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Melatonin Priming Enhances Potassium Dichromate Stress Tolerance and Morpho-Physiological Performance via Genetic Modulation in Melon (*Cucumis melo* L.) Plant

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ABSTRACT: Heavy metal accumulation in agricultural soil is primarily driven by pesticides, polluted water, and industrial gas emissions, which pose threats to sustainable crop production. Chromium (Cr) stress has an adverse impact on plant development and metabolism, but approaches to reduce its toxicity and enhance plant resistance remain limited. Melatonin is a potent antioxidant involved in regulating various morpho-physiological functions of plants under different abiotic stresses. In this study, we investigated the impact of exogenous melatonin to mitigate the negative effects of potassium dichromate (PD) stress in melon plants and analyzed genetic modulation of morphological, physiological, and biochemical parameters. The obtained results revealed that melatonin treatment (100 $\mu\text{mol L}^{-1}$) considerably improved seed germination rate, promoted plant growth, and stabilized chloroplast ultrastructure of leaves under PD-stress. This physiological resilience was similarly reflected by maintained photosynthetic efficiency and significantly stabilized photochemical parameters (e.g., Fv/Fm and NPQ). At the molecular level, quantitative polymerase chain reaction (qPCR) analysis confirmed that melatonin treatment maintained organelle integrity by upregulating primary metabolism indices and hindering Cr accumulation. Specifically, melatonin reduced the Cr-induced downregulation of chlorophyll biosynthesis genes [*CmHEMA* (MELO3C006296.2), *CmGOGAT* (MELO3C008481.2), and *CmPOR* (MELO3C016714.2)], restoring chlorophyll content by up to 5.08 $\text{mg}\cdot\text{g}^{-1}$, increased by 67.11%. The expression level of genes [*CmSPS* (MELO3C003715.2), *CmPEPC* (MELO3C018724.2), and *CmRubisco* (MELO3C012180.2)] showed an effective upsurge in carbohydrate synthesis. Moreover, melatonin significantly enhanced the antioxidant system [e.g., increasing SOD (46.13%), POD (35.85%), and APX (25.00%) activities] and promoted the accumulation of lignin and metallothionein [via upregulation of *Cm4CL* (MELO3C002346.2) and *CmMet* (MELO3C016513.2) genes], which restricted Cr translocation from the root to the shoot. To summarize, exogenous melatonin application could serve as an effective strategy for mitigating Cr-induced stress in melon by stabilizing basic photosynthetic processes and secondary metabolism through biochemical and molecular defensive mechanisms, thereby preventing Cr translocation by activating the accumulation of secondary metabolites (e.g., lignin and metallothionein) and photo-respiration elements. Our findings provided new perspective to understand melatonin as a viable, multidimensional bio-regulator for improving crop resilience in Cr-polluted agricultural systems.

KEYWORDS: *Cucumis melo*; growth indices; heavy metal; melatonin; stress; toxicity



1 Introduction

Since the onset of industrialization, the biosphere has experienced a significant accumulation of pollution from the presence of lethal heavy metals, for example, copper (Cu), cadmium (Cd), lead (Pb), and chromium (Cr) in soil, sediments, and groundwater. This pollution primarily results from various human activities, including mineral degradation, effluents from the textile dye, paper, and paint industries, urban sewage, and the use of agricultural chemicals [1,2]. In general, the toxicity mechanism of heavy metals is known for the main interaction process at the biotic and abiotic levels.

Cr is a hazardous heavy metal that negatively impacts plant development and significant growth factors, e.g., seed germination, photosynthetic capacity, and total biomass [3]. The chlorosis of leaves and necrosis of tissues are the primary morphological alterations induced by Cr stress, indicating its negative effect on plant growth that ultimately leads to plant death in severe cases [4,5]. This type of induced stress causes cytological changes in cellular organs and disrupts the ultrastructure of the chloroplast envelope as well as the degradation of membrane systems in plants [6]. The direct toxic effect of Cr on plants becomes continuous due to its persistent property, which will accumulate along the food chain and then cause irreversible damage [7]. Cr stress also kills plants by interfering with mineral element absorption by disturbing pigment production, as well as modifying the enzyme activity contributed to breakdown of glucose and antioxidant systems, resulting in disrupted photosynthesis and respiration [8,9]. Further, heavy metals have a negative impact on plants because they generate reactive oxygen species (ROS) and stimulate oxidative stresses [10,11].

Photosynthesis is an essential process for green pigmentation and relevant fluorescence indices in plants. It is the main source for the transformation of received light energy into chemical energy stored in organic compounds [4]. Photochemical parameters in plants are measures used to evaluate the efficiency and health of the photosynthetic machinery, particularly the Photosystem II (PSII) apparatus. The ratio of variable fluorescence (Fv) and maximum fluorescence (Fm) represents the major efficiency of light conversion in the photosynthetic reaction center II (PSII), whereas the quenching coefficient (qP) denotes the quantity of light energy absorbed for electron transfer in the PSII antenna pigment, which reflects the PSII reaction center's activity. NPQ is the absorbed quantity of light energy received by the PSII pigmentation that is not utilized for photosynthetic electron transport and is converted into heat, which positively protects the photosynthetic apparatus [12].

Chloroplast is a main cell organelle of plants that performs photosynthesis activities and produces redox oxygen species (ROS) [13]. The accumulation of ROS under adverse environmental stress damages the stability and intrinsic function of complex internal structures and functional disorders, including the double membrane system [14,15]. The evolutionary resistive mechanism led to the maintenance of the plant membrane, which acts as a defensive barrier between the cytoplasm and exterior stress factor by retaining its components as well as organization through various membrane lipids. However, lipid composition frequently varies in response to environmental stresses, particularly the remodeling of the primary components of the green tissue membrane, e.g., monogalactosediacyl glycerol (MGDG) and digalactosyldiacylglycerol (DGDG) [16]. Furthermore, increased metal chelation and lignification of cell walls favorably decreased heavy metal accumulation and restricted translocation inside plants [17,18].

Melatonin (N-acetyl-5-methoxytryptamine), an endogenous indole tryptamine phytohormone, was discovered in plants in 1995. It is known to govern the positive regulation of several aspects of plant development, including root and leaf senescence and biomass output [19]. It is a free radical scavenger with substantial antioxidant characteristics, which is twice that of vitamin E, four times that of glutathione, and fourteen times that of mannitol [20–22]. The tiny size of the molecule, along with its high lipidicity and hydrophilicity, makes it simpler to penetrate cells and decrease hydrogen peroxide while minimizing

oxidative damage under stressful conditions [23]. Further, exogenous melatonin treatment has been demonstrated to significantly increase pigment synthesis and photosystem II (PSII) performance, thereby increasing photosynthetic efficiency during stress [24]. Recently, the application of melatonin has emerged as a promising approach to coping with plants from different types of biotic and abiotic stresses.

Melon is widely regarded as a model plant for studying the complex biological processes that determine plant-specific traits [25]. In northeast China, agricultural black soil is rich in organic matter, which is strongly connected with specific heavy metal accumulations [26] and is extensively spread over the Songnen Plain. Due to the growth of the leather tanning industry in countries like China, Pakistan, and India, around 40 million liters of wastewater containing chromium (Cr) are being produced each year, damaging the texture of agricultural land [27]. In 2022, the Ministry of Ecology and Environment of China narrated in the Ecological Environment Bulletin that heavy metals are serious contaminants affecting the environmental quality of agricultural land [28]. However, there is a lack of knowledge on the identification of the metabolic processes involved in heavy metal resistance during plant growth dynamics in melon plants under Cr stress. The precise genetic and physiological role of melatonin in response to Cr stress is also crucial for understanding the evolution of melon species and their resilience to abiotic stresses.

