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RETRACTED: The Transcriptome and Metabolome Reveal the Mechanism by Which Melatonin Enhances Drought Tolerance in *Platycrater argutae*

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Abstract

Drought stress severely impacts the survival of *Platycrater arguta*, an endangered tertiary relict plant. This study investigated the mechanism by which exogenous melatonin enhances drought tolerance in *P. arguta* seedlings through integrated physiological, transcriptomic, and metabolomic analyses. Under 30% PEG-6000-induced drought, seedlings exhibited leaf wilting, reduced relative water content (RWC: 78.6% vs. 84.8% in controls), and elevated oxidative damage (malondialdehyde increased by 62.94%, H_2O_2 by 83.78%). Exogenous melatonin application, particularly at 100 μM (T3), significantly alleviated drought symptoms, restoring RWC to 83.7%, reducing membrane permeability (relative electrical conductivity 1.38-fold vs. CK vs. 2.55-fold in PEG), and lowering oxidative markers (MDA and H_2O_2 accumulation by 28.33% and 27.84%, respectively). T3 treatment also enhanced osmolyte synthesis, increasing proline content by 90.14% and soluble sugars by 47.25% compared to the controls. Transcriptome sequencing revealed 31,870 differentially expressed genes in T3, predominantly enriched in carbohydrate metabolism, oxidative phosphorylation, and tryptophan metabolism pathways. Metabolomic profiling demonstrated that 100 μM melatonin elevated endogenous melatonin levels 19.62-fold and modulated tryptophan-derived metabolites, including indole derivatives and benzoic acid compounds. These findings indicate that melatonin mitigates drought stress by enhancing antioxidant capacity, osmotic adjustment, and metabolic reprogramming, with 100 μM as the optimal concentration. This study provides critical insights into melatonin-mediated drought resistance mechanisms, offering a theoretical foundation for conserving and restoring *P. arguta* populations in arid habitats.

Keywords: *Platycrater argutae*; drought relief; melatonin; tryptophan

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1. Introduction

As a critically endangered tertiary relic, the deciduous shrub *Platycrater arguta* (Hydrangeaceae) faces survival challenges due to combined pressures from biotic and abiotic stress factors. This species is the sole extant member of its genus [1,2], representing a unique lineage with significant evolutionary and conservation value. Studying such relic species offers insights into historical biogeography, adaptation mechanisms, and the impacts of contemporary climate change on vulnerable biodiversity. Among these, drought stress

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stands as one of the most prevalent abiotic stressors in natural ecosystems and serves as a critical environmental factor influencing plant growth and development [3–5]. For *P. arguta*, whose current fragmented distribution is closely linked to specific microclimates, particularly water availability, understanding drought tolerance mechanisms is paramount for its survival and effective conservation.

Drought stress directly impacts plant physiological processes by disrupting photosynthetic characteristics, reactive oxygen species (ROS) metabolism, and osmotic regulation [4,6,7]. These disruptions lead to cellular dehydration, plasmolysis, and damage to cell membranes and enzyme systems, ultimately resulting in metabolic imbalance, growth inhibition, and reduced productivity. Studies have demonstrated that water availability is a pivotal determinant of *P. arguta*'s geographical distribution [1]. Plants deploy complex physiological and molecular responses to counter drought, including osmotic adjustment, enhanced antioxidant defense, and stress signaling pathways.

Melatonin (MT), an indoleamine compound ubiquitously present in animals and plants, functions both as an endogenous phytohormone synthesized by plants and as an exogenous regulator absorbed and accumulated from the environment [8–10]. Melatonin, a highly effective scavenger of free radicals and secondary antioxidant, is essential for mediating stress responses in plants [11,12]. It modulates multiple metabolic pathways to alleviate damage caused by diverse abiotic stresses, including heavy metal toxicity, drought, and extreme temperatures [13–15].

Focusing on the threatened relict species *P. arguta*, this study specifically investigates the efficacy of exogenous melatonin in mitigating drought stress in this vulnerable plant. Given its unique phylogenetic position and high conservation urgency, understanding its stress response mechanisms is ecologically significant. The findings could inform conservation strategies not only for *P. arguta* but potentially for other endangered species facing similar climatic pressures. This study elucidates the protective mechanisms of melatonin supplementation (100~200 μM) against water deficit-induced alterations in biomass allocation, photosynthetic efficiency, and redox homeostasis in *P. arguta* juveniles. The findings will provide critical insights into whether exogenous melatonin mitigates drought-induced stress damage in *P. arguta*, while establishing a theoretical foundation for enhancing the conservation and field population expansion of this endangered species.

2. Materials and Methods

2.1. Experimental Materials and Treatments

Two-year-old *P. arguta* seedlings with uniform growth and no signs of pests or diseases were selected for this study. The experiment was conducted in a controlled environment at the Wenzhou Jingshan Plant Resource Nursery, with temperatures maintained between 16 °C and 29 °C. Drought stress was induced by irrigating each pot daily with 100 mL of 30% polyethylene glycol 6000 (PEG-6000, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) solution. To prevent leakage, the pots were placed in blue plastic trays. Prior to the experiment, the seedlings were thoroughly watered for three days and subjected to no further irrigation during the trial. Six treatments were established, each with five pots and three biological replicates:

(1) CK: normal watering;
(2) T1: drought stress (PEG);
(3) T2: drought stress + 50 μM melatonin (PEG + MT50)
(4) T3: drought stress + 100 μM melatonin (PEG + MT100)
(5) T4: drought stress + 200 μM melatonin (PEG + MT200)

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Melatonin solutions (Merck KGaA, Darmstadt, Germany) were uniformly sprayed onto both leaf surfaces using a fine mist sprayer at 17:00–18:00 for five consecutive days before drought induction. The control groups received equivalent volumes of distilled water.

