ^a	23,395.67 $\pm$ 2,152.25 ^{bc}	24,716.67 $\pm$ 935.23 ^{bc}	26,715.5 $\pm$ 788.10 ^b	26,219.67 $\pm$ 1,632.31 ^b	22,841.33 $\pm$ 1,949.34 ^c
DI ₀ /CS _m	6,453.33 $\pm$ 564.13 ^{bc}	7,658.67 $\pm$ 74.67 ^a	6,399 $\pm$ 229.59 ^{bc}	5,991 $\pm$ 403.82 ^c	6,452.67 $\pm$ 1,003.50 ^{bc}	7,225.67 $\pm$ 506.18 ^{ab}
TR ₀ /CS _m	24,103.33 $\pm$ 2,359.35 ^a	15,737.00 $\pm$ 2,191.93 ^c	18,317.67 $\pm$ 1,111.26 ^b	20,724.5 $\pm$ 387.94 ^b	19,767.00 $\pm$ 683.79 ^b	15,615.67 $\pm$ 1,451.61 ^c
ET ₀ /CS _m	14,352 $\pm$ 1,058.57 ^a	8,139 $\pm$ 1,136.36 ^c	9,947 $\pm$ 510.65 ^{cd}	11,904.75 $\pm$ 616.6 ^b	10,883.67 $\pm$ 1,551.54 ^{bc}	8,507 $\pm$ 833.78 ^{de}