2 Materials and Methods

2.1 Plant Materials and Experimental Treatments

An oriental melon variety (longqing) was chosen as experimental material based on its susceptibility to biotic and abiotic stresses and weaker plant growth during its lifespan. The varietal seeds were offered by the Heilongjiang Academy of Agricultural Sciences (HAAS), Daqing Branch, China, and entire experiment was carried out in a controlled condition in the aseptic laboratory at HAAS. An exogenous melatonin treatment was checked on seed germination traits and morphological and physiological parameters of seedlings grown under heavy metal-induced stress. To sort out the treatments of melatonin and PD stress, a pre-experiment was conducted at small scale in lab and the actual experiment was conducted.

For evaluating the seed germination status and related parameters (sprout length, average diameter, and surface area), the seeds were gently sterilized in a solution of potassium permanganate (KMnO_4 , 10%) for one minute, then washed with double distilled water (ddH_2O), and allowed to dry. Later, a total of 50 melon seeds were spread on a 9-cm-wide autoclaved petri dish having 4 layers of wet qualitative slow filter paper, and moisture was regularly retained with a total of 12 mL of test solution. Finally, a total of four treatment groups along with 3 replications of each treatment were used [e.g., (i) control with no stress (CK, just ddH_2O), (ii) 100 μM of melatonin (M) application, (iii) 40 mg L^{-1} of potassium dichromate (PD) application as a stress, and (iv) melatonin treatment (M) + potassium dichromate stress (PD)]. The respective concentrations of potassium dichromate and melatonin treatment were referenced from previous research [29–31]. The treated melon seeds in petri dishes were incubated for 60 h at 28°C, and seed germination status as well as linked indices [sprout length (cm), average diameter (mm), and surface area (cm^2)] were tested. The germination rate of 50 melon seeds of comparative treatments was calculated to determine the efficiency of melatonin using the equation as follows:

Seed germination rate = (germinated seeds-total seeds) $\times$ 100%;

Melatonin efficiency = ([M + PD] rate-[PD] rate)/[PD] rate $\times$ 100%.

2.2 Cultivation of Seedlings and Tissue Sampling

To cultivate proper melon seedlings and test the effect of exogenous melatonin treatment to mitigate the PD stress, the treated seeds were allowed to grow in sterilized plastic pots (diameter 6 cm and depth

7.5 cm) in controlled conditions using completely randomized design (CRD) geometry with three repetitions of each treatment. Briefly, seeds were put in muslin bags and allowed to germinate for two days at 28°C before being transferred to plastic pots filled with 3% organic matter soil and potting compost with a pH of 7–7.5. The characteristics of the compost are as follows: organic carbon (OC) = 20% (weight percentage on a dry product basis), organic nitrogen (ON) = 1%, organic matter (OM) = 35%, and pH = 6–7.

The emerging seedlings at the 4-true-leaf stage were divided into four groups. (i) spraying ddH₂O as a control (without any stress), (ii) spraying 100 µM of melatonin (M), (iii) irrigating 40 mg L⁻¹ of potassium dichromate solution as PD stress, and (iv) melatonin treatment (M) + potassium dichromate (PD) stress, each treatment was carried out with equal volume (40 mL) daily. The leaf samples from each treatment group were collected on the 1st day, 3rd day, and 5th day to determine the variation in physio-chemical indicators, as well as for transcriptional regulation through qPCR analysis; however, the morphological and ultrastructural observations were recorded on the 5th day.

2.3 Transmission Electron Microscopy (TEM)

The leaf tissues of raised seedlings sampled from each treatment were checked for cytological observations using a transmission electron microscopy analysis at different magnification levels (1.5k×, 8k×, and 20k×), as reported in a previous study of Liu et al. [32]. Briefly, the true leaf tissues from normal (control, without stress) and stress-induced obvious symptoms were sampled from melon seedlings cultivated under four different treatments (above-mentioned). The sampled leaves were sliced into 2 mm-wide pieces and fixed with a 2.5% (v/v) solution containing 1% osmium tetroxide. Then, all samples were embedded in epoxy resin embedding medium (EMBed-812), sliced in thin sections, dried using acetone solutions, viewed with a high-resolution transmission electron microscope, and photographed.

2.4 Determination of Physiological and Biochemical Indices

A total of 1–2 g (g) of the fresh leaves from the top of the grown seedlings were randomly sampled from three biological repetitions of different treatment groups, and the physiological and biochemical indices [e.g., peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX)] were estimated based on the following instructions of commercially available enzyme-linked immunosorbent assay (ELISA) kits, Michy Biology Company (Suzhou, China). The level of PD-induced changes in membrane lipid peroxidation was also deliberated by quantifying the malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents using ELISA test kits according to the briefed protocol. The lipid composition in the cell membrane of seedling leaves was checked through the electrospray ionization (ESI) mass spectrometry method with a slight modification to the earlier reported protocol as Narayanan et al. [33].

The lignin content was determined using the same method as Shao et al. [34]. In short, the collected leaf tissues from various treatments and replications were standardized in a mortar with 10 mL of 95% ethanol (v/v) at 4°C, then centrifuged at a relative force of 5000× g for 4 min. The formed supernatant were separated and washed double with ethanol (80% v/v) and one time with acetone before being thoroughly dried in an oven at 60°C. The dry samples were incubated at 80°C for 2 h in a 4:1 (v/v) acetyl bromide (CH₃COBr) and acetic acid (CH₃COOH) before cooling to 25°C and being transferred to 50 mL glass bottles containing 5 mL of acetic acid and 2 M sodium hydroxide (NaOH). The reaction was ended through adding the 7.5 M hydroxylamine hydrochloride (NH₂OH·HCl), and the final volume was adjusted with acetic acid. In the end, the lignin was measured using the absorbance rate at 480 nm. Metallothionein (Met) was measured through ELISA test kits, following the same technique of Zhang et al. [35].

2.5 Estimation of Total Chlorophyll Contents

Chlorophyll of the leaf samples taken for each treatment and replication were also measured using the ethanol-acetone extraction technique as Dong et al. [36]. Briefly, a sharp cutting razor was used to cut a total of 0.30 g of fresh weight (FW) leaf samples from the main vein of leaves joined at the same node. All samples were submerged in a 10 mL solution of ethanol and acetone (1:1) for 24 h. The NanoDrop™ One/OneC Microvolume UV Spectrophotometer (Thermo Fisher Scientific, Delaware, USA) was used to measure the extract's absorbance rate at 440, 663, and 645 nm. Total chlorophyll was deliberated based on the formula:

Total chlorophyll ($\text{mg} \cdot \text{g}^{-1}$ FW) = $(8.04 \times A_{663} + 20.29 \times A_{645}) \times V/W \times 1000$; W = weight, V = volume, and A = absorbance rate, individually.

2.6 Determination of Photosynthetic Fluorescence Indices

The chlorophyll fluorescence emission from the aerial surfaces of the intact plant leaves were monitored as described in a previous study [36]. The samples were collected from different treatment groups in slightly cloudy conditions, and the leaves were kept in fully dark conditions for 20 min prior to measuring fluorescence indices [e.g., light-adapted minimum fluorescence (F₀), photochemical quenching coefficient (qP), maximum photochemical efficiency of PSII (F_v/F_m), and non-photochemical quenching coefficient (NPQ)].

2.7 Quantification of CR and Translocation Ratio

The samples of melon root, stalk, and leaf from different treatments were collected and completely dried. A 0.50 g (g) of every sample was homogenized with 7.50 mL of nitric acid (HNO₃) and 2.50 mL of Perchloric Acid (HClO₄) and kept overnight. Afterward, 5 mL of HNO₃ was added to the mixture for full digestion and endogenous Cr was quantified by the same method of Zaheer et al. [37].

Translocation ratios of Cr in different organs were estimated based on the formulas of previous Luo et al. [38]: e.g., T roots to stalk (%) = $C_{\text{stalk}}/C_{\text{roots}} \times 100$ (1); T stalk to leaves (%) = $C_{\text{leaves}}/C_{\text{stalk}} \times 100$ (2); T roots to leaves (%) = $C_{\text{leaves}}/C_{\text{roots}} \times 100$ (3), where C_{leaves} is concentration of Cr ($\mu\text{g/g}$) in leaves; C_{roots} is in roots ($\mu\text{g/g}$); C_{stalk} is in stalk ($\mu\text{g/g}$).