On the 10th day of treatment, fully expanded functional leaves (3rd–4th from the apex of current-year branches) were collected at 7:30 AM, sealed with parafilm, and immediately transported to the laboratory. Fresh leaves were used to measure cell membrane permeability, chlorophyll content, relative water content, and leaf mass per area. The remaining samples were stored at −80 °C for metabolomic, transcriptomic.

2.2. Determination of Physiological Indicators

The measurements of MDA, H_2O_2, SS, and Pro all require the use of crude enzyme extract. The preparation method for crude enzyme extract is as follows: The veins of *P. arguta* leaves were removed, and the leaves were cut into small pieces. One gram of the leaf pieces was placed in a pre-cooled mortar and ground into a homogenate under liquid nitrogen. The homogenate was then poured into a test tube, and nine milliliters of phosphate buffer solution with a concentration of 0.1 mol·L^{-1} and a pH of 7 was added. The centrifuge was set to a temperature of 4 °C and a speed of 10,000 r/min, and the mixture was centrifuged for 10 min. After the mixture was stratified, the supernatant, which served as the crude enzyme extract, was collected and stored in a refrigerator at 4 °C for future use.

The MDA content was measured using the thiobarbituric acid (TBA) colorimetric method [16]. The SS content was determined using the anthrone colorimetric method [17]. The Pro content was assayed using the acidic ninhydrin colorimetric method [18].

2.3. Transcriptomic Sequencing and Analysis

Total RNA was extracted from frozen leaf tissues using MJzol Reagent (Magen, Shanghai, China) and purified using magnetic bead-based methods. RNA quality was assessed using a Nanodrop2000 (NanoDrop Technologies LLC, Waltham, MA, USA), agarose gel electrophoresis, and an Agilent 2100 Bioanalyzer (RIN $\geq$ 7.0) (Agilent, Santa Clara, CA, USA). Following poly(A) mRNA selection using oligodeoxythymidine-coated magnetic beads, RNA fragments of ~300 nucleotides were generated through chemical fragmentation for subsequent cDNA synthesis. Library preparation was performed with the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs (Beijing) Ltd., Beijing, China), followed by paired-end sequencing (2 × 150 bp) on an Illumina NovaSeq 6000 sequencing system (BGI, Shenzhen, China).

Raw sequencing data underwent quality control filtering prior to genome alignment via HISAT2 (v2.2.1) against the GRCh38 reference assembly. Differentially expressed genes (DEGs) were identified using DESeq2 (v1.30.1) based on read counts mapped to genes. Genes were defined as DEGs using the default significance thresholds: a false discovery rate (FDR) < 0.05 and an absolute $\log_2$ fold change > 1.0 (equivalent to a fold change > 2.0 or < 0.5).

GO term enrichment analysis for gene sets was performed using Goatools (v1.2.3). Fisher's exact test was employed, and GO terms with an adjusted *p*-value < 0.05 were considered significantly enriched. KEGG pathway enrichment analysis was conducted using KOBAS (v3.0) with similar statistical principles to the GO analysis. Fisher's exact test was applied, followed by multiple testing corrections using the Benjamini–Hochberg (BH) procedure (FDR) to control the false positive rate. KEGG pathways with a corrected *p*-value (FDR) < 0.05 were defined as significantly enriched within the DEGs.

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2.4. Metabolomic Profiling

Cryopreserved *P. arguta* leaf samples were pulverized into fine powder using a grinding mill (30 Hz, 1 min). Aliquots (50 mg) of homogenized tissue were mixed with 10 µL of internal standard mixture solution (100 ng/mL) and 1 mL of extraction solvent (methanol/water/formic acid, 15:4:1, $v/v/v$). After vortexing for 10 min, the mixtures were centrifuged at 12,000 rpm for 5 min (4 °C). The supernatants were transferred to new tubes, concentrated under reduced pressure, and reconstituted in 100 µL of 80% methanol/water. Finally, the samples were filtered through 0.22 µm membranes into HPLC vials for LC-MS/MS analysis.

Chromatographic separation was performed on a Waters ACQUITY UPLC HSS T3 (Jitai Biotechnology Co., Ltd., Shanghai, China) C18 column (1.8 µm, 100 × 2.1 mm i.d.) maintained at 40 °C. The mobile phase consisted of (A) ultrapure water with 0.04% acetic acid and (B) acetonitrile with 0.04% acetic acid. A gradient elution program was applied at 0.35 mL/min as follows: 0–1.0 min, 95% A; 1.0–8.0 min, 95%→5% A; 8.0–9.0 min, 5% A; 9.0–9.1 min, 5%→95% A; 9.1–12.0 min, 95% A. The injection volume was 2 µL.

Analysis was conducted on a Q-Trap 6500+ system equipped with an electrospray ionization (ESI) source. Key parameters included the following: ESI temperature 550 °C; ion spray voltage 5500 V (positive mode) or −4500 V (negative mode); curtain gas (CUR) 35 psi. Compound-specific detection was achieved through multiple reaction monitoring (MRM) using optimized declustering potentials (DP) and collision energies (CE) for each ion transition.

