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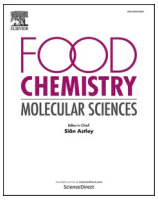

Effects of melatonin on inhibiting quality deterioration of postharvest water bamboo shoots

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Melatonin (PubChem CID: 896)
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Trichloroacetic acid (PubChem CID: 6421)
Acetylacetone (PubChem CID: 31261)
Tris (PubChem CID: 6503)
Acetone (PubChem CID: 180)
Ammonia aqueous (PubChem CID: 14923)
N, N-dimethylformamide (PubChem CID: 6228)
Methanol (PubChem CID: 887)

ABSTRACT

Water bamboo shoots (*Zizania latifolia*) is prone to quality deterioration during cold storage after harvest, which causes the decline of commodity value. Chlorophyll synthesis and lignin deposition are the major reasons for quality degradation. This paper studied the influence of exogenous melatonin (MT) on the cold storage quality of water bamboo shoots. MT treatment could delay the increase in skin browning, hardness and weight loss rate, inhibit chlorophyll synthesis and color change of water bamboo shoots, while maintain the content of total phenols and flavonoids, and inhibit lignin deposition by inhibiting the activity and gene expression of phenylpropanoid metabolism related enzymes as PAL, C4H, 4CL, CAD, and POD. The results indicate that exogenous MT treatment can effectively inhibit the quality degradation of cold stored water bamboo shoots.

1. Introduction

Water bamboo (*Zizania latifolia*), a perennial aquatic herb of gramineous family, is one of the popular aquatic vegetables in China. The fresh shoot of water bamboo is rich in protein, flavone, minerals and crude fiber, which are beneficial for human health. In addition, the crispy and tender texture is attractive to the consumer. However, the lignification (Yang et al., 2022) and browning, greening (Zhang, Murtaza, et al., 2021) rapidly happen in postharvest water bamboo shoots, which lead to the quality deterioration.

Lignin is a complicated aromatic polymer made up primarily of guaiacyl (G), p-hydroxyphenyl (H), and syringyl (S) lignins. (Zheng et al., 2023). Lignin is mainly deposited in the secondary thickened cell wall of plants, which is crucial for mechanical support and water transport (Luo, Xu, & Yan, 2008a; Zhao, Shi, et al., 2020). In postharvest shoots of water bamboo, lignin deposition is the major cause for the lignification (Yang et al., 2023). Lignin deposition is regulated by lignin biosynthesis pathway, which involves phenylpropane metabolism pathway (Luo, Xu, & Yan, 2008b). The first step in the production of lignin is triggered by phenylalanine ammonia-lyase (PAL), which deaminates L-phenylalanine to cinnamic acid (Qin et al., 2022). In addition to PAL, some other enzymes involved in lignin production include cinnamic acid 4-hydroxylase (C4H), carotene 3-hydroxylase (C3H), ferulate-5-hydroxylase (F5H), 4-coumarate-CoA ligase (4CL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD). In green asparagus (*Asparagus officinalis* L.), the lignin deposition is enhanced after harvest, which promoted the lignification of fresh shoots (Wang & Fan, 2019). It was shown that the lignin biosynthesis enzyme activities of PAL, CAD and POD were positively linked with the lignin accumulation in asparagus during postharvest storage (Lwin et al., 2020). In addition, it was

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further suggested that cell wall thickening was one of the deciding elements leading to lignification of asparagus (Pu et al., 2020). In water bamboo shoots, we previously found that CAD, POD and laccase (LAC) were activated during the process of postharvest lignification (Qi et al., 2020).

Color change is also one of the signs of plant aging. The main cause of browning is the degradation of chlorophyll (Xu et al., 2019). There are many types of chlorophyll, such as chlorophyll *a*, b, c, and d, among which higher plants related to food are mainly composed of chlorophyll *a* and b. Chlorophyll helps to maintain the appearance quality of fruits, but greening also indicated the quality deterioration of postharvest water bamboo shoots. The occurrence of green color in post harvested water bamboo shoots is related with chlorophyll content (Wen et al., 2019).

Melatonin (MT), a tryptophan derivative found in many animals and plants. In animals, MT synthesis occurs in the pineal gland, whereas in plants, MT synthesis occurs in a variety of organs (Aghdam et al., 2022; Zhang et al., 2020). MT participates in progress and growth, delays senescence of leaves and facilitate fruit maturity (Debnath et al., 2019). MT treatment has been widely applied to delay chilling injury, senescence and preserve various fruits and vegetables. In addition, as an antioxidant, MT can enhance tolerance of postharvest crops to abiotic and biological stressors by directly removing reactive oxygen species stresses, and can improve the efficiency of other antioxidants by activating the antioxidant system (Hernández-Ruiz & Arnao, 2018). It is reported that exogenous MT can delay the postharvest senescence of broccoli by reducing the respiratory rate and enhancing the activities of superoxide dismutase (SOD), catalase (CAT) and POD (Wei et al., 2020). Further research has shown that exogenous MT, as an efficient ROS scavenger, inhibits ROS accumulation and delays lignification in bamboo shoots (Li, Suo, et al., 2019) and chlorophyll degradation in Pak choi (Song et al., 2023).

Lignification and greening are the two most serious factors which limit the postharvest quality of water bamboo shoots. In present research, the influences of MT on inhibiting lignification and color change were analyzed and the mechanisms involved in these processes were investigated. The results will enrich the knowledge of fruit quality deteriorate and provide theoretical insight for the application of MT as a preservative of harvested crops.

2. Materials and methods

2.1. Plant materials

The fresh shoots of water bamboo (*Zizania latifolia* cv. 'Xiaolatai') with same size and without any injury, pests, diseases were randomly divided into two groups of 60 each. The shoots were immersed in water (control) and 100 μM MT solution (MT), respectively, for 20 min. The samples were air-dried and placed at 4 ± 1 °C, 85–90 % humidity for 28 d, then moved to 25 ± 1 °C for 3 d to imitate its shelf life. The samples (Middle part of water bamboo shoots, about 1 cm width of skin and flesh tissue) were chopped by stainless steel knife and collected at 0, 7, 14, 21, 28 and 28 + 3 d, then stored at −80 °C for subsequent physiological indicators and molecular experiments. All treatments and index determination were triply replicated.