2.8 Transcription Expression of Putative Genes

To investigate the genetic regulation mechanism of photosynthetic and physiological indices in melon, the annotated putative genes were obtained from previously reported studies, e.g., *4CL* [34], *HEMA* [39], *GOGAT* [40], *POR* [41], *PAO* [42], *SPS* [43], *PEPC* [44], *ruBisCo* [45], *PSB* [46], *Hcf136* [47], *APX* [48], *POD* [49], *SOD* [50], *Met* [51], and the housekeeping gene “*Actin*” [32], as shown in the Supplementary File. The functional annotation of selected genes was predicted in the referenced genome of melon [(v3.6.1, DHL92); Cucurbit Genomics Database, <http://cucurbitgenomics.org>].

The transcription expression of putative genes contributing in key metabolic pathways in melon plants were analyzed in response to melatonin treatment under PD-induced stress using qPCR verification. For the molecular experiment, total RNA was firstly extracted by RNeasy Pure Plant Kit (Qiagen Biotech, Beijing, China) according to the instructions, the integrity was checked through NanoDrop 1000 (Thermo Fisher Scientific, Delaware, USA), and the concentration index of A260/A230 ratio >2.0 was used for complementary DNA (cDNA) synthesis. The first strand cDNA was synthesized by the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara Bio, Kusatsu, Japan) using 2 μL of total RNA. The cDNA products were diluted 5-fold with ddH₂O before experimental use. For the qPCR analysis, a total of 10 μL of reaction mixture containing 1 μL of cDNA, 0.2 μL of primer, 5 μL of 2 $\times$ SYBR Green PCR Master Mixture, and 3.8 μL of ddH₂O was used

to examine expression level in the Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., California, USA) [32]. The detailed information about the molecular sequences (forward and reverse) of exported primers of predicted annotated genes in the melon genome and the referenced *actin* gene is available in the Supplementary File (Table S1).

2.9 Statistical Data Analysis

All the experimental data was regularly collected, and the numerical values were computed using the Microsoft Excel Worksheet (v2019) [32]. The statistical differences among treatments within each measurement of time series data including physiological parameters were evaluated by two-factor repeated measures ANOVA (Analysis of Variance) and Tukey's HSD ($p < 0.05$) using GraphPad Prism (v10.2.0), while one-way ANOVA with Tukey's HSD ($p < 0.05$) was employed for the morphological parameters. The endogenous Cr quantification and translocation rates were compared using an independent samples *t*-test. The data were assessed for normality and homogeneity of variances prior to analysis.

3 Results

3.1 Analysis of Seed Germination Status

We noticed that seed germination rate followed an S-shaped pattern under stress treatment, with an early slow phase followed by a rapid increase and a downward tendency at the end (Table 1). However, the observed S-shaped pattern varied between treatments. At the initial stage, the melatonin-treated seeds showed a quicker and higher germination rate (about 13.94%) during the first 36 h compared to the control (CK) group. However, as the time progressed, the germination rate differences decreased between the CK and melatonin-treated groups. It is noticeable that the saturation in germination under melatonin treatment is 12 h earlier than the control (CK), indicating that exogenous melatonin has the beneficial function of promoting the germination of seeds.

Table 1: The germination rate of melon seeds under different treatments and multiple time series; Mean $\pm$ SD ($n = 3$). Different uppercase letters (A, B, C, D) within a column indicate significant differences among treatments at given time points, whereas the lowercase letters (a, b, c, d) within a row denote significant differences among given time points within the same treatment (Tukey's HSD, $p < 0.05$).

Treatments	Time Duration				
	12 h Mean $\pm$ SD	24 h Mean $\pm$ SD	36 h Mean $\pm$ SD	48 h Mean $\pm$ SD	60 h Mean $\pm$ SD
CK	26.00 $\pm$ 3.64 ^{dB}	55.33 $\pm$ 2.40 ^{cB}	81.33 $\pm$ 2.40 ^{bB}	92.67 $\pm$ 3.71 ^{aA}	96.00 $\pm$ 2.00 ^{aA}
M	35.33 $\pm$ 2.40 ^{cA}	63.33 $\pm$ 2.40 ^{bA}	92.67 $\pm$ 0.67 ^{aA}	96.67 $\pm$ 1.76 ^{aA}	98.67 $\pm$ 1.33 ^{aA}
PD	9.33 $\pm$ 1.33 ^{cC}	22.67 $\pm$ 1.33 ^{bD}	27.33 $\pm$ 2.67 ^{abD}	28.00 $\pm$ 3.06 ^{abC}	38.73 $\pm$ 2.91 ^{aC}
M + PD	25.33 $\pm$ 3.53 ^{cB}	48.00 $\pm$ 2.00 ^{bC}	64.67 $\pm$ 2.40 ^{aC}	67.33 $\pm$ 1.76 ^{aB}	71.33 $\pm$ 2.91 ^{aB}

The germination rate in PD-stress treated groups exhibited a sluggish trend, with the highest rate reaching only 38.73% at 60 h, which represents a 59.66% decrease relative to the CK group. However, exogenous melatonin application significantly alleviated the detrimental impact of potassium dichromate (PD)-stress on germination rate, which increased by 84.17%. PD stress significantly impaired the development condition of melon seed sprouts, as demonstrated by lower sprout length (27.59%), average diameter (19.54%), and surface area (37.50%) compared to the CK group (Fig. 1). However, exogenous melatonin treatment effectively reversed the negative effects of PD stress on these phenotypic parameters and even enhanced more when compared to CK treatment.