Qualitative identification was performed against the MetWare Database (MWDB) established using authentic standards. Quantitative analysis was conducted in MRM mode with triple quadrupole detection. Chromatographic peaks of all target compounds were integrated, and concentrations were determined using standard calibration curves.

2.5. Data Analysis and Statistical Methods

Data were processed using Microsoft Excel 2010. One-way analysis of variance (ANOVA) was conducted to assess the significance of differences across various indices. Figures were generated using Origin 2020. A comprehensive approach involving Duncan's method, Tukey's HSD test, and the aov function from the R package agricolae (v1.3-7) was employed for multiple comparisons and ANOVA.

3. Results

3.1. Effects of Exogenous Melatonin on the Phenotype of P. arguta

Under normal watering conditions (CK), plants exhibited healthy growth with normal leaf color and no signs of wilting. In contrast, all seedlings subjected to 30% PEG-6000 treatment displayed varying degrees of leaf wilting. Exogenous melatonin application at different concentrations differentially alleviated drought-induced wilting, resulting in distinct phenotypic responses. Among the treatments, 100 nM melatonin (PEG+MT100) showed the most significant mitigation effect, followed by 200 nM (PEG+MT200), while 50 nM (PEG+MT50) provided minimal relief. Specifically, leaves treated with 100 nM melatonin displayed slight wilting and curling at the margins, with limited affected areas. At 200 nM, the leaves exhibited curling but no wilting or drooping. In contrast, seedlings treated with 50 nM melatonin experienced severe leaf wilting, desiccation, and even abscission, accompanied by pronounced water loss (Figure 1).

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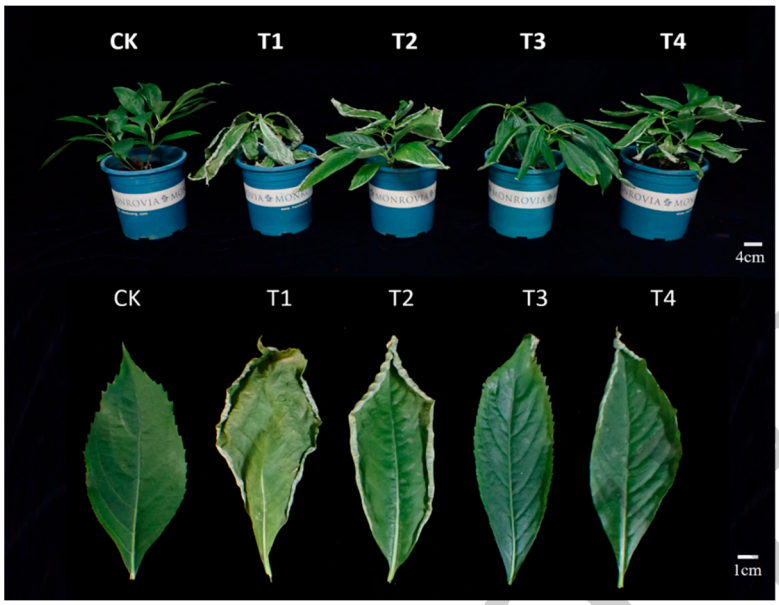
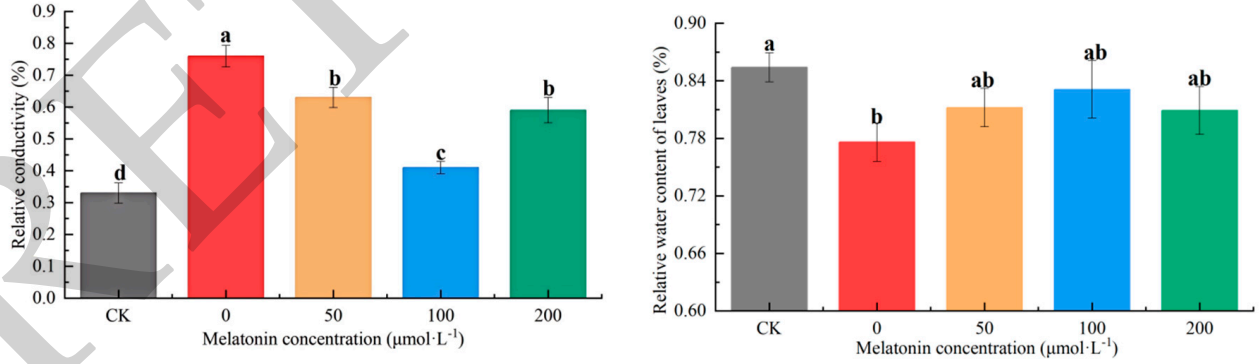


Figure 1. The hydrotoponic response of *P. arguta* to PEG-6000-induced osmotic perturbation was characterized through comprehensive morphometric analysis under whole-basin growth conditions.

After 10-day drought exposure, leaf RWC—a key parameter reflecting cellular hydration status—showed a progressive decline across experimental groups. The most severe dehydration occurred in PEG-treated specimens (78.6% RWC), representing a 7.3% reduction compared to well-watered controls (CK: 84.8%). Exogenous melatonin application significantly attenuated this dehydration effect, with 100 μM demonstrating superior efficacy (83.7% RWC) over both lower (50 μM: 81.4%) and higher (200 μM: 80.4%) concentrations in maintaining membrane-bound water (Figure 2a). Relative electrical conductivity (REC), reflecting cell membrane damage, increased substantially in drought-stressed plants. The untreated PEG group reached 76.6% REC, 2.55-fold higher than the CK group. In contrast, REC values for 50, 100, and 200 μM melatonin treatments were 2.10-, 1.38-, and 2.07-fold higher than CK, respectively. These results demonstrate that exogenous melatonin application significantly reduced membrane damage, with 100 μM showing the most pronounced protective effect (Figure 2b).