2.2. Lignin staining

The cross-sections were cut from the base of each shoot. Phloroglucinol-HCL method was used for lignin staining (Qian et al., 2023). 200 μL 1 % phloroglucinol solution was added evenly on the cross section of water bamboo shoots, and add concentrated HCl after 3 min of staining to complete the Wiesner reaction.

2.3. Determination of color aberration, hardness and weight loss

The color aberration was measured with a hand-held color difference meter. Using white board as a control, determine L, a, b values of middle part of water bamboo shoots, ΔE represents the change of color difference of samples during storage.

The hardness was determined using a GY-1 hardness tester with a 0.4 cm probe diameter. The hardness was measured at the middle section of the water bamboo shoots based on 0.5 cm of the hardness tester entering the tissue, and expressed in $kg{\cdot}cm^{-2}$.

The weight loss rate was measured as [(weight of samples before storage−weight of samples after storage) × 100/weight of samples before storage].

2.4. Determination of ALA, PBG, ProtoIX, Mg-ProtoIX and Pchl contents

For 5-aminolevulinic acid (ALA) determination, the sample (1 g) was powdered in 4 % trichloroacetic acid (5 mL), 8000 × *g* centrifuged for 15 min. 2.35 mL of sodium acetate (1 M) and 0.15 mL of acetylacetone were mixed with 5 mL supernatant. After boiling for 10 min in water, the combinations cooled to room temperature. For porphobilinogen (PBG) determination, the sample (0.5 g) was crushed in 5 mL extraction solution (0.6 M Tris, 0.1 M EDTA, pH 8.2), 10000 × *g* centrifuged for 10 min. The content of ALA and PBG was determined using method of Zhao, Hao, et al. (2020). 2 mL of mixtures were added with 2 mL of Ehrlich Hg and placed in total darkness for 15 min. The absorbance at 553 nm was measured. Then the contents of ALA and PBG were estimated with the standard curve.

The determination of ProtoIX, Mg-ProtoIX, and Pchl were slightly modified the method reported (Aras et al., 2021). Sample (0.5 g) was powdered in 5 mL of 80 % alkaline acetone, 8000 × *g* centrifuged for 15 min at 4 °C. All supernatant was diluted to 25 mL using 80 % alkaline acetone. The contents were calculated using the following formulas.

$$\text{ProtoIX} = 0.18016OD_{575} - 0.04036OD_{628} - 0.04515OD_{590}$$

$$\text{Mg} - \text{ProtoIX} = 0.06077OD_{590} - 0.01937OD_{575} - 0.003423OD_{628}$$

$$\text{Pchl} = 0.03563OD_{628} + 0.007225OD_{590} - 0.02955OD_{575}$$

2.5. Determination of chlorophyll contents

Sample (0.5 g) was homogenized in 3 ml of N, N-dimethylformamide (DMF), 10000 × *g* centrifuged for 10 min. The content of chlorophyll was calculated using the method of Li, Zhang, Li, et al. (2019).

$$\text{Chl a} = 12.70OD_{664.5} - 2.79OD_{647}$$

$$\text{Chl b} = 20.70OD_{647} - 4.62OD_{664.5}$$

$$\text{Total Chl} = 17.90OD_{647} + 8.08OD_{664.5}$$

2.6. Determination of total phenol and flavonoid contents

Sample (2 g) was homogenized in 5 mL of 5 % HCL methanol solution. An absorbance of 765 nm was measured. The standard curve was made with gallic acid, the total phenol content was estimated using tissue per gram of fresh weight (Zhang, Yang, et al., 2021).

The total flavonoid contents was done using the method of Tao et al. (2020). Frozen tissue extract (0.2 mL) was diluted to 5 mL. After 5 min, 0.3 mL 5 % $NaNO_2$ was added to the diluent, followed by 0.6 mL 10 % $AlCl_3$, 2 mL NaOH (1 M) and 2.1 mL distilled water. Absorbance at 510 nm was measured. The standard curve was drawn with rutin, and total flavonoid contents were estimated with the tissue per gram of fresh weight.

2.7. Determination of lignin content

The lignin content was analyzed by L-3000 high performance liquid chromatography (HPLC) (RIGOL, Suzhou, China) method developed by Qian et al. (2023). The sample (0.5 g) was extracted twice with ethyl acetate, dried in nitrogen, and redissolved in 0.5 mL methanol. Elution of the 10 μL sample was performed using a 25:75 (A:B, v/v) ratio of methanol (A; 100 %) and 0.03 M sodium acetate buffer (B; pH 3) at a flow rate of 1 mL·min^{-1}. Each lignin's concentration was calculated by comparing peak area to standard value.

2.8. Determination of enzyme activity related to lignin synthesis

Frozen tissue (2 g) was homogenated in 5 mL sodium borate buffer (0.2 M, pH 8.7, containing 0.1 % β-Mercaptoethanol and 1 % PVPP), 10000 × *g* centrifuged for 15 min at 4 °C. Supernatant (0.5 mL) was added to 2.5 mL Tris-HCL buffer (50 mM, pH 8.9, 16 mM L-phenylalanine, 3.6 mM NaCl) and incubated at 37 °C for 1 h. The change of 0.01 OD_{290}/min was defined as one unit (U) of PAL (Aghdam et al., 2020).

Frozen tissue (4 g) was homogenated in 10 mL Tris-HCL buffer solution (0.1 M, pH 7.5, 25 % glycerol, 0.1 M dithiothreitol), 10000 × *g* centrifuged at 4 °C for 15 min. For C4H activities determination, the mixture (3 mL) included 50 mM PBS buffer solution (pH 7.5), 2 mM *trans*-cinnamic acid, 0.5 mM NADPH, and 0.5 mL the supernatant. The change of 0.01 OD_{290}/min was defined as one unit (U) (Li, Zhang, Xu, et al., 2019). For 4CL activities determination, the mixture (2.5 mL) included Tris-HCL buffer (pH 7.5), 0.2 mM p-coumaric acid, 7.5 mM $MgCl_2$, 1 mM ATP, 40 mM CoA and 0.5 mL enzyme extract. The change of 0.01 OD_{333}/min was defined as one unit (U) (Li, Zhang, Xu, et al., 2019).