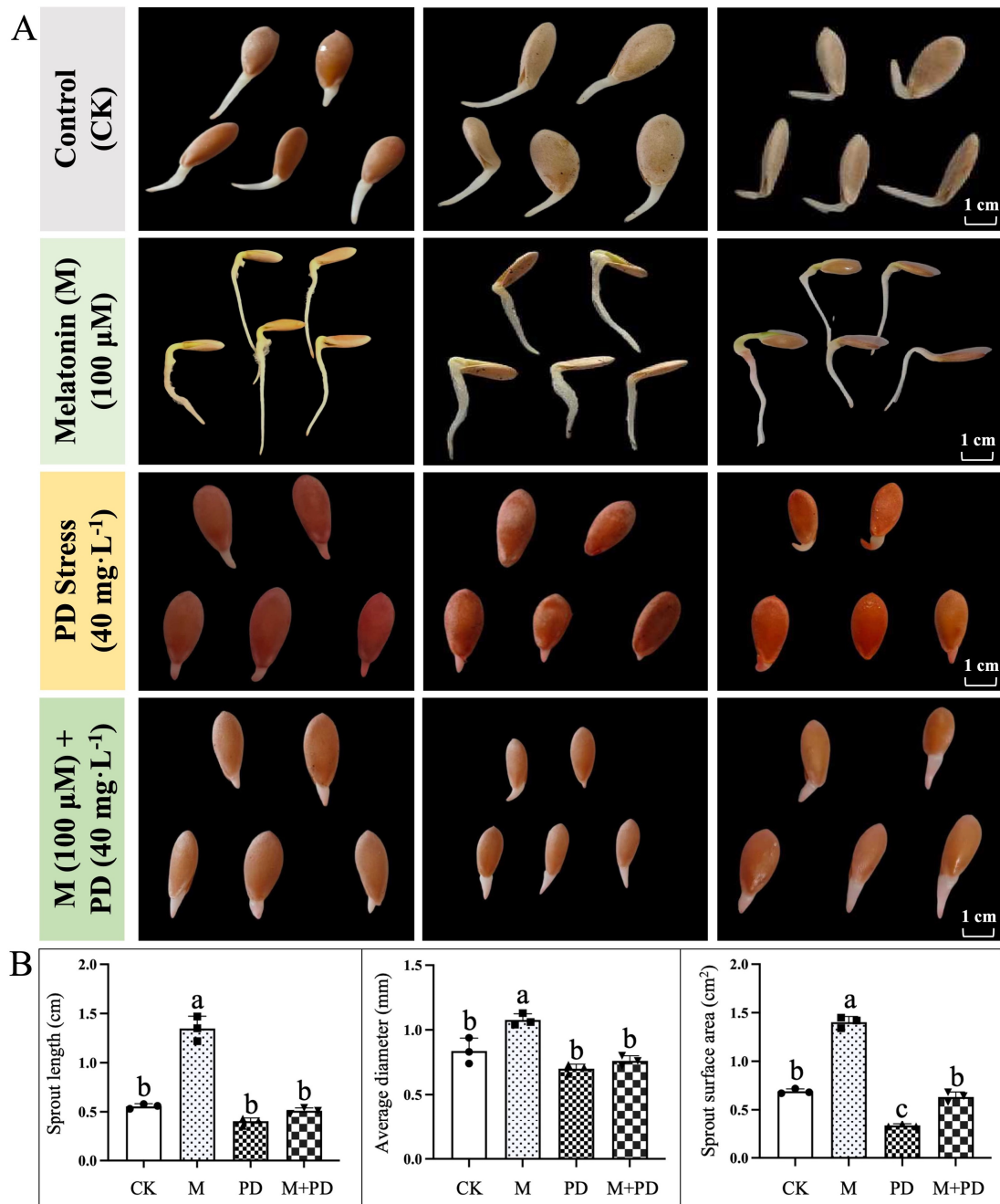


Figure 1: General observation of melon seed germination in response to different treatments of melatonin and PD stress. (A) Germination status and (B) Studied attributes associated with germination of melon seeds under different treatments. The graph bars represent the mean values, and error bars represent the Standard Deviations (SD) of the three replicates for each treatment, and dissimilar statistical letters (a, b, c) placed above the bars indicate the outcome of the Tukey's HSD test, $p < 0.05$.

3.2 Analysis of Morphological and Cytological Observations

The inhibitory effect of PD stress and the enhancing effect of melatonin on the morphological characteristics of melon were evaluated. The treated seedlings exhibited typical stress symptoms, but melatonin showed the improved leaf morphology, as illustrated in the figure (Fig. 2A). Statistical analysis

consistently showed that seedling growth and development were most severely affected under PD stress; however, extensive dehydration, chlorosis, and necrosis were observed on the leaf surfaces, indicating that PD stress induced significant osmotic stress and chlorophyll degradation in melon leaves compared to the melatonin and control (CK) treatment.

Transmission electron microscopy (TEM) of the internal leaf organelles in melon seedlings subjected to various treatments revealed significant structural variations, as shown in the figure (Fig. 2B). PD-stressed samples exhibited swollen, spherical chloroplasts with indistinct and irregular thylakoid structures, supplemented by a reduced number of starch granules. In contrast, exogenous melatonin treatment effectively preserved the developmental integrity of melon leaves by stabilizing the chloroplast ultrastructure, maintaining normal thylakoid organization, and supporting regulated metabolic processes under stress.

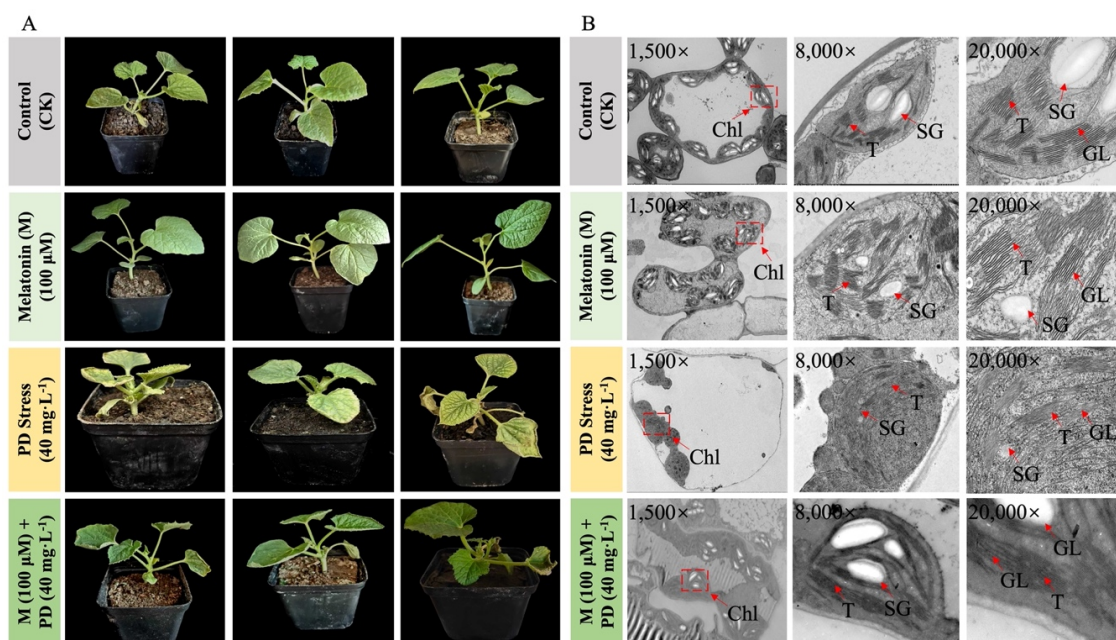


Figure 2: Phenotypic and cytological appearance of melon seedling leaves under different treatments of melatonin and PD-stress. **(A)** Phenotypic observations of seedling leaves from three repetitions and **(B)** Cytological observations of endogenous chloroplast ultrastructure of sampled leaves at 60 h of time. Microscopic observation at dissimilar magnification levels of 1.5k×, 8k×, and 20k×. T, thylakoid; CM, chloroplast membrane; Chl, chloroplast; SG, starch granule; and GL, granal lamellae.

3.3 Analysis of Physiological Parameters

Regarding the physiological indices, PD-stressed samples exhibited substantially higher levels of MDA and H₂O₂ compared to the control (CK) on the 5th day, as shown in the figure (Fig. 3A,B). However, exogenous melatonin treatment effectively reduced MDA and H₂O₂ levels by 25.10% and 32.29% on the 3rd day, and by 35.72% and 21.98% on the 5th day, compared to PD-stressed samples. Additionally, SOD, POD, and APX activities increased by 88.94%, 105.26%, and 410.00% on the first day of PD stress compared to CK at the same time point, as illustrated in the figure (Fig. 3C–E).

As stress duration increased, the activity of these antioxidants significantly declined during anaphase, decreasing by 23.17%, 7.02%, and 9.43% on the 5th day compared to the 3rd day. In contrast, melatonin-treated samples exhibited continuous improvement, with antioxidant levels rising by 46.13%, 37.74%, and 25.00% relative to PD-stressed samples on the 5th day. Analysis of membrane lipid composition showed that the molar percentages of DGDG and MGDG accounted for over 60% of total leaf glycolipids. PD stress reduced

MGDG and DGDG concentrations by 8.09% and 11.03%, respectively, while increasing the MGDG to DGDG ratio by 3.15%, indicating that PD stress adversely affected plastid membrane structure and composition, as illustrated in the figure (Fig. 3F).