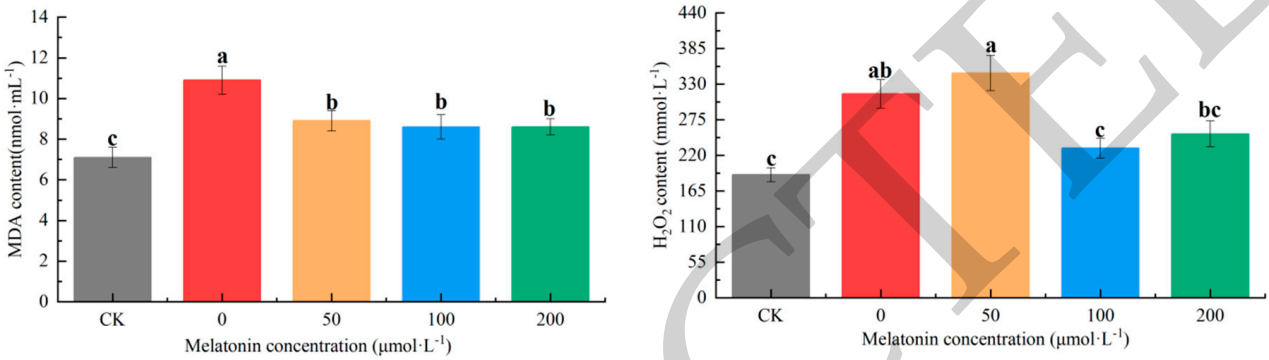
(**a**) (**b**)

Figure 2. Dose-dependent effects of exogenous melatonin on leaf physiological parameters: (**a**) relative electrolyte leakage (REC) and (**b**) relative water content (RWC) across MT concentration gradients. Alphabetic superscripts (a–d) denote statistically distinct clusters identified through Tukey's HSD test ($\alpha = 0.05$), where non-overlapping letters reflect membrane integrity–water retention coordination under osmotic stress.

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3.2. Effects of Exogenous Melatonin on Peroxide Accumulation in P. arguta

As the final byproduct generated through polyunsaturated fatty acid peroxidation in cellular membranes, malondialdehyde (MDA) concentration directly reflects the degree of oxidative damage in biological systems. In this experiment, the CK group exhibited a significantly lower MDA content compared to all other treatments. The untreated drought-stressed group (0 μM melatonin) showed a 62.94% increase in MDA relative to CK, while 50, 100, and 200 μM melatonin treatments resulted in 33.78%, 28.33%, and 28.47% increases, respectively. Notably, MDA levels in melatonin-treated groups showed no significant differences among concentrations, with values intermediate between CK and untreated drought-stressed plants (Figure 3a).



(**a**) (**b**)

Figure 3. (**a**) MDA content (reflecting membrane oxidative damage); (**b**) H_2O_2 accumulation across all experimental groups. Alphabetic superscripts (a–c) denote statistically distinct clusters identified through Tukey's HSD test ($\alpha = 0.05$), where non-overlapping letters reflect membrane integrity–water retention coordination under osmotic stress.

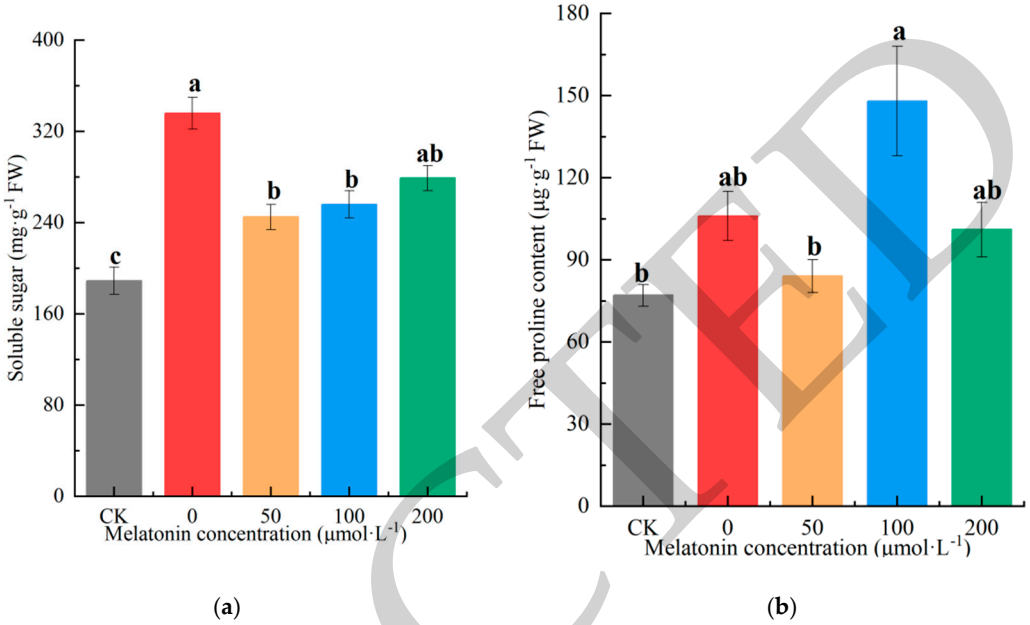
Hydrogen peroxide (H_2O_2) content, which rises under drought stress and induces cytotoxicity at elevated levels, was significantly lower in the CK- and 100 μM melatonin-treated groups compared to other treatments. The 200 μM melatonin group showed moderate H_2O_2 accumulation, while the untreated (0 μM) and 50 μM melatonin groups exhibited the highest levels, exceeding CK by 83.78% and 97.21%, respectively. The 50 μM melatonin treatment group exhibited a 2-fold increase in hydrogen peroxide accumulation compared to the control (CK), demonstrating significant drought-induced oxidative injury at the cellular level. In contrast, the 100 and 200 μM melatonin treatments limited H_2O_2 increases to 27.84% and 41.35% above CK, respectively, demonstrating a concentration-dependent mitigation effect (Figure 3b).