Frozen tissue (3 g) was homogenated in 5 mL Tris-HCL buffer (200 mM, pH 7.5), 10000 × *g* centrifuged at 4 °C for 15 min. The 3 mL mixture included 100 mM sodium phosphate buffer (pH 6.5, 3 mM NADP+, 3.2 mM *trans*-cinnamic acid) and 0.5 mL enzyme extract. The change of 0.01 OD_{340}/min was defined as one unit (U) of CAD (Suo et al., 2018).

Sample (3 g) was homogenated in 5 mL sodium phosphate buffer (200 mM, pH 6), 10000 × *g* centrifuged at 4 °C for 15 min. For G-POD activities determination, the mixture (3 mL) included 50 mM sodium phosphate buffer (pH 6.0), 5 mM guaiacol, 5 mM H_2O_2 and 0.5 mL enzyme extract. The change of 0.01 OD_{470}/min was defined as one unit (U) (Qian et al., 2023). For S-POD activities determination, the mixture (3 mL) included 50 mM sodium phosphate buffer (pH 6.0), 0.05 mM syringolin, 0.2 mM H_2O_2 and 0.5 mL enzyme extract. The change of 0.01 OD_{530}/min was defined as one unit (U) (Qian et al., 2023).

2.9. Transcriptome analysis

Transcriptome analysis was performed on the water bamboo shoots stored for 14 days (control, melatonion). Total RNA isolation, integrity analysis, library detection, and sequencing followed Qian et al. (Qian et al., 2023).

2.10. qRT-PCR experiment

Relevant genes (Table S1) for *qRT-PCR* were chosen based on the sequencing results. Primer Premier 6.0 software was used to construct the primers (Table S1) and synthesized by GENEWIZ (Suzhou, China). Action (He et al., 2020) was the internal reference gene. For *qRT-PCR*, ChamQ Universal SYBR qPCR Master Mix kit (Vazyme, Nanjing, China) was used.

2.11. Data processing and analysis

SPSS 24.0 was used for variance analysis and Turkey method for difference significance. A significant difference was indicated by $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Effects of MT treatment on the appearance, lignin deposition, weight loss and hardness

As shown in Fig. 1A and D, the skin wilting and color change (browning dan greening) of water bamboo shoots happened during storage. In addition, the lignin deposition was found in the shoots after 7 d storage, it was enhanced as the elongation of storage. The weight loss and hardness of water bamboo shoots increased during storage, especially during the shelf life (Fig. 1B and C). MT treatment significantly decreased the ratio of weight loss and hardness in comparison with control, and effectively inhibited the skin wilting, greening and lignin deposition, especially after 14 d storage (Fig. 1).

3.2. Differential expressed genes analysis (C14 vs M14)

In this study, differential expressed genes (DEGs) analysis was performed between the control and MT-treated water bamboo shoots at the 14th d of cold storage. The C14 vs M14 DEGs included 5979 genes, 2112 of which were up-regulated and 3867 down-regulated. The most prevalent KEGG pathways with up-regulated DEGs were plant hormone signal transduction, starch and sucrose metabolism and MAPK signaling (Fig. 2A). Most of the down-regulated DEGs were related to plant-pathogen interaction, plant hormone signal transduction and MAPK signaling (Fig. 2B). In addition, MT-treated shoots also downregulated plant-pathogen interaction, plant hormone signal transduction, MAPK signaling pathway-plant, starch and sucrose metabolism, protein processing in endoplasmic reticulum, carbon metabolism, phenylpropanoid biosynthesis and other pathways (Fig. 2C).

3.3. Effects of MT treatment on chlorophyll metabolism

This study examined the chlorophyll biosynthesis pathway to reveal the influences of MT treatment on genes involved in chlorophyll metabolism. L-Glutamate is used as the basic material in the biosynthesis of chlorophyll to produce ALA. Porphobilinogen synthase (ALAD) catalyzed the conversion of ALA to PBG. Protoporphyrin III oxidase (PPOX), Mg protoporphyrin IX monomethyl ester cyclase (CHlE), protochlorophyllide reductase (POR), and other enzymes are then responsible for producing protochlorophyllate (Pchl), Mg protoporphyrin IX (Mg-ProtoIX), and eventually chlorophyll *a* (Fig. 3A). *ZlSGR* and *ZlPAO* expression decreased in MT-treated water bamboo shoots compared to the control (Fig. 3A).

The ALA content increased during the first 14 d of storage and then decreased till the end (Fig. 3B). The PBG concentration of water bamboo shoots gradually decreased during the early storage period, and gradually increased after 7 d of storage, reaching a peak at the 14th day. At this time, the PBG content in MT treated water bamboo shoots was 0.691 times that of the control, significantly lower than the control (Fig. 3C). The content of Mg-ProtoIX decreased in all shoots during storage, except a sharp increase exhibited in MT treated shoots during shelf life (Fig. 3D). The ProtoIX content decreased to a valley at the 21th d of storage, the MT treatment declined the content during whole storage (Fig. 3E). The content of Pchl showed stability in the early and middle storage stage and a dramatic decrease in the later stage. MT treatment could inhibit the accumulation of Pchl during the early stage of cold storage, and sudden content increase appeared during shelf life (Fig. 3F). Chlorophyll *a*, b and total chlorophyll content all displayed upward trend, and the contents exhibited sharp rises during shelf life in all shoots. Throughout the storage period, the MT treated shoots had lower levels of chlorophyll *a*, b and total chlorophyll than the control (Fig. 3G, H and I).