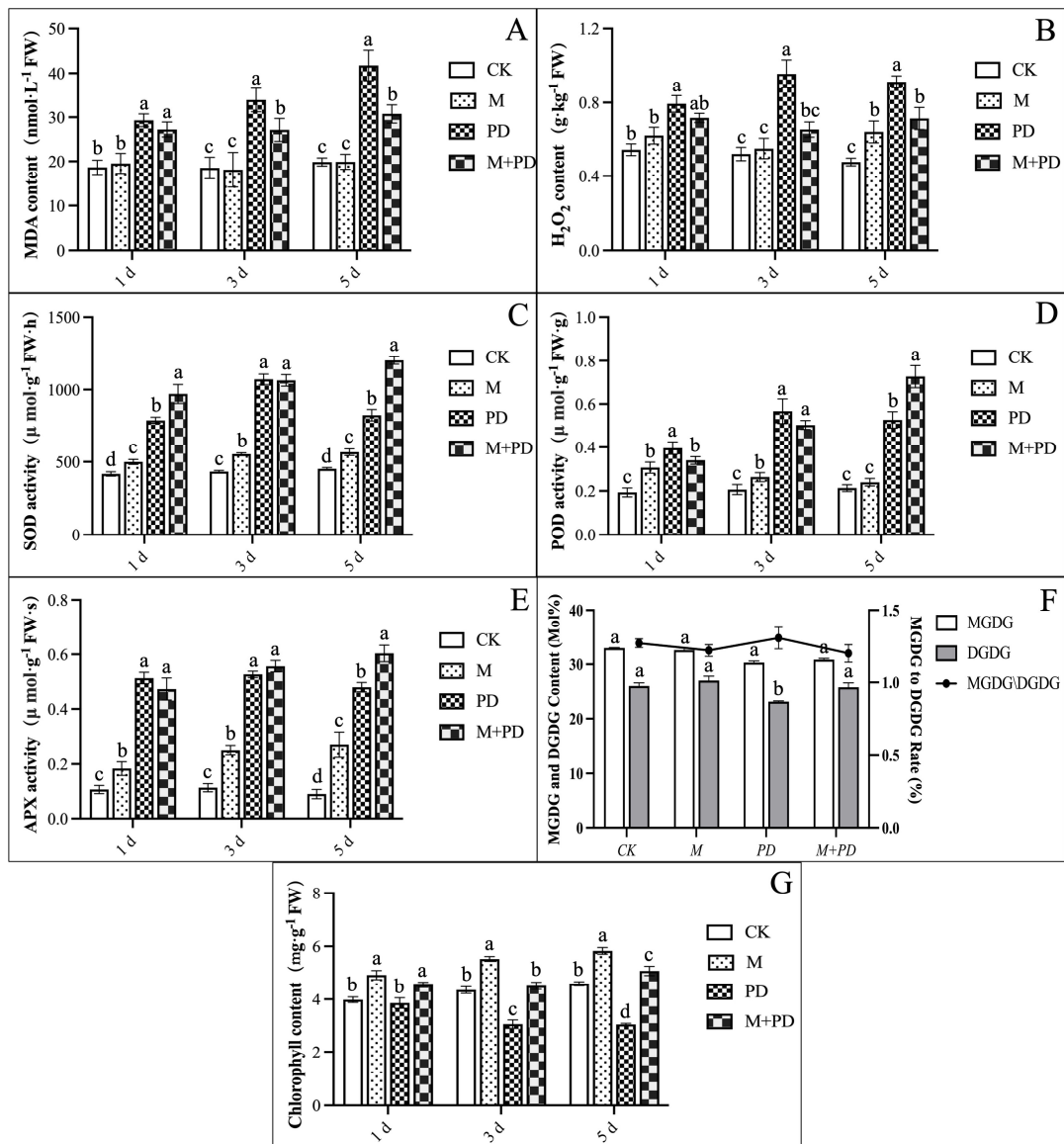


Figure 3: Effects of melatonin and PD stress treatments on the physiological parameters of melon seedlings at different time points. (A) MDA content, (B) H_2O_2 content, (C) SOD activity, (D) POD activity, (E) APX activity, (F) Molar percentage of MGDG and DGDG content, and (G) Chlorophyll content. The graph bars represent the mean values, and error bars represent the Standard Deviations (SD) of the three replicates for each treatment, and dissimilar statistical letters (a, b, c, d) placed above the bars indicate the outcome of the Tukey's HSD test, $p < 0.05$.

3.4 Analysis of Photosynthetic Status

In terms of photosynthetic-linked activity, total chlorophyll (Chl) contents were noticed to be decreased significantly by 29.82% on the 3rd day and 33.77% on the 5th day as shown in the figure (Fig. 3G), revealing the significant upsurge in superoxidative photosynthesis apparatus, implying that PD stress negatively affected the photosynthesis apparatus, so that caused the significant degradation of chlorophyll. On the

other hand, exogenous melatonin application efficiently retained the Chl contents and homeostasis of MGDG and DGDG levels, which increased by 1.78% and 11.45% compared to PD-stressed samples on the 5th day, and lowered the MGDG to DGDG ratio by 9.17% at the same time period. It was also discovered that the protective mechanism of exogenous melatonin to chlorophyll synthesis is not limited to stabilizing the relative content under PD-stressed conditions, which increased by 47.71% on the third day and 67.11% on the 5th day; the chlorophyll content in melatonin-treated samples showed an obvious increasing trend, with improvements of 26.83% on the 3rd day and 27.02% on the 5th day, respectively.

3.5 Analysis of Chlorophyll Fluorescence Parameters

Chlorophyll-linked photosynthesis fluorescence parameters [F0 (minimal fluorescence yield of chlorophyll), Fv (variable fluorescence), and Fm (maximum fluorescence)] were checked to evaluate the PD stress-induced photoinhibition and the photoprotective function of melatonin. Chlf results showed that PD-stress induced a 17.38% reduction in F0 and 18.29% in Fv/Fm as compared to CK on the 5th day as shown in the figure (Fig. 4A,B), denoting the inhibited photosynthetic electron transport rate. Likewise, PD-stress caused a significant reduction in qP (18.01%) and NPQ (26.79%) (Fig. 4C,D), indicating the comprehensively restrained photochemical electron transfer under PD-induced stress treatment. However, the beneficial effect of melatonin on photosynthesis was further confirmed by the enhanced Chlf parameters, which increased 13.73% in F0, 18.65% in Fv/Fm, 12.81% in qP, and 30.26% in NPQ on the 5th day compared to that of the PD-stressed samples, respectively.

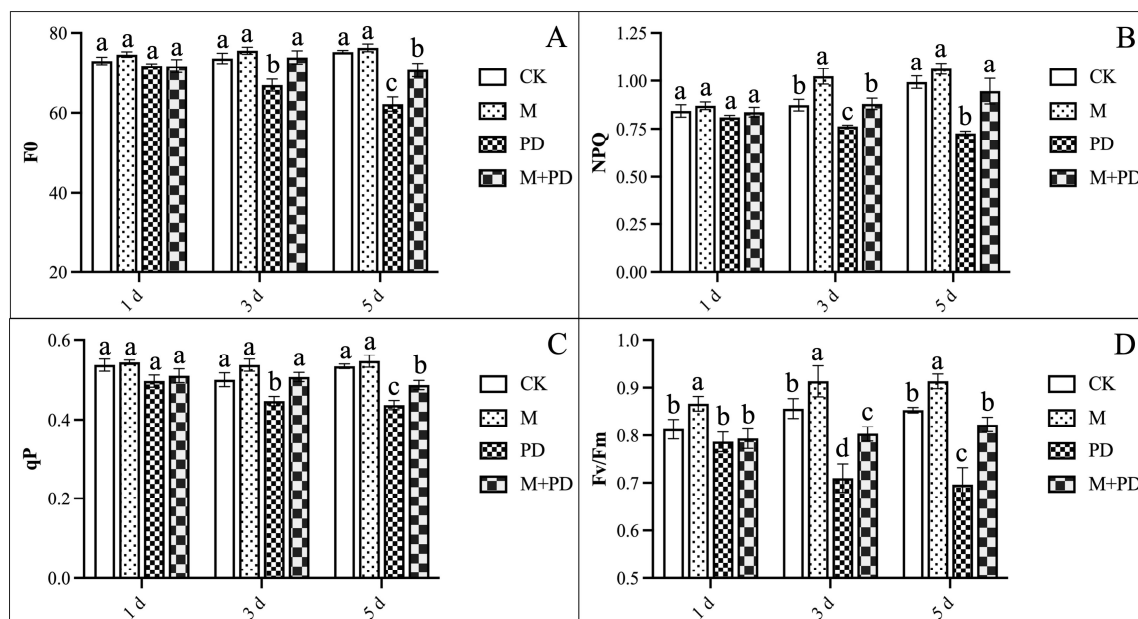


Figure 4: Effects of melatonin and PD stress treatments on chlorophyll fluorescence parameters of melon seedling leaves at different time points. (A) F0, (B) NPQ, (C) qP, and (D) Fv/Fm. The graph bars represent the mean values, and error bars represent the Standard Deviations (SD) of the three replicates for each treatment, and dissimilar statistical letters (a, b, c) placed above the bars indicate the outcome of the Tukey's HSD test, $p < 0.05$.