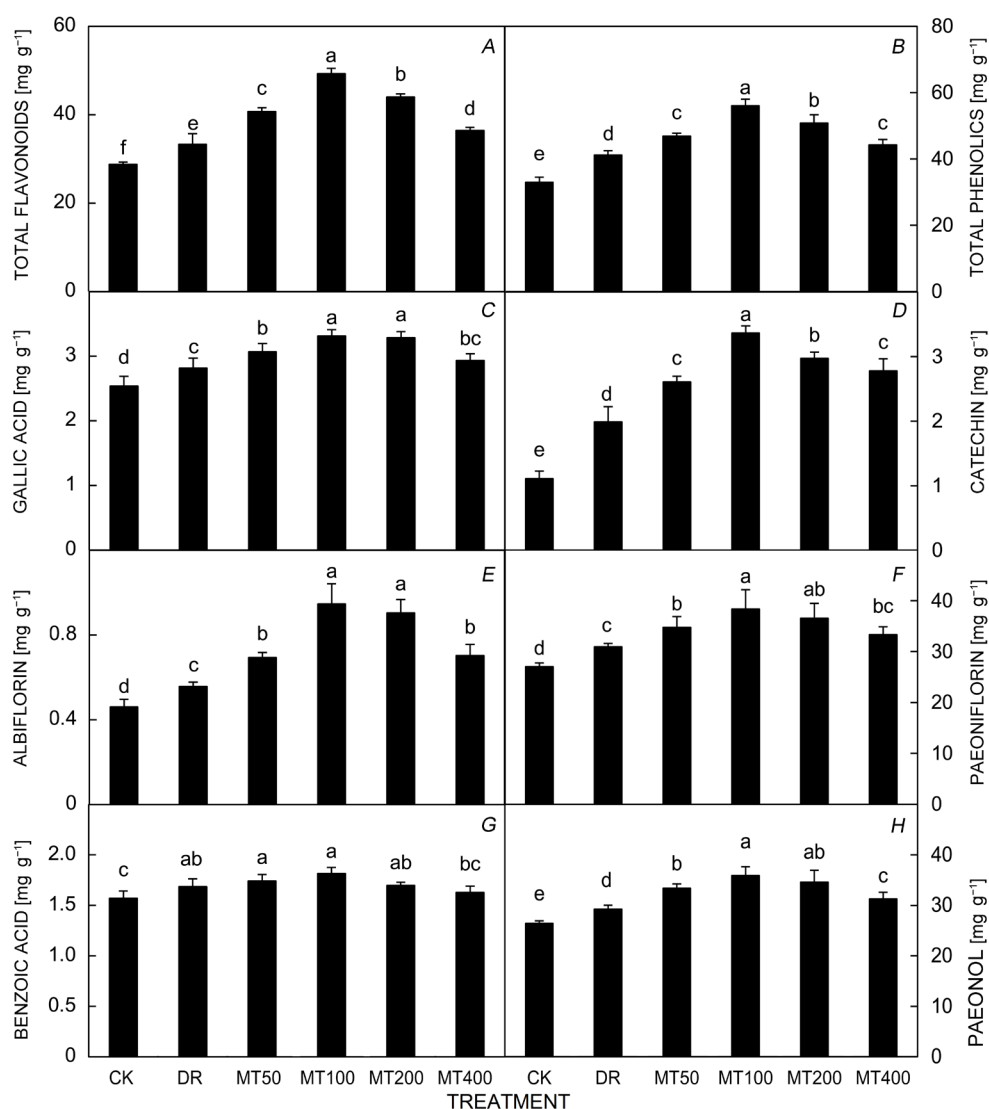


Fig. 5. Alterations in medicinal constituent accumulation in *Paeonia ostii* root bark after leaf-sprayed melatonin (MT) treatment under drought conditions. Total flavonoid (A), total phenolics (B), gallic acid (C), catechin (D), albiflorin (E), paeniflorin (F), benzoic acid (G), and paenol (H). Values are expressed as means $\pm$ SD ($n = 3$). Different lowercase letters over the bars indicate significant differences between treatments at $P < 0.05$ based on Duncan's multiple range test. CK – Control; DR – Drought; MT50 – 50 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT100 – 100 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT200 – 200 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$; MT400 – 400 $\mu\text{mol}(\text{MT}) \text{ L}^{-1}$.

in *P. ostii* remains unverified. Thus, their possible contribution to the observed changes in antioxidant activity and osmoprotectant accumulation should be tested through future transcriptomic, proteomic, enzymatic, or genetic analyses. Similar coordinated improvements in antioxidant enzymes (SOD, POD, CAT) and osmoprotectants have been reported in drought-stressed *Fagopyrum esculentum* following melatonin application (Tian *et al.* 2025), and are consistent with previous findings in *P. ostii* by Fan *et al.* (2020), further supporting the pivotal role of melatonin in enhancing the drought tolerance. Although these findings support a potentially conserved protective role of melatonin across plant species, the molecular components and regulatory relationships in *P. ostii* may differ from those reported in buckwheat, maize, or other species and require independent verification.

Variations of photosynthetic pigment contents in drought-stressed *P. ostii* with melatonin treatment:

The marked reductions in chlorophyll *a*, chlorophyll *b*, and carotenoids in drought-stressed *P. ostii* leaves might be associated with ROS overproduction. Excess ROS can promote pigment loss by damaging thylakoid membranes (Meng *et al.* 2014) and altering the transcriptional regulation of pigment metabolism, including the activation of degradation-related genes and repression of biosynthesis-related genes (Sezgin *et al.* 2019). However, MT treatment partially counteracted these drought-associated reductions, maintaining photosynthetic pigment contents under water deficit. Similar protective mechanisms have been well documented in other plant species, where melatonin stabilizes the chloroplast apparatus by downregulating key senescence and catabolic genes such as pheophorbide *a*

oxygenase (*PAO*) and chlorophyllase (*CHLASE*), and stimulating pigment synthesis pathways during abiotic stress (Zhang *et al.* 2015, Sharma *et al.* 2020). The greater retention of photosynthetic pigments in MT-treated plants coincided with lower ROS and MDA contents. These concurrent responses suggest a possible association between reduced oxidative stress and pigment stability, although pigment biosynthesis and degradation were not directly measured.

Alterations in leaf gas exchange and OJIP chlorophyll fluorescence traits triggered by exogenous melatonin under water deficit: The concurrent decrease in net photosynthetic rate (P_N) and stomatal conductance (g_s) alongside an elevated intercellular CO_2 concentration (C_i) suggests that nonstomatal limitations may have contributed substantially to the decline in photosynthesis. When C_i remains sufficient, such photosynthetic inhibition typically reflects direct impairment of the photosynthetic apparatus and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity rather than mere CO_2 restriction (Campos *et al.* 2019). MT treatment increased P_N and reduced C_i relative to DR, a response consistent with partial alleviation of nonstomatal limitations.

Fluctuations in JIP-test parameters (elevated W_K , V_j , M_0 , and reduced ϕE_0) were consistent with impaired donor-side function and restricted electron transport beyond Q_A . Such impairment of photosynthetic electron transport has been proposed to increase the likelihood of electron leakage to oxygen, potentially contributing to ROS formation and oxidative membrane damage (Chalanika De Silva and Asaeda 2017). Although electron leakage itself was not directly measured, the concurrent changes in ROS contents, MDA content, and electrolyte leakage are consistent with oxidative stress and membrane damage. Conversely, lower W_K and V_j and higher ϕE_0 in MT-treated plants indicated less severe impairment of donor-side function and electron transport beyond Q_A , which aligns with observations in maize (Guo *et al.* 2020). By upregulating antioxidant enzyme activities (Fan *et al.* 2020) and chemically quenching free radicals, melatonin shields essential components such as the PSII reaction-center D1 protein from oxidative degradation (Moustaka *et al.* 2024).