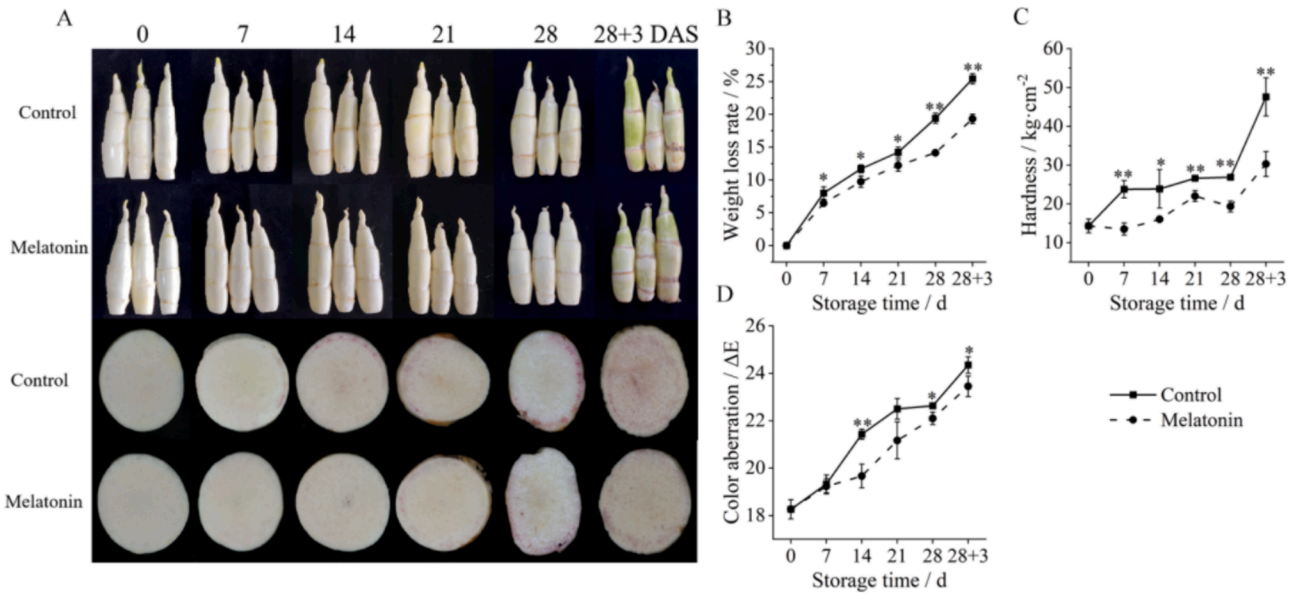

Fig. 1. Effect of MT treatment on the appearance, lignin staining and sensory quality of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

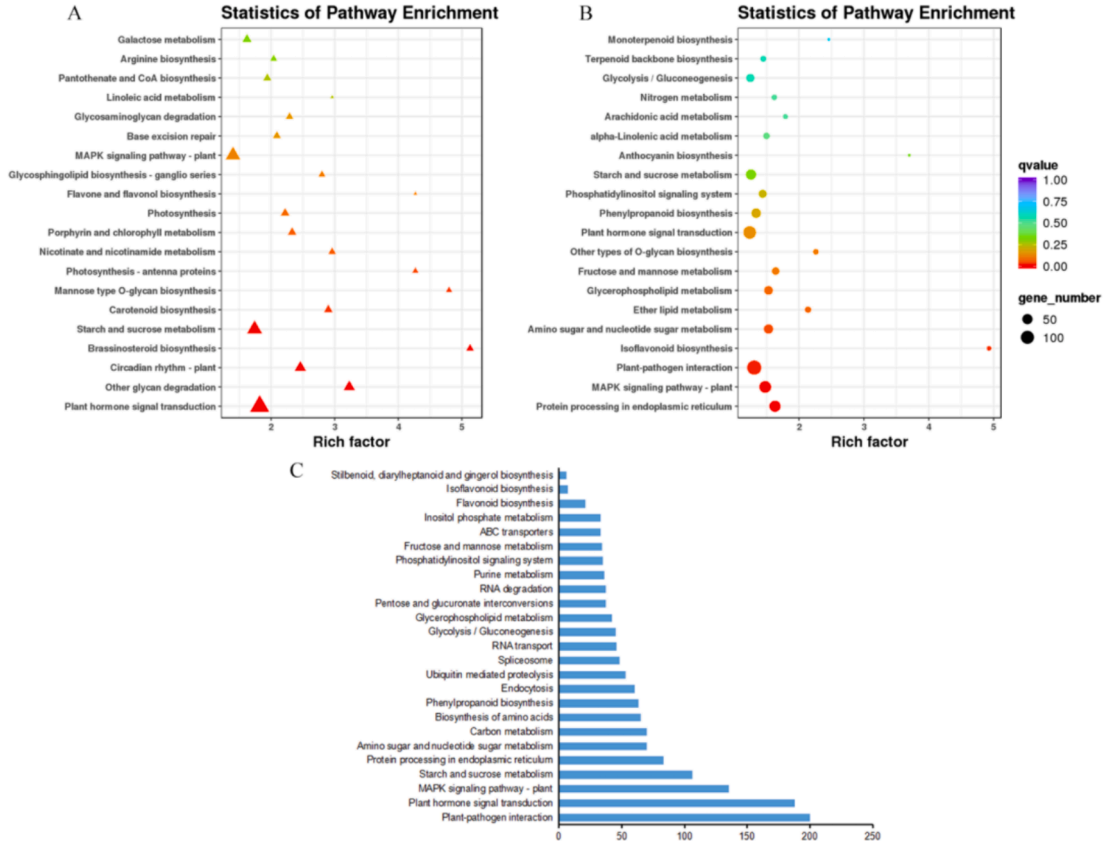

Fig. 2. Gene expression differences between the control and MT treated groups during 14 days storage of water bamboo shoots. Up (A) and down (B) regulated metabolic pathways and the number of genes associated with these pathways. (C) The main metabolic pathways of commonly down-regulated genes as well as the number of DEGs in each pathway in the MT treated group compared with the control.

3.4. Effect of MT treatment on relative expression level of chlorophyll metabolism related genes

Pheophorbide a oxygenase (PAO) can oxidize chlorophyll, thereby turning green into colorless. *ZlPAO1*, *ZlPAO2* and *ZlPAO3* expression first dropped and subsequently increased during cold storage, peaks exhibited at 28th (*ZlPAO1*), 21th (*ZlPAO2* and *ZlPAO3*) d of storage, MT group showed lower expression level of *ZlPAO* in water bamboo during storage than control (Fig. 4A, 4B and 4C). CH1E can affect the production of divinylprotochlorophyllide. The *ZlCH1E* expression exhibited a