3.6 Analysis of Met and Lignin Contents

The significant variations in metallothionein (Met) and lignin levels were observed across different tissues (leaf, stalk, and root) subjected to various treatments, with these differences becoming more evident as the duration of PD-stress treatment increased. In leaves, Met and lignin content surged by 26.98%

and 4.64% on the 1st day and by 24.26% and 19.23% on the 5th day compared to CK, as shown in the figure (Fig. 5A,B). On the 1st day, Met and lignin contents increased by 23.33% and 2.52% under PD-stress, respectively, whereas there was a 37.84% and 14.03% increase on the 5th day, as shown in the figure (Fig. 5C,D). In roots, Met and lignin accumulation increased by 25.47% and 2.43% on the 1st day and by 29.90% and 23.80% on the 5th day, compared to CK treatment, as shown in the figure (Fig. 5E,F). Interestingly, Met content in leaf, stalk, and root also increased by 8.57%, 1.69%, and 1.56% on the 5th day, compared to tissues under PD-stress; however, there was no obvious change at the initial stage. Additionally, the lignin content in the same tissues followed a similar increasing trend by 10.79%, 6.68%, and 2.54%, respectively.

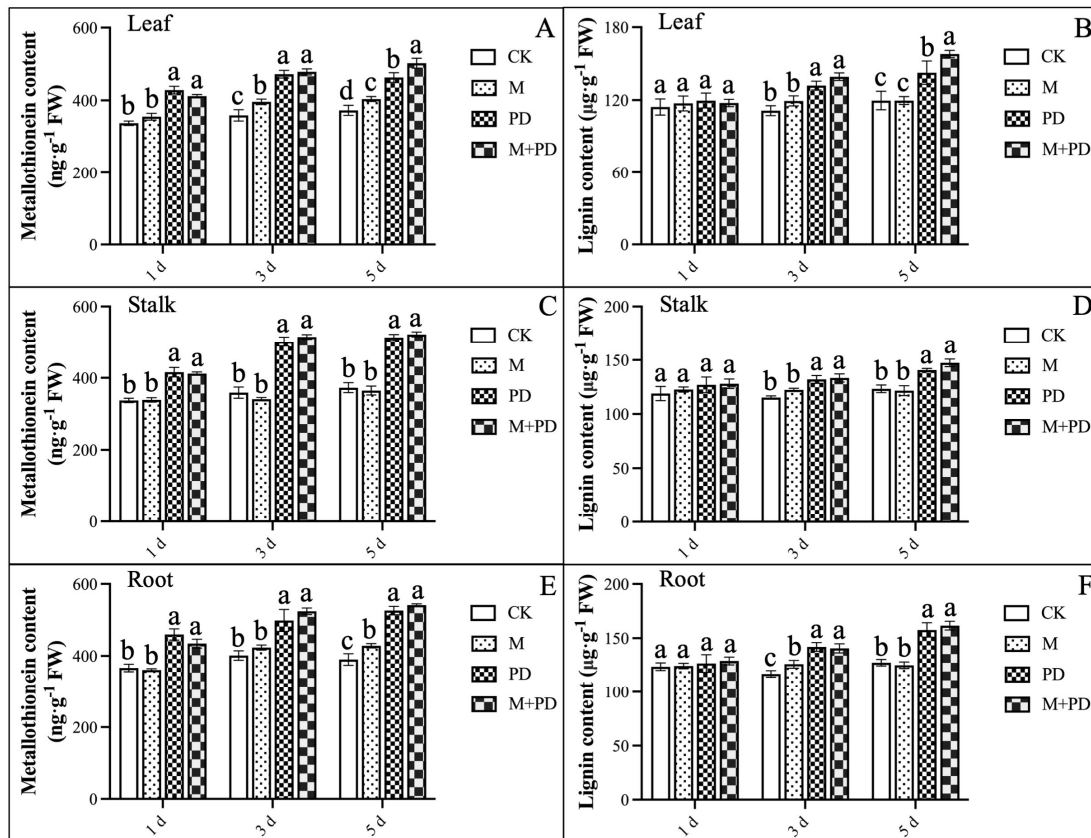


Figure 5: Effects of melatonin and PD stress treatments on Met and lignin contents in different tissues of melon seedlings at different time points. (A,B) Met and lignin contents in leaf, (C,D) in stalk, and (E,F) in root. The graph bars represent the mean values, and error bars represent the Standard Deviations (SD) of the three replicates for each treatment, and dissimilar statistical letters (a, b, c, d) placed above the bars indicate the outcome of the Tukey's HSD test, $p < 0.05$.

3.7 Analysis of Quantified Cr and Translocation Rate

We observed that exogenous melatonin treatment decreased endogenous Cr concentration ($\mu\text{g/g DW}$) in leaf, stalk, and root by 71.53%, 11.45%, and 2.43%, under PD-induced stress. Furthermore, the observed decreasing trend of Cr translocation rate (%) between various organs was consistent with the endogenous quantified Cr. However, exogenous melatonin treatment reduced Cr translocation rates by 9.28% and 67.71% from root to stalk and stalk to leaf, with the greatest inhibition rate seen in root to leaf, which dropped by 70.83% (Table 2).

Table 2: Effects of melatonin and PD stress treatments on Cr quantification and translocation ratio in various tissues (leaf, stalk, and root) of melon seedlings. CK, Control; M, Melatonin; PD, Potassium dichromate. “–” notation denotes the no stress treatment; Mean $\pm$ SD ($n = 3$). Dissimilar statistical letters within a column indicate the differences among the treatments using independent samples t -test ($p < 0.05$).

Treatments	Endogenous	Quantified Cr ($\mu\text{g/g DW}$)	Translocation Rate (%)	Root to Stalk	Stalk to Leaf	Root to Leaf
	Leaf	Stalk	Root			
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
CK	–	–	–	–	–	–
M	–	–	–	–	–	–
PD	23.92 $\pm$ 1.65 ^a	110.26 $\pm$ 4.56 ^a	383.39 $\pm$ 2.76 ^a	28.77 $\pm$ 1.39 ^a	21.68 $\pm$ 0.88 ^a	6.24 $\pm$ 0.47 ^a
M + PD	6.81 $\pm$ 0.95 ^b	97.63 $\pm$ 3.20 ^b	374.06 $\pm$ 2.65 ^b	26.10 $\pm$ 0.89 ^b	7.00 $\pm$ 1.16 ^b	1.82 $\pm$ 0.26 ^b

3.8 Analysis of Gene Transcriptional Regulation Trend

To investigate the genetic effectiveness of melatonin in alleviating the inhibitory effect of Cr stress, the transcriptional regulation trends of the selected putative melon genes associated closely with chlorophyll and carbohydrate synthesis and a series of key metabolism pathways were examined by qPCR analysis as shown in the figure (Fig. 6). We noticed that the expression trend of the *GOGAT* gene (*MELO3C008481.2*) encoding ferredoxin-dependent glutamate synthase 1 and the *HEMA* gene (*MELO3C006296.2*) encoding glutamyl-tRNA reductase (GluTR), which catalyzes the biosynthesis of glutamate and tetrapyrrole in plastid, triggering the chlorophyll biosynthesis, reduced by 16.00% and 7.00% under PD stress as compared with CK, indicating that the limited accessibility of the substrate for chlorophyll synthesis. Meanwhile, the upregulated *PAO* gene (*MELO3C023571.2*) induced by PD stress, which increased by 202.00%, was consistent with the ever-increasing chlorophyll degradation to its final product.