Drought stress increased ABS/RC , DI_0/RC , TR_0/RC , and ET_0/RC and decreased RC/CS_m in *P. ostii* leaves, suggesting that water deficit inactivated a substantial proportion of PSII reaction centers. The resulting redistribution of excitation energy to the remaining active centers promoted light harvesting and energy trapping per RC (Chang *et al.* 2025), but the overall energy-processing capacity at the cross-section level was still markedly reduced because of the substantial loss of functional RCs. To prevent photoinhibition under restricted electron flow, stressed leaves dissipated surplus excitation energy as heat, reflected by the marked rise in thermal dissipation parameters (DI_0/RC , DI_0/CS_m , and ϕD_0). Melatonin application normalized this energy imbalance. In MT-treated leaves, higher RC/CS_m was accompanied by lower energy flux per active reaction center and

lower dissipation parameters, indicating that melatonin effectively distributed the energy load, minimized wasteful thermal dissipation, and restored overall photon capture and electron transport performance. This regulatory pattern aligns with the optimization of photosynthetic energy flow reported in *Carya cathayensis* (Sharma *et al.* 2020), demonstrating that melatonin can sustain light-energy conversion efficiency in *P. ostii*. The responses observed in *P. ostii* were broadly consistent with melatonin-associated improvements in PSII performance and reductions in oxidative damage reported in maize (Guo *et al.* 2020) and pecan (*C. cathayensis*) (Sharma *et al.* 2020). However, these cross-species parallels provide comparative context only and do not demonstrate that identical mechanisms operate in *P. ostii*. Species-specific differences in photosynthetic regulation, antioxidant capacity, melatonin metabolism, and drought sensitivity may limit direct extrapolation to tree peony.

Effects of melatonin on function and coordination of PSII and PSI: Efficient photosynthesis relies on the functional integrity and synchronized electron transfer between PSII and PSI (Sharma *et al.* 2020). In our study, drought-induced photoinhibition in *P. ostii* was clearly manifested by drastic declines in F_v/F_m and PI_{abs} , two highly sensitive indicators of PSII photochemical performance and energy trapping (Liu *et al.* 2024, Ren *et al.* 2025). Under drought stress, the changes in $\Delta I/I_0$ and $\Phi_{PSI/PSII}$ were consistent with altered coordination between PSI- and PSII-related photochemical processes. Their partial recovery following MT treatment, together with improvements in other photosynthetic parameters, suggests that MT treatment was associated with better balance between the two photosystems. However, intersystem electron flux and PSI acceptor-side limitation were not directly measured; therefore, the proposed effect on photosynthetic electron transport requires further verification.

Following MT treatment, concurrent improvements in gas exchange, chlorophyll fluorescence, and 820-nm modulated reflectance showed that the recovery of net photosynthetic rate (P_N) coincided with improved PSI- and PSII-related photochemical performance. These parallel responses suggest a possible association between photosystem coordination and P_N recovery, although the present data do not establish a direct causal relationship.

Effects of melatonin on secondary metabolites in *P. ostii* under drought: Secondary metabolites are indispensable for plant adaptation to abiotic stresses, acting as crucial determinants for both stress resistance and medicinal quality (Kumar *et al.* 2021). In our study, water deficit significantly enriched diverse bioactive compounds in *P. ostii* root bark, including total phenolics, flavonoids, gallic acid, catechin, benzoic acid, paeonol, paeoniflorin, and albiflorin. This inherent metabolic shift primarily relies on the activation of the phenylpropanoid pathway, a core metabolic route highly responsive to dehydration (Dias *et al.* 2022). The stress-induced accumulation of these components closely parallels previous observations

in tree peony, where drought triggered the upregulation of upstream biosynthesis genes [such as phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), and chalcone synthase (*CHS*)] to sustain cellular homeostasis (Zhao *et al.* 2019).

Interestingly, exogenous melatonin did not merely preserve baseline metabolism but actively amplified this stress-responsive chemical defense, pushing concentrations of these medicinal components well beyond levels induced by drought alone. As a systemic signaling molecule, melatonin is known to interact with transcription factor networks [e.g., *MYB* and basic helix–loop–helix (*bHLH*) transcription factors] to stimulate secondary metabolism (Jafari and Shahsavar 2021). The increases in phenolic and flavonoid contents observed in this study were consistent with previous reports of melatonin-associated changes in phenylpropanoid metabolism in other plant species. *PAL* regulation has been reported in grape and hickory (Xu *et al.* 2017, Sharma *et al.* 2020), providing comparative context for the present observations. However, as *PAL* expression, enzyme activity, and metabolic flux were not measured in the present study, whether similar regulatory processes occur in *P. ostii* requires further investigation. The increased accumulation of monoterpene glycosides, including paeoniflorin and albiflorin, suggests possible changes in terpenoid-related metabolism following MT treatment. However, because precursor abundance, pathway-specific gene expression, enzyme activity, and metabolic flux were not measured, the involvement of the mevalonate (MVA) pathway or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway cannot be determined from the present data and requires further investigation.