3.3. Effects of Exogenous Melatonin on Osmolyte Accumulation in P. arguta

Soluble sugars and proline, key osmoregulatory substances, are critical for maintaining cellular osmotic balance under drought stress. In this study, the observed differential in soluble carbohydrates—with consistently elevated levels across drought treatments relative to CK—reflects active osmotic adjustment as a key physiological adaptation strategy. The untreated drought-stressed group (0 μM melatonin) exhibited a 77.12% increase in soluble sugar content compared to CK, while 50, 100, and 200 μM melatonin treatments resulted in increases of 40.92%, 47.25%, and 60.18%, respectively. Notably, the 50 μM melatonin group showed the lowest soluble sugar accumulation among melatonin-treated plants, with values closer to CK (Figure 4a). Proline content varied markedly across treatments, peaking in the 100 μM melatonin group. The untreated drought-stressed group (0 μM) and 200 μM melatonin group displayed moderate proline levels, whereas the CK and 50 μM

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melatonin treatments showed the lowest accumulation. Specifically, proline content in the 0, 50, 100, and 200 μM melatonin groups exceeded CK by 30.38%, 9.86%, 90.14%, and 29.61%, respectively. This highlights the superior efficacy of 100 μM melatonin in enhancing proline synthesis to counteract osmotic stress (Figure 4b).



(**a**) (**b**)

Figure 4. (**a**) Soluble sugar content; (**b**) free proline content. Alphabetic superscripts (a–c) denote statistically distinct clusters identified through Tukey's HSD test (α = 0.05), where non-overlapping letters reflect membrane integrity–water retention coordination under osmotic stress.

3.4. Transcriptome Sequencing Alignment, Annotation, and Differential Gene Analysis

The de novo transcriptome reconstruction using the Trinity assembler yielded 316,538 RNA isoforms (301.34 Mbp total length), exhibiting broad size distribution from 952 bp average to 17.2 kb maximum contigs. These spliced variants underwent systematic functional characterization through six-tiered bioinformatic pipelines (Non-Redundant Protein, Swiss-Prot/UniProtKB, Pfam domain architecture, Clusters of Orthologous Groups, GO, and KEGG), with cross-database annotation landscapes quantitatively profiled in Figure 5.

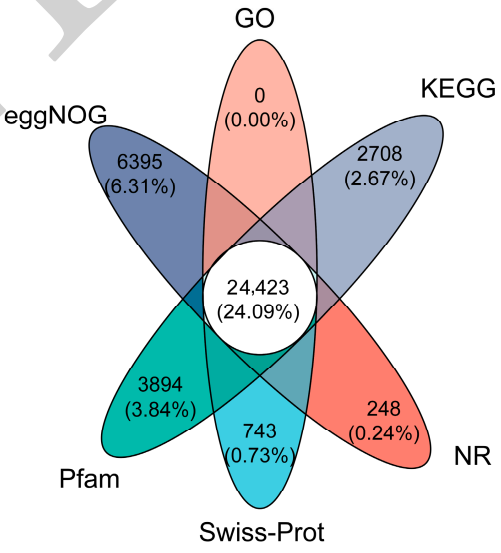


Figure 5. Gene annotation of each database.

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Differential expression analysis identified distinct gene sets across comparisons. Specifically, 720 (2.49%), 206 (0.71%), 9728 (33.58%), and 149 (0.51%) genes were uniquely expressed in T1 vs. CK, T2 vs. CK, T3 vs. CK, and T4 vs. CK, respectively. Comparisons between melatonin-treated and drought-stressed groups revealed 30 (0.10%), 46 (0.16%), and 517 (1.78%) unique genes in T2 vs. PEG, T3 vs. PEG, and T4 vs. PEG, respectively. Cross-treatment comparisons yielded 114 (0.39%), 80 (0.28%), and 1,019 (3.52%) unique genes in T2 vs. T3, T2 vs. T4, and T3 vs. T4, respectively.

When T3 was compared to CK, a total of 31,870 differentially expressed genes (DEGs) were detected, with 22,324 being upregulated and 9546 downregulated. In the T3 versus T1 comparison, 27,068 DEGs were identified, consisting of 229 upregulated and 26,839 downregulated genes. Similarly, the contrast between T3 and T2 yielded 11,832 DEGs, including 4462 upregulated and 7370 downregulated transcripts. Finally, the T3 vs. T4 comparison displayed 22,598 DEGs, of which 5618 were upregulated and 16,980 downregulated. These results underscore the pronounced transcriptional reprogramming induced by 100 μM melatonin, aligning with its superior efficacy in enhancing drought tolerance.