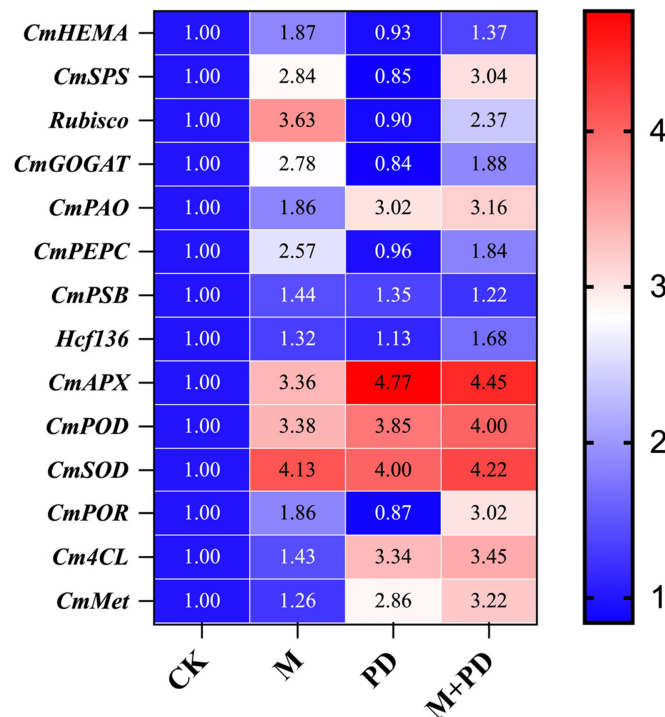


Figure 6: Heatmap of transcriptional expressions of the genes related to chlorophyll synthesis, antioxidant enzymes, metallothionein, and lignin synthesis in melon leaves under different treatments.

Similarly, the severely downregulated expression of the *SPS* gene (*MELO3C003715.2*) (15.00%), the *PEPC* gene (*MELO3C018724.2*) (4.00%), and the *Rubisco* gene (*MELO3C012180.2*) (10.00%), which catalyzed the sucrose synthesis and carbon fixation, further confirmed the reduced starch content. However, exogenous melatonin treatment showed the genetic improvement by increasing the expression level of the antioxidant-related genes, e.g., the *POD* gene (*MELO3C002242.2*, by 3.89%) and the *SOD* gene (*MELO3C017624.2*, by 5.50%) as compared with PD-stressed samples, and the *Rubisco* (*MELO3C012180.2*), *HEMA*, and *SPS* genes were upregulated by 163.33%, 47.31%, and 257.65%, respectively. Likewise, the expression level of the Met synthesis-related gene in melon (*CmMet*, *MELO3C016513.2*) and the lignin synthesis-related gene (*Cm4CL*, *MELO3C002346.2*) were also seemed to be enhanced by 3.29% and 12.59% under melatonin treatment. These results significantly showed the genetic improvement of photosynthetic, physiological, and biochemical traits of melon in response to different combination of treatments.

4 Discussion

Melon is a commercial Cucurbitaceae fruit crop at global scale but numerous abiotic stress factors greatly affect its plant growth, development, yield, and productivity [25,32]. Chromium, one of the heavy metal elements used in modern industry, has exhibited a major threat to agricultural productivity, particularly in organic matter-rich areas that are positively associated with heavy metal ions [48–52]. The significant accumulation of high heavy metal concentrations produce a variety of deleterious effects on plants activities, including growth inhibition, metabolic issues, and reproductive disorders [53–56]. Understanding the genetic basis of plant stress responses is critical for generating improved stress-tolerant cultivars for sustainable agriculture.

Seed is the fundamental component of the plant life cycle; nevertheless, seed germination is a well-known early stage that is vulnerable to chemical and adverse rhizosphere conditions [57]. Although the seed coat can operate as a protective barrier against the detrimental effects of heavy metal ions, most seeds coat surface triggers the dormancy phase and exhibit a decrease in associated germination and vigor in response to heavy metal stress [58–62]. According to Parr et al. [63] and Samrana et al. [64], chromium suppresses the germination rate as well as physiological, cytological, biochemical, and molecular responses in cotton seeds and acacia beans. According to Akinci et al. [65], the rate of melon seed germination and early seedling development decreased as the Cr content increased. However, the use of melatonin has been used for its essential and protective potential in modulating stress-induced germination [21,24,38]. Lei et al. [66] found that melatonin improved seed germination by increasing reserve mobilization, enhancing scavenging, and lowering ROS generation under Cr stress conditions. Li et al. [67] discovered that exogenous priming with melatonin reduced Cr stress during rice (*Oryza sativa*) seed germination. In our experiment, 100 μ M of melatonin treatment considerably increased the germination rate and related parameters of melon seeds under Cr-induced stress, which is a direct confirmation of melatonin's role as a potent growth-promoting, an effective bio-regulator and stress-alleviating agent endowing the sprout with a larger surface area and longer sprout length, assuring better germination as shown in the figure (Fig. 1). These findings strongly imply that melatonin has high antioxidant characteristics for reducing the detrimental stress impacts on sprouting and its further growth while also positively promoting the germination rate and growth status of melon seeds.

Photosynthesis activity in plants serves as the primary process for plants growth activities and organic matter accumulation, and is regulated by various genes and enzymes, but this process is highly sensitive to Cr stress [68,69]. Fv/Fm, NPQ, and qP are photochemical parameters used to measure the efficiency and health of the chlorophyll fluorescence and photosynthetic process in plants. They are calculated from the fluorescence emitted by chlorophyll molecules within Photosystem II (PSII) [12,32,70]. In the present

research, the Cr stress-induced hypoallergic plants showed a severe reduction in total chlorophyll content as shown in the figure (Fig. 3) and associated photochemical parameters (F_0 , F_v/F_m , and NPQ) as shown in the figure (Fig. 4), which broadly confirms the destruction level of photosynthetic efficiency as stated in the previous studies [37,70]. These abnormal events triggered endogenous physiological, molecular, and cytological (ultrastructural) changes in the chloroplast and thylakoid membrane as shown in the figure (Fig. 2) and similarly showed that Cr stress negatively affected the light-dependent photosynthetic reactions, as confirmed by the decreased MGDG and DGDG contents shown in the figure (Fig. 3), which are the primary components of the green tissue membrane [16]. However, the upregulated pheide a oxygenase (PAO) which catalyze the first step in the chlorophyll degradation pathway from pheide a to primary fluorescent chlorophyll catabolite along with the downregulated key genes (*HEMA* and *GOGAT*) catalyzed the synthesis of glutamate and tetrapyrrole in plastids, which trigger the total chlorophyll biosynthesis, as well as Rubisco and PEPC in the carbon fixation cycle, vastly limiting the substrate accessibility for chlorophyll synthesis and also causing degradable starch granules. In contrast, our application of melatonin significantly released the inhibitory effect on melon seedlings growth under Cr stress by continuing the leaf expansion and reducing leaf chlorosis as shown in results (Fig. 1, Table 1), preserved the intrinsic endogenic ultrastructure of melon leaves as shown in the figure (Fig. 2), and increased chlorophyll content at the molecular level, indicating a healthier chlorophyll cycle under stress. These results are consistent with the previous research findings [71–73], which demonstrated the protective role of melatonin in proper photosynthesis and pigmentation by retaining regular chloroplast structures and sustaining energy transfer efficiency under Cr stress.

It has been stated that the accumulation of Cr in melon seedlings is the initial stage of heavy metal ions entering the food chain, which requires great concern to find out effective methods in limiting Cr assimilation [74]. In our experiment, Cr accumulation occurred in the following organ sequence: root > stalk > leaf as shown in table (Table 2), possibly owing to its preferential property to accumulate in the root and the protective mechanism of shoots through hindering Cr mobility to aerial tissues of plant [2], but melatonin significantly reduced Cr concentration and translocation rate through root to shoot. These results are similar to the earlier findings in *Oryza sativa* and *Zea mays* cultivation under cadmium as well as chromium stress [75,76]. Besides, we assumed from our findings that the effectiveness of melatonin in reducing the toxicity of Cr is not limited to preventing the heavy metal ion transport but also relies on the specialization of cellular structure and detoxification protein by regulating primary and secondary metabolism. However, plants can reduce heavy metal permeation by immobilizing the cell wall when exposed to heavy metal stress.