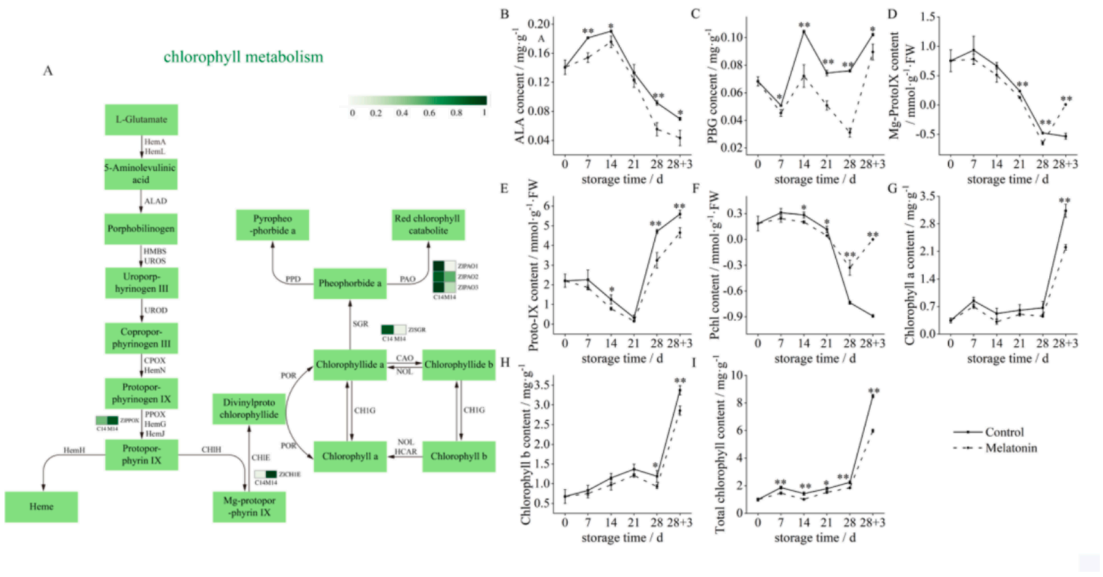

Fig. 3. Effect of MT treatment on chlorophyll metabolism of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

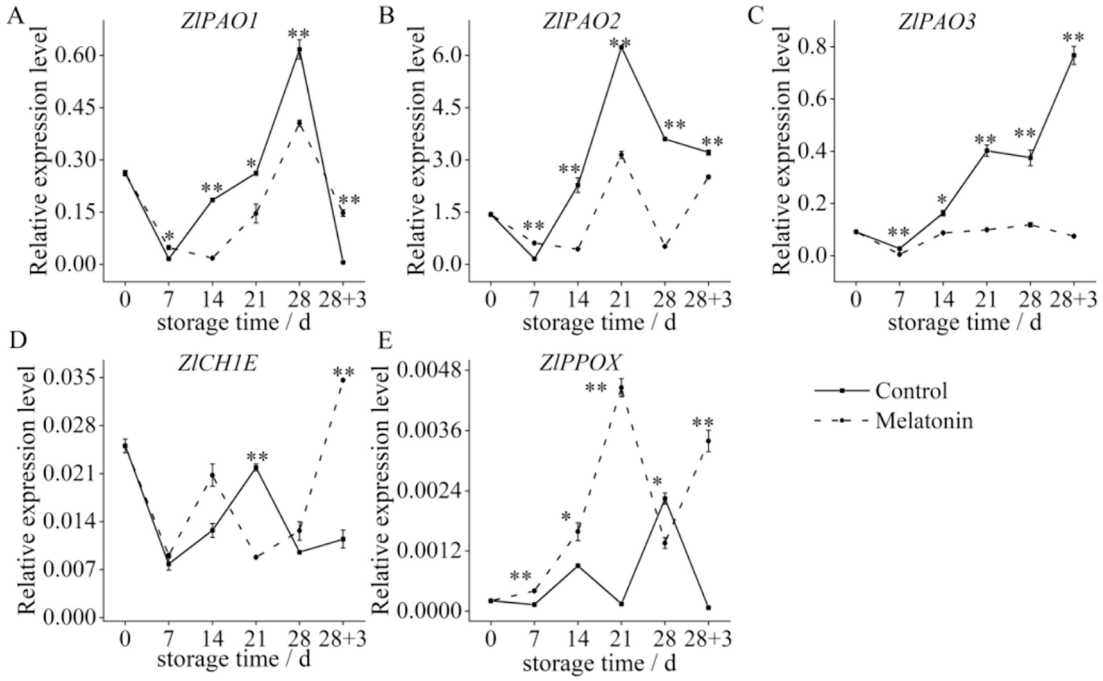

Fig. 4. Effect of MT treatment on expression level of DEGs in chlorophyll metabolism of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

"W" pattern, MT treatment advance the peak from 21th d (control) to 14th d (MT), and dramatically enhanced *ZlCH1E* expression during shelf life (Fig. 4D). PPOX can catalyze the oxidation of protoporphyrinogen III to protoporphyrinogen IX. The *ZlPPOX* expression level increased to peaks at 14th d (control) and 21th d (MT), MT treatment increased *ZlPPOX* expression level during storage, except the end of cold storage (Fig. 4E).

3.5. Effect of MT treatment on the lignin biosynthesis

This study investigated the lignin biosynthesis pathway to determine how MT treatment affected lignin biosynthesis genes. Based on KEGG, eight lignin biosynthesis enzymes were identified: PAL, C4H, 4CL, caffeoyl-CoA O-methyltransferase (CCoAOMT), cinnamoyl-CoA reductase (CCR), CAD and POD. Under the action of C3H, 4CL, CCoAOMT, CCR, F5H, caffeic acid 3-O-methyltransferase (COMT), CAD, and POD, phenylalanine deaminates to trans cinnamic acid and hydroxylation to trans 4-coumaric acid. As showed in Fig. 5, MT treatment downregulated *ZlPALs*, *ZlC4Hs*, *Zl4CLs*, *ZlCCRs*, *ZlPODs*, and *ZlCADs*.