3.5. Functional Enrichment Analysis of Differentially Expressed Genes

In the T3 vs. CK comparison, GO enrichment analysis revealed distinct functional categories. For biological processes (BP), DEGs were predominantly enriched in macromolecule modification (836 genes), phosphorus metabolic processes (761), cellular protein modification (757), protein modification (757), and phosphorus compound metabolism (728). Cellular component (CC) terms included cell part (7334 genes), cellular anatomical entity (6880), membrane-bounded organelle (2810), intracellular membrane-bounded organelle (2798), intrinsic membrane component (2552), membrane part (2534), and membrane (1496). Molecular functions (MF) were enriched in catalytic activity (1340 genes), kinase activity (701), phosphotransferase activity (630), and DNA binding (621).

KEGG pathway analysis highlighted distinct metabolic reprogramming across comparisons. Pathway enrichment analysis revealed distinct metabolic and regulatory patterns across the comparisons (Figure 6). In T3, relative to CK, the predominant pathways were associated with "Biosynthesis of cofactors," "Oxidative phosphorylation," and "Glycolysis/Gluconeogenesis." When contrasting T3 with T1, the most significantly enriched pathways included "Starch and sucrose metabolism," "Peroxisome," and "Pentose and glucuronate interconversions." For T3 versus T2, DEGs were primarily linked to "Plant hormone signal transduction," "Protein processing in endoplasmic reticulum," and "Plant-pathogen interaction." Meanwhile, the comparison between T3 and T4 highlighted pathways such as "Ribosome," "Oxidative phosphorylation," and "Carbon fixation in photosynthetic organisms."

Integrated analysis demonstrated that T3-specific DEGs were primarily associated with carbohydrate metabolism (e.g., glycolysis, starch/sucrose metabolism, pentose/glucuronate interconversions) and oxidative phosphorylation. Cross-group comparisons further revealed enrichment in pathways such as "Plant hormone signal transduction," antioxidant metabolism ("Oxidative phosphorylation," "Peroxisome"), and carbon metabolism ("Carbon fixation"). These findings align with melatonin's role in enhancing drought resilience through the coordinated regulation of energy metabolism, stress signaling, and redox homeostasis.

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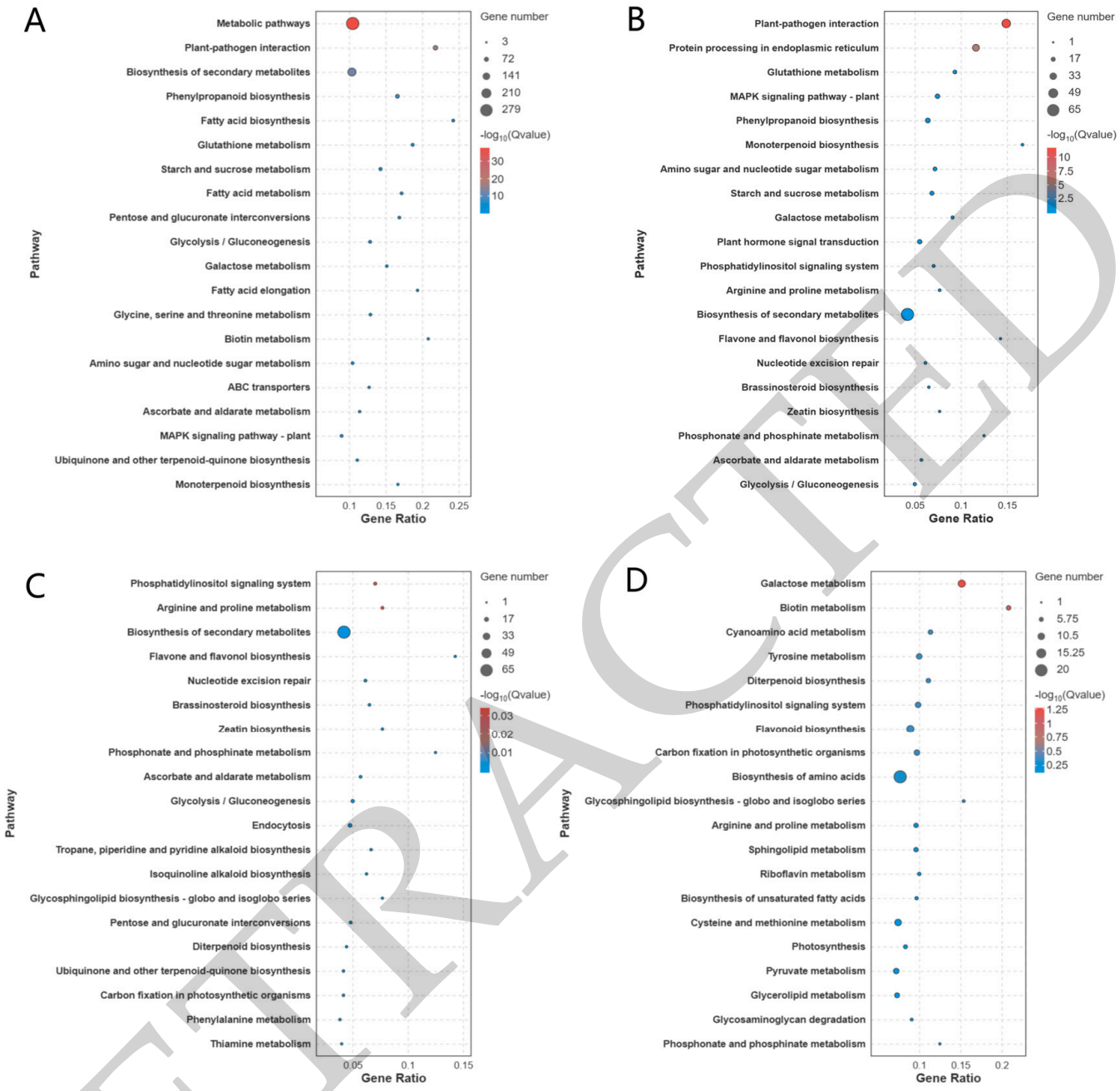


Figure 6. Enrichment analysis of KEGG differential genes in different treatment groups of *P. arguta*. (**A**) CK VS PEG; (**B**) CK VS PEG + MT50; (**C**) CK VS PEG+MT100; (**D**) CK VS PEG+MT200.