Lignin acts as a key component in maintaining cell wall integrity by thickening the walls to restrict the entry of heavy metal ions. Its synthesis primarily depends on the phenylpropanoid pathway [77]. This pathway begins with phenylalanine, which is catalyzed by phenylalanine ammonia-lyase (PAL) to initiate the reaction, followed by 4-coumarate CoA ligase (4CL), which provides substrates for subsequent reactions. These processes ultimately lead to the production of various phenylpropanoid compounds, including lignin, flavonoids, and isoflavones [78]. In this study, physiological and transcript-level analyses showed that melatonin treatment increased lignin content and upregulated the expression of the *Cm4CL* gene, potentially enhancing resistance to chromium toxicity by promoting the accumulation of secondary metabolites that reinforce the cell wall, as illustrated in Fig. 7. Metallothionein (Met), as a cysteine-rich protein, chelates heavy metals due to their thiol groups, which significantly form intracellular chelation and detoxification when exposed to heavy metal stress [78]. We also found that PD stress induced Met accumulation across all organs (leaves, roots, and stalks) of melon seedlings, but exogenous melatonin treatment alleviated the accumulated Met as shown in the figure (Fig. 5). This finding aligns with a previous

report that melatonin application upsurges biosynthesis of Met in radish (*Raphanus sativus* L.) in response to cadmium stress [79]. Additionally, our melatonin application similarly mitigates the Cr toxicity through triggering the transcriptional level of the *CmMet* gene for biosynthesis of Met in melon as shown in the figure (Fig. 6), thereby restricting Cr transport within the plant organs.

SOD, POD, and APX are the main antioxidant enzymes protecting plants against stress-induced damages [32]. Most plants evolve defense mechanisms through the accumulation of antioxidant enzymes, which act as rapid protection mechanisms for scavenging excessive ROS under stress conditions [37]. Our study results exhibited the enhanced SOD, POD, and APX activities under Cr stress as shown in the figure (Fig. 3), especially for APX, increased 410.00% exceptionally exposed to PD stress on 1st day, reflecting an urgent and robust antioxidant response compared with SOD and POD, indicating that APX may be a priority antioxidant enzyme induced by PD stress, rapidly activating to mitigate acute oxidative damage in the early stress stage, which was similar to the previous researches [80,81]; however, the response of the antioxidants exposed to Cr stress differs by melatonin treatment. As the stress intensified, the antioxidant activities indicated a time-dependent reaction, with an initial upsurged followed by declined tendency.

MDA and H₂O₂ are the products of membrane lipid peroxidation under stress conditions, which also act as the vital signs to expose the peroxidation intensity induced through reactive oxygen species (ROS) as shown in the figure [82]. The overproduced ROS induced crucial oxidative destruction to the cell membrane is preliminary activity which happens in stressed plants. While in our study, application of melatonin significantly controlled the level of H₂O₂ and MDA by enhancing the antioxidant enzyme activities as results shown in the figure (Fig. 3), which may be indicative of the effectiveness of melatonin in releasing the adjust-limitation induced by Cr stress and strengthening the antioxidative defenses process to pursue ROS activity. This result also aligns with the previous research on melatonin application in mitigating oxidative stress in response to chromium and additional heavy metal-induced stresses [71,83]. Our current research evidence also suggested that the antioxidant property of these enzymes could act as a rapid response mechanism in plants to resist the stress conditions, while the application of exogenous melatonin serves as a supplementation that effectively enhances enzymatic activities to provide a continuous protective function to maintain metabolite homeostasis.

Additionally, the exogenous melatonin treatment enhanced the antioxidant activities, which is shown as attributed to the transcriptional regulation. Herein, our functional validation showed that the upregulated transcription levels of genes are catalyzing the biosynthesis of APX, SOD, and POD in response to increased Cr stress as shown in the figure (Fig. 6), which is related to the former results of Smeets et al. [84] and Zhang et al. [85]. Similarly, Altaf et al. [86] reported that exogenic melatonin can upregulate the antioxidant genes in pepper plant in response to chromium, nickel, cadmium, and vanadium-induced stress. Malik et al. [75] found that melatonin treatment enhanced the genetic modulation of antioxidant enzyme systems in response to Cr-induced stress in maize (*Zea mays* L.). The mechanism of melatonin treatment in controlling Cr concentration within melon seedlings is significant, while the efficiency of melatonin treatment varied between shoots and roots, and it is noteworthy that the protective property of melatonin to aboveground parts is more pronounced than to roots under PD stress [84–86]. In addition, our schematic diagram (Fig. 7) clearly illustrates the proposed model for morpho-physiological regulation via genetic modulation. It shows how melatonin alleviates chromium-induced oxidative damage by enhancing antioxidant enzyme activity and upregulating transcript levels. Meanwhile, the recent molecular evidences also provided new perspective for further exploring the mechanism of specific gene and pathways by genome editing and yeast-two-hybrid analysis to determine the hub gene in regulating Cr stress tolerance in crop plants [87,88].

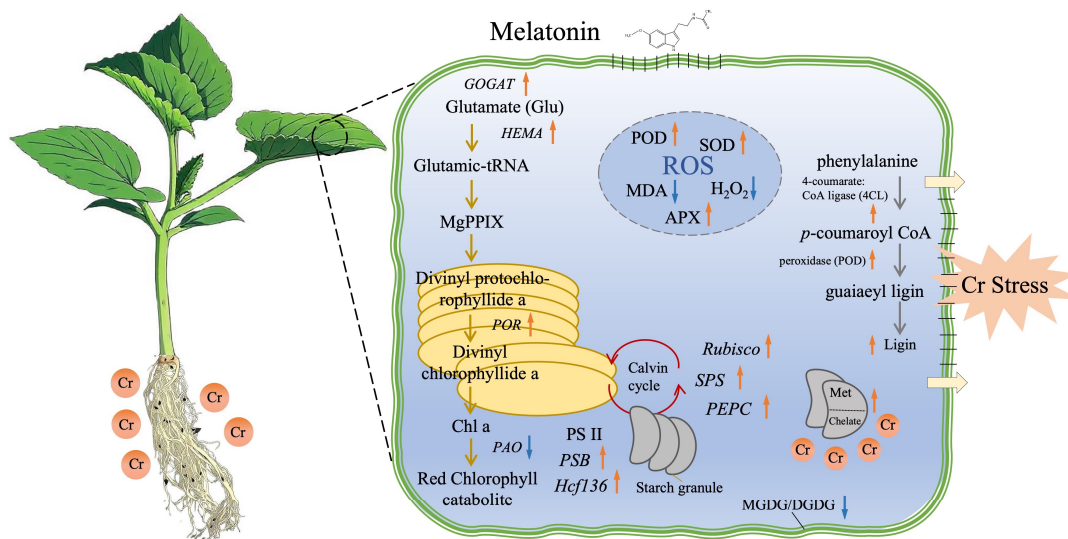


Figure 7: The schematic diagram illustrates the proposed regulatory network mechanism through which melatonin treatment enhances chromium (Cr) stress tolerance in melon plants. Orange arrows indicate an increase, while purple arrows represent a decrease in the indicators and genes associated with metabolite production.

5 Conclusion

In summary, our findings demonstrate that melatonin treatment effectively alleviates heavy metal toxicity and induced stress in melon plants through transcriptional regulation of gene. This regulation enhances growth dynamics and membrane oxidative status, reduces oxidative damage, promotes lignification, and inhibits chromium uptake. Consequently, melatonin treatment presents a promising and sustainable strategy for mitigating heavy metal toxicity in agricultural soils. However, further comprehensive studies integrating breeding, molecular genetics, and omics approaches are essential to gain deeper insights into functionally validated hub genes involved in genetic regulatory networks.

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