3.6. Effect of MT treatment on the total phenol content, flavonoid content and lignin content

Further screening of KEGG pathways related to total phenol and total flavonoid metabolism revealed that significantly more downregulated genes than upregulated genes were found in C14 vs M14 (Fig. 5). The

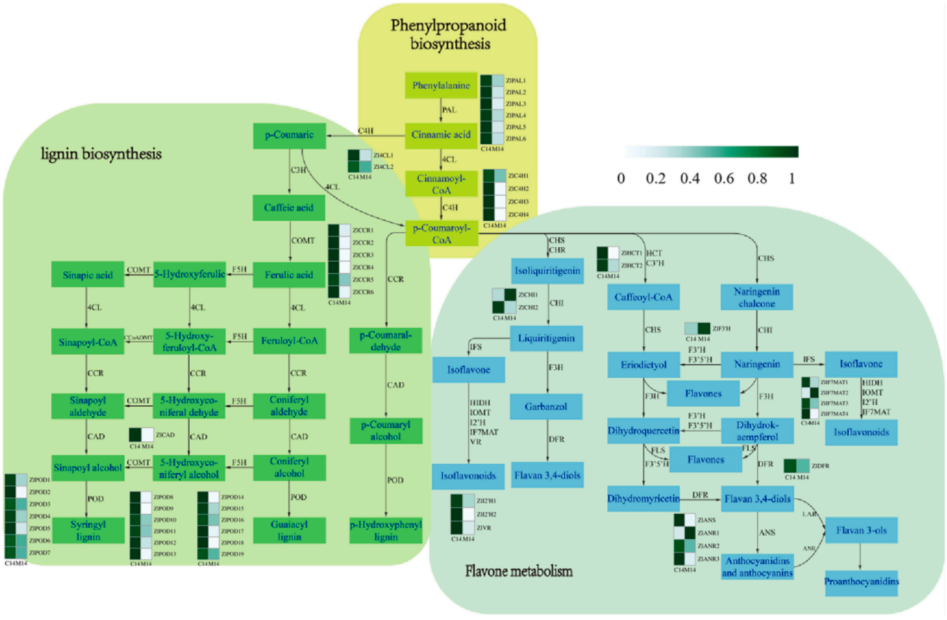

Fig. 5. Effect of MT treatment on lignin biosynthesis of water bamboo shoots during cold storage.

phenol content of the control decreased during storage, especially during the first 7 d of cold storage, while that of the MT treatment group increased and then decreased (Fig. 6A). Total flavonoid content of control group declined persistently, whereas the content of MT group is much higher and increased after 21 d of storage (Fig. 6B). MT treatment delays total phenol and flavone breakdown during cold storage in water bamboo shoots (Fig. 6A and B). Total lignin content of water bamboo shoots exhibited an upward trend during cold storage. The most abundant lignin was G-lignin, accounting for more than 80 % of the total lignin, while S-lignin was the least, accounting for less than 5 %. The MT treatment inhibited lignin synthesis, especially the synthesis of G-lignin. This was consistent with phloroglucinol-HCL staining of water bamboo shoot cross sections (Fig. 6C, D, E and Fig. 1A).

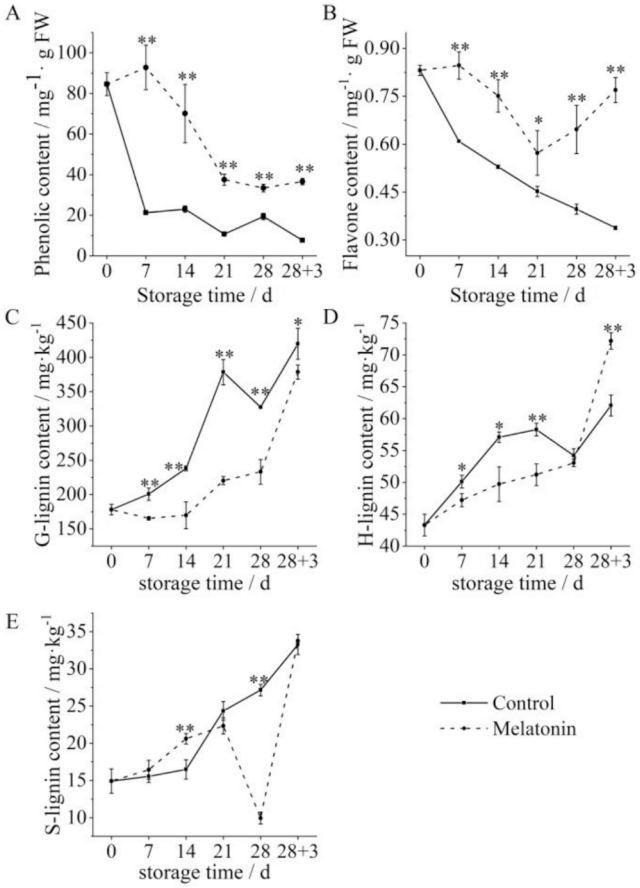

Fig. 6. Effect of MT treatment on total phenol, flavonoid and lignin contents of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

3.7. *Effects of MT treatment on lignin synthesis related enzymes activities*

The synthesis of lignin in plants involves phenylpropane metabolism pathway, which is catalyzed by a series of enzymes, including PAL, C4H, 4CL, CAD, POD, etc. PAL activity in two groups both showed an upward trend during storage, the control reached a peak at the 21st d of cold storage, while MT treatment significantly reduced PAL activity, and delayed the peak to the 28th d (Fig. 7A). The C4H activity in two groups was both declined and peaks showed at the 14th d, and MT could inhibit C4H activity, except it significantly increased the activity at the end of cold storage (Fig. 7B). The 4CL activity in all shoots declined to the 21th day and then increased, and MT treatment effectively restrained the activity (Fig. 7C). CAD activity in control group increased somewhat over the first 14 d of cold storage, then significantly increased and peaked at the 21th day. The MT treatment dramatically reduced CAD activity and delayed the development of the peak value. The inhibition rate of CAD in the MT treatment group was 50.7 % after 21 d of cold storage compared to control (Fig. 7D). S-POD activity initially reduced and then increased to peaks at the 14th (MT group) and 21th (control group) d during cold storage, with MT group S-POD activity considerably higher than control at the peak (Fig. 7E). The G-POD activity of control group decreased after 7 d of cold storage, but then increased rapidly and maintained its activity. MT treatment could reduce the G-POD activity and inhibit its increase. The G-POD activities of MT group were much lower than that in control during the middle and late storage (Fig. 7F).