3.6. Tryptophan Metabolite Analysis

In this experiment, 31 tryptophan metabolic pathway-related metabolites were quantitatively analyzed. A total of 16 metabolites across 7 classes were detected with differential expression between treatments: aniline compounds (1 species: 2-aminophenol, 2-AF), benzoic acid derivatives (1 species: 2-aminobenzoic acid, 2-AA), indole derivatives (8 species: 6-hydroxymelatonin, 6-HMLT; tryptamine, TRM; N-acetylserotonin, NAS; melatonin, MLT; L-tryptophan, L-TRP; DL-indole-3-lactic acid, ILA; 5-hydroxytryptophol, 5_HTOL), medium-chain keto acids (1 species: 2-ketoadipic acid, 2-KA), carbonyl compounds (1 species: L-kynurenine, L-KYN), pyridine derivatives (2 species: picolinic acid, PA; nicotinic acid, N-Acid), and quinoline carboxylic acids (2 species: xanthurenic acid, XA; quinolinic acid, QA).

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Among the indole derivatives, two melatonin-related compounds, MLT and 6-HMLT, exhibited a relatively high abundance. The MLT contents in the CK~T4 treatment groups were 1.3518 ng/g, 2.0171 ng/g, 11.0537 ng/g, 29.5711 ng/g, and 67.6499 ng/g, respectively. Significant differences were observed between the CK/T1 groups and the T2/T3/T4 groups. The CK group had the lowest MLT content, accounting for only 2.00%, 2.98%, 16.34%, and 43.71% of the other treatment groups. The treatment groups sprayed with exogenous melatonin showed significantly higher MLT content than the CK and PEG groups, with levels 5.84-, 9.76-, and 19.62-fold higher than those of the CK group. These results indicate that higher exogenous melatonin concentrations lead to greater endogenous melatonin accumulation. Additionally, the indole derivative IGA displayed substantial variation among treatments, with contents of 20.8052 ng/g, 51.7191 ng/g, 18.0867 ng/g, 24.2732 ng/g, and 19.1119 ng/g across the groups. The T2 group had the lowest IGA content, while the other groups showed 1.15-, 2.86-, 1.34-, and 1.06-fold higher levels compared to T2.

Among the benzoic acid derivatives, 2-AA exhibited significant variations across the treatment groups. The 2-AA content was notably lower in melatonin-sprayed groups (14.37 ng/g, 12.30 ng/g, and 20.98 ng/g), with the lowest observed in the T3 group, representing only 17.64%, 15.97%, 85.60%, and 58.64% of the levels in the other treatment groups. In contrast, the PEG-stressed group showed the highest 2-AA accumulation.

Overall, exogenous melatonin application modulated the content of tryptophan-related compounds in *P. arguta* leaf tissues, with melatonin-treated groups displaying intermediate levels between the CK control and PEG-stressed groups. Notably, the T2 and T3 treatments outperformed T4 in balancing tryptophan metabolism under drought stress.

3.7. KEGG Enrichment Analysis of Tryptophan-Related Differential Metabolites

Experimental evaluation of exogenous melatonin dosage variations on drought-stressed *P. arguta* demonstrated that the 100 μM/L concentration (T3) optimally enhanced both phenotypic resilience and physiological homeostasis among all treatments. Combined with the analysis of tryptophan-related compound contents, this further confirmed that 100 μM/L melatonin is the optimal concentration for enhancing drought resistance in *P. arguta*. Therefore, the KEGG functional annotation and enrichment analysis of tryptophan-related differential metabolites focused on comparing the enrichment differences between the T3 and other treatment groups.

In the comparison between T3 and CK, the pathways with the most enriched differentially expressed genes were 'biosynthesis of secondary metabolites' and 'tryptophan metabolism'. Between T3 and T1, the most enriched pathways were 'tryptophan metabolism', 'nicotinate and nicotinamide metabolism', and 'metabolic pathways'. For T3 versus T2, the predominant enriched pathways were 'metabolic pathways' and 'tryptophan metabolism'. Similarly, in T3 versus T4, the top enriched pathways were 'metabolic pathways' and 'tryptophan metabolism'.

Collectively, the differentially expressed genes between the T3 and other treatment groups were primarily enriched in 'metabolic pathways' and 'tryptophan metabolism'. Pairwise comparisons among other treatment groups revealed additional enriched pathways, including 'nicotinate and nicotinamide metabolism', 'biosynthesis of secondary metabolites', and 'biosynthesis of amino acids'. These results underscore the central role of tryptophan metabolism and broader metabolic reprogramming in the drought resistance mechanisms regulated by melatonin in *P. arguta*.