In the present study, the increases in paeonol, paeoniflorin, albiflorin, gallic acid, benzoic acid, catechin, and the measured total phenolic and flavonoid contents indicate changes in the chemical composition of the root bark of *P. ostii*. Whether these compositional changes directly reflect enhanced pharmacological activity or medicinal value remains to be further confirmed. Subsequent studies incorporating bioactivity evaluation, interactions among constituents, and bioavailability assessment would help clarify this question more comprehensively.

Concentration-dependent responses to melatonin:

The promotive effects of MT were clearly concentration-dependent, but not in a simple dose–response manner. Of all the concentrations examined, 100 $\mu\text{mol L}^{-1}$ gave the greatest and most consistent improvements across the measured traits, while the responses at 400 $\mu\text{mol L}^{-1}$ were noticeably weaker for several parameters. This pattern indicates that increasing MT concentration beyond a certain point does not necessarily enhance its effectiveness under the conditions of this experiment. The reason for the diminished response at the highest concentration cannot be determined here, since we did not assay direct phytotoxicity markers, endogenous MT contents, hormonal crosstalk, or redox-regulatory pathways. It should also be noted that no concentrations below 50 $\mu\text{mol L}^{-1}$ were included, so we cannot rule out the possibility that even lower doses might perform as well or better, particularly

under milder stress or different experimental settings. Accordingly, 100 $\mu\text{mol L}^{-1}$ melatonin produced the most pronounced responses among the tested concentrations, but this is not necessarily a universal optimum. Further work employing a wider and more finely spaced concentration gradient will be required to establish the effective dose range and to clarify why higher concentrations become less effective.

Study limitations and future perspectives: Several limitations should be considered when interpreting these findings. The study relied mainly on physiological, biochemical, chlorophyll-fluorescence, and targeted metabolite measurements. These results support a beneficial role of exogenous MT in the drought response of *P. ostii* ‘Fengdan’, but they do not establish the underlying molecular mechanisms. Because gene expression, protein abundance, the activities of key enzymes involved in secondary-metabolite biosynthesis, transcription-factor activity, endogenous hormone contents, and pathway intermediates were not examined, the proposed involvement of *MYB*-related regulation, MAPK signaling, *PAL*-mediated phenylpropanoid metabolism, and MVA-dependent terpenoid biosynthesis remains hypothetical and requires direct validation in *P. ostii*. The experiment was also limited to four-year-old potted ‘Fengdan’ plants exposed to the controlled drought treatment and foliar MT application. Responses may vary among developmental stages, cultivars, and genotypes, as well as under field conditions with different soil, temperature, light, and water regimes. Moreover, only a limited range of MT concentrations and application schedules was tested. Although 100 $\mu\text{mol L}^{-1}$ produced the strongest responses under the conditions used here, its effectiveness under other conditions and the broader effective dose range remain to be determined. The increased concentrations of selected root-bark secondary metabolites reflect changes in chemical composition but do not by themselves demonstrate improved pharmacological efficacy, bioavailability, therapeutic value, yield, or commercial medicinal quality. These implications should therefore be interpreted cautiously. Future research should integrate molecular and enzymatic analyses with endogenous hormone measurements, comprehensive metabolite profiling, and direct bioactivity assays. Multi-season, multi-location field trials using different cultivars will also be needed to determine the reproducibility and practical value of MT application.

Conclusion: Under the conditions tested, drought increased oxidative-stress and membrane-injury indicators and impaired gas exchange and PSI- and PSII-related photochemical performance in four-year-old potted *P. ostii* ‘Fengdan’. However, foliar application of 100 $\mu\text{mol L}^{-1}$ melatonin coincided with lower oxidative damage, enhanced antioxidant enzyme activities, and improved osmotic adjustment. The greater antioxidant capacity observed in MT-treated plants coincided with lower ROS accumulation, greater pigment retention,

and improved PSI- and PSII-related photochemical performance. Beyond physiological rescue, foliar MT treatment was associated with increased root-bark concentrations of several phenolic compounds and monoterpene glycosides. In summary, under the conditions tested, foliar MT treatment resulted in reduced drought-induced physiological injury, improved PSI- and PSII-related photochemical performance, and increased accumulation of several medicinally relevant secondary metabolites in the root bark of pot-grown *P. ostii* 'Fengdan'. Among the concentrations tested, 100 $\mu\text{mol L}^{-1}$ produced the strongest overall response. These findings were obtained from a single controlled drought experiment using four-year-old potted plants; therefore, the effectiveness of this treatment across other developmental stages, cultivars, drought regimes, and field conditions requires further validation.

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