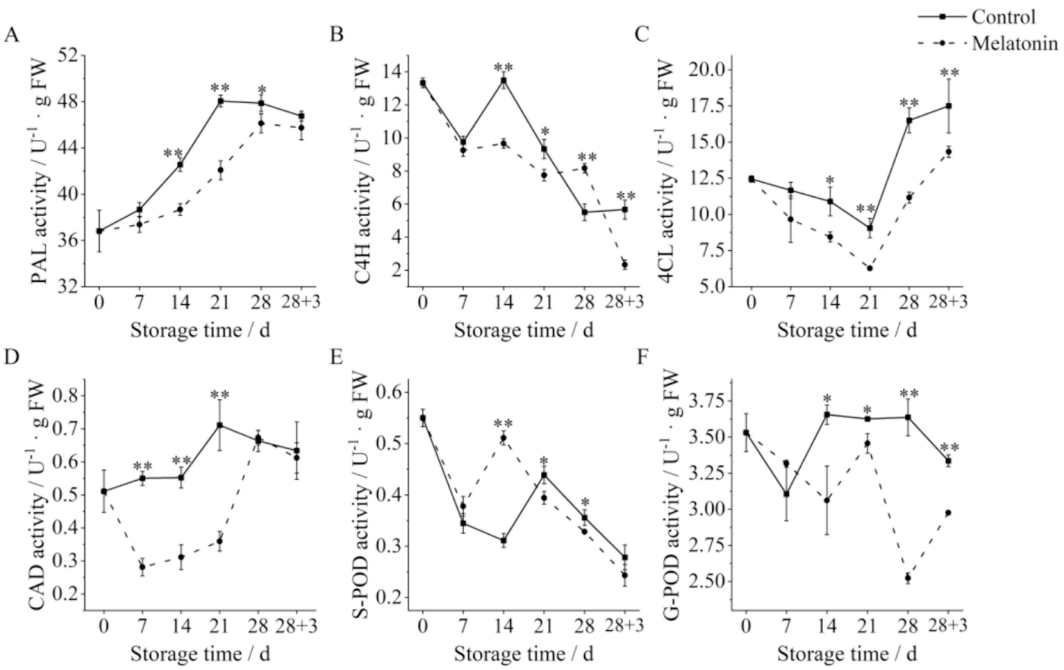

Fig. 7. Effect of MT treatment on enzymes related to lignin synthesis of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

3.8. Effects of MT treatment on relative expression level of phenylpropane metabolism related genes

The lignin concentration of postharvest water bamboo shoots increased continually, PAL, 4CL, C4H, CAD, POD, and other enzymes, as well as their gene expression, regulated lignin synthesis and accumulation. The expression of *ZlPAL* in all shoots increased to peaks at the 21th d of cold storage and both sharply enhanced during shelf life, MT treatment significantly inhibited the expression of *ZlPAL* (Fig. 8A). The expression of *Zl4CL* of control increased to a peak at the 14th day of cold storage, while MT treatment delayed the peak production and inhibited the expression of *Zl4CL* in the early and middle storage period (Fig. 8B). The expression level of *ZlC4H* in water bamboo shoots waved and increased during shelf life (Fig. 8C). The expression of *ZlCAD* increased to peaks at the 14th d of cold storage (Fig. 8D). The expression of *ZlPOD1*and *ZlPOD2* in the control group increased about 9.1 times and 4.5 times respectively during the whole storage (Fig. 8E and 8F). During storage, MT treatment slightly reduced the expression level of *ZlC4H*, *ZlCAD*, *ZlPOD1* and *ZlPOD2* (Fig. 8C, D, E and F).

4. Discussion

MT, an indoleamine hormone derived from tryptophan, is found in many organisms (Sati et al., 2022). In plants, MT is regarded as a novel type of preservative capable of greatly delaying senescence. Exogenous MT has been shown to postpone quality deterioration in sweet cherries (*Prunus avium* L.) (Wang et al., 2019), as well as to increase the quality of yellow-flesh peach fruit during storage (Wu et al., 2023). In addition,

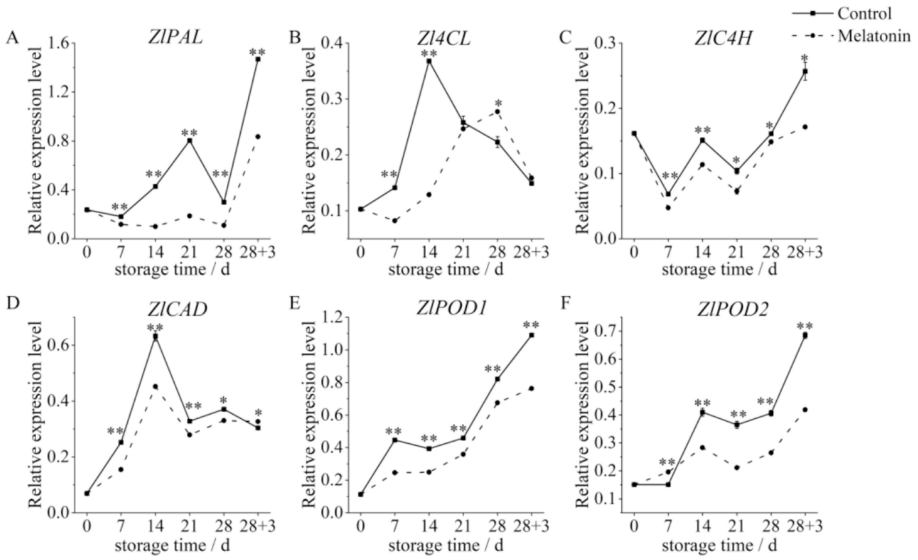

Fig. 8. Effect of MT treatment on expression level of DEGs in lignin biosynthesis of water bamboo shoots during cold storage. * indicates significance at the 0.05 level; ** indicates significance at the 0.01 level.

MT treatment could inhibit lignification of postharvest bamboo shoot during cold storage, as expressed in this research (Li, Suo, et al., 2019). In this work, exogenous MT administration prevented chlorophyll metabolism and lignin deposition in postharvest refrigerated water bamboo shoots (Fig. 1).

The greening of the epidermis is the most direct factor that used to evaluate the commercial value of water bamboo. The UV-C treatment significantly reduced chlorophyll production in water bamboo, and the increase in green color was directly proportional to chlorophyll concentration (Wen et al., 2019). Chlorophyll biosynthesis includes the production of chlorophyll *a* and *b*. PPOX is the last enzyme in the chlorophyll biosynthesis pathway. The up-regulated chlorophyll synthesis genes were crucial to promote the synthesis of chlorophyll in citrus peels (Xie et al., 2019). ALA and PBG are precursors of chlorophyll biosynthesis. In this study, the ALA, Mg-protoIX, Pchl showed similar change trends with chlorophyll during early storage stage (Fig. 3), implied the ALA, Mg-protoIX, Pchl were direct factor that affected the chlorophyll synthesis. The sharp decline of ALA, Mg-protoIX, Pchl content during late storage (Fig. 3), indicated great consumption used in the accumulation of chlorophyll.