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4. Discussion

Melatonin is synthesized and transported in plants, with a mechanism similar to that in animals, both using tryptophan as the precursor. The biosynthesis of melatonin in plants follows a four-step enzymatic cascade starting from tryptophan, with the involvement of six key enzymes: tryptophan decarboxylase initiates the pathway, followed by tryptophan hydroxylase, and tryptophan 5-hydroxylase for hydroxylation. Subsequently, serotonin N-acetyltransferase catalyzes acetylation, while N-acetylserotonin methyltransferase and caffeic acid O-methyltransferase mediate the final methylation steps to yield melatonin. Therefore, studying the tryptophan metabolic pathway is crucial for understanding the role of melatonin in drought resistance mechanisms of *P. arguta*.

P. arguta exhibited distinct morphological, peroxidative, and osmoregulatory responses under drought stress with exogenous melatonin treatments. Observations in *Rosa sertata* × *R. rugosa* showed that PEG-induced drought stress significantly inhibited plant growth, causing leaf curling and wilting, and a decrease in relative water content [19]. Exogenous melatonin application alleviated these symptoms, improving RWC in a dose-dependent manner. Similar studies on *Zanthoxylum bungeanum* demonstrated that melatonin (200 μM) effectively restored leaf turgor and chlorophyll content under drought, highlighting its species-specific optimal concentration for stress mitigation [20].

Drought-induced cellular damage, marked by elevated malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and relative electrical conductivity (REC), was mitigated by melatonin in *P. arguta*. This aligns with findings in *Z. bungeanum*, where melatonin reduced MDA and H_2O_2 levels by 33% and 30%, respectively, under drought [21]. The reduction in membrane permeability and oxidative markers underscores melatonin's role in scavenging reactive oxygen species (ROS) and stabilizing membrane integrity, a conserved mechanism across plant species [22–24].

Carbohydrate metabolism, which is critical for osmotic adjustment and energy homeostasis, was significantly enriched in drought-stressed *P. arguta*, as revealed by transcriptomic analysis. Similar patterns were reported in *Agropyron mongolicum*, where drought-responsive genes were enriched in starch/sucrose metabolism pathways [25,26]. These findings suggest a universal adaptive strategy where plants prioritize carbon allocation to sustain cellular functions under water deficit [27]. Furthermore, melatonin's interaction with phytohormones, such as auxin and abscisic acid (ABA), may coordinate stress signaling. For instance, in *Arabidopsis*, tryptophan metabolism modulates auxin-ABA crosstalk, balancing growth and stress responses [28]. Drought-induced H_2O_2 accumulation suppresses tryptophan synthase activity, reducing auxin synthesis while promoting ABA accumulation—a dynamic interplay potentially regulated by melatonin [29–31].

Transcriptomic enrichment in metabolic pathways, particularly tryptophan metabolism, in the T3 group (100 μM melatonin) highlights melatonin's dual role as a metabolic regulator and antioxidant. Studies on *Arabidopsis* TSB1 (tryptophan synthase β-subunit 1) revealed its involvement in abiotic stress adaptation by fine-tuning auxin and ABA homeostasis [32]. In *P. arguta*, melatonin likely mitigates drought-induced oxidative stress by modulating tryptophan-derived metabolites, thereby influencing H_2O_2 levels and hormone signaling. This mechanistic overlap suggests that melatonin enhances drought resilience through conserved pathways involving redox balance and hormone coordination [33].

Growing evidence emphasizes phytohormones as pivotal mediators of drought adaptation. Melatonin, acting synergistically or antagonistically with hormones like ABA, ethylene, and jasmonic acid, orchestrates complex signaling networks to optimize stress responses. Our findings corroborate this paradigm, positioning melatonin as a master regulator bridging metabolic reprogramming and hormonal crosstalk under drought.

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This study highlights the potential application of exogenous melatonin in enhancing drought tolerance in *P. arguta.* Melatonin is commercially available through chemical synthesis or microbial fermentation, with relatively high costs for plant applications. However, considering its significant benefits in improving drought resistance, the cost is acceptable, especially for endangered species or regions prone to drought. Additionally, foliar spraying of melatonin has shown promising results, but its effectiveness may vary under different environmental conditions. Future studies should focus on optimizing spray frequency, concentration, and timing to maximize its benefits in diverse conditions, particularly in arid areas. Thus, melatonin holds great potential for agricultural and ecological applications, especially for plants in drought-prone regions.

5. Conclusions

This study demonstrated the pivotal role of exogenous melatonin in enhancing drought stress tolerance in *P. arguta* through physiological and transcriptomic analyses. Under drought stress, *P. arguta* plants exhibited growth inhibition, elevated peroxide content, and reduced antioxidant enzyme activity. However, exogenous melatonin treatment mitigated leaf dehydration, elevated relative water content in leaves, and improved drought tolerance through the upregulation of antioxidant enzymes to eliminate reactive oxygen species (ROS), alongside the accumulation of osmoprotectants to reduce osmotic potential. Transcriptomic profiling revealed that differentially expressed genes in melatonin-treated leaves were predominantly enriched in pathways related to carbohydrate metabolism (including ribosome, glycolysis/gluconeogenesis, starch and sucrose metabolism, fructose and mannose metabolism, pentose and glucuronate interconversions), antioxidant metabolism, and carbon metabolism.

It is noteworthy that this research focused primarily on melatonin-mediated metabolic and transcriptional responses in leaves, while root development and regulatory mechanisms under drought stress remain unexplored. Future studies should integrate phenotypic and physiological assessments with genomic-level analyses to establish a comprehensive evaluation system for accurately quantifying drought resistance in *P. arguta*, thereby advancing the development of standardized resistance indicators.

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