MT has been found in previous research to lower ALA and PBG levels (Menezes et al., 2023). In addition, MT may destroy the structure and function of chloroplasts, leading to the reduction of chlorophyll concentration (Sarropoulou et al., 2012). The MT treated water bamboo shoots showed lower chlorophyll synthesis intermediates (ALA, PBG, Mg-protoIX, Proto-IX, Pchl) content, and caused lower chlorophyll content (Fig. 3). The MT treatment significantly declined chlorophyll content during shelf life, when great accumulation of Mg-protoIX and Pchl appeared (Fig. 3). This phenomenon implied the great restrain of ALA accumulation by MT treatment in water bamboo shoot during storage may the main cause of chlorophyll synthesis was inhibited. The up-regulation of synthetases (*PPOX*, *CHIE*) and down-regulation of degrading enzymes (*PAO*, *SGR*) (Figs. 3, 4) did not lead to the enhanced accumulation of chlorophyll, because lack of substrate (ALA).

Lignin was involved in the cross-linking of lignin polymers, cell wall polysaccharides and proteins (Feijao et al., 2021), which may lead to the accumulation of lignin complexes, thus increasing the hardness of water bamboo shoots. This study discovered that MT treatment after harvest can successfully reduce the increase of hardness and lignin content (Figs. 1, 6), which is consistent with report that ozone treatment inhibited the hardness and lignin accumulation of postharvest water bamboo shoots (Liu et al., 2022). The biosynthesis of lignin is crucial for the lignification process. Phenols, as precursors of lignin synthesis, play a key role in lignin deposition (Fig. 5). The reduction in total phenol concentration in water bamboo shoots coincided with the increase in hardness and lignification (Figs. 1, 6), which was similar with previous findings that phenol oxidation could enhance lignin formation (Pan et al., 2020). MT delayed the reduction of total phenol content of water bamboo shoots during cold storage in contrast to the control group, which was similar to EAWP (electrostatic atomized water particles) delayed the reduction of phenol content, and the high total phenol content in asparagus reduced the deposition of lignin during storage, indicated that transformation of phenols to lignin was inhibited (Lwin et al., 2020). Flavonoids are also phenolic chemicals (Sun et al., 2019). MT greatly inhibited the reduction of flavonoids in cold stored water bamboo shoots (Fig. 6), similar to report that MeJA delayed the reduction of total flavonoids in medlar fruit (Ozturk et al., 2019). The change trend of phenolic content of all water bamboo shoot during cold storage was similar with the change trend of flavone content (Fig. 6).

Phenylpropane metabolic pathway is the most important pathway in lignin biosynthesis. In phenylpropane metabolism pathway, phenylalanine is deaminated under the action of PAL to form trans cinnamic acid, and hydroxylated under the action of C4H to form *trans*-4-coumaric acid (Qian et al., 2023). Then, three lignin monomers, H-lignin, G-lignin and S-lignin, are generated under the action of C3H, 4CL, CCoAOMT, CCR, F5H, COMT, hydroxycinnomoyl transferase (HCT), Chalcone synthase (CHS), CAD and POD. The enzymes PAL, 4CL, C4H, CAD, and POD are essential for lignin production (Fig. 5). Transcriptome analysis identified 43 lignin biosynthesis genes, most of which showed an increased trend, such as *ZlPAL*, *Zl4CL*, *ZlCAD*, *ZlPOD1*, and *ZlPOD2* (Fig. 8). These findings demonstrated that lignin biosynthesis reached an active stage after 14 d of cold storage, with a substantial number of lignin monomers produced. The up regulation of *ZlPAL*, *ZlCAD* and *ZlPOD* expression level were consistent with the change of corresponding enzyme activity and lignin accumulation trends (Figs. 7, 8), which indicated that PAL, CAD and POD regulate lignin biosynthesis at the transcription level. The expression levels of *AgCAD* and *AgPAL* in three celery types (*Apium graveolens* L.) were positively linked with lignin content (Ding et al., 2020). The decrease of PAL activity might result in the decrease of lignin content (Hu et al., 2017).

MT is implicated in various plant responses to biotic and abiotic stress. The phenylpropanoid pathway is the primary secondary metabolic pathway in plants for stress resistance (Li, Xu, et al., 2019). MT treatment suppressed the activity of essential lignin production enzymes (PAL, 4CL, CAD, and POD), significantly delaying lignification of loquat (*Eriobotrya japonica* Lindl.) (Wang et al., 2021). MT treatment significantly reduced the hardening of water bamboo shoots during cold storage, delayed lignin accumulation, and inhibited PAL, 4CL, C4H, CAD, and POD activity (Figs. 1, 6, 7). The expression level of phenylpropanoid metabolism-related genes in water bamboo shoots treated with MT was significantly lower than the control throughout storage (Figs. 5, 8), which was consistent with the report that MT treatment delayed the senescence of asparagus after harvest, hindered PAL and POD activities, reduced the lignin content, and thus delayed the increase of hardness (Boonsiriwit et al., 2021). Thus, MT treatment effectively inhibited lignin deposition by the down-regulation of lignin deposition related genes, thus delayed the process of lignification during storage.

5. Conclusions

The effects of exogenous MT on cold storage quality of water bamboo shoots were investigated in this study. The results indicated that MT treatment could effectively inhibit the increase of weight loss, hardness and color aberration, so maintained the storage quality of water bamboo. Transcriptome and metabolic pathway analysis revealed MT treatment suppressed the synthesis of chlorophyll and lignin by inhibiting synthetic substrate content, enzyme activity and gene expression related to chlorophyll and phenylpropanoid metabolism. Further research on how exogenous MT regulate chlorophyll metabolism and lignin biosynthesis will aid in understanding the unique role of MT in the postharvest preservation of water bamboo shoots.

CRediT authorship contribution statement

Chunlu Qian: Methodology, Conceptualization. **Yan Sun:** Writing – original draft, Investigation. **Bei Zhang:** Investigation. **Yuyang Shao:** Investigation. **Jun Liu:** Visualization, Validation. **Juan Kan:** Investigation. **Man Zhang:** Investigation. **Lixia Xiao:** Conceptualization. **Changhai Jin:** Conceptualization. **Xiaohua Qi:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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