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Impact of melatonin application on lignification in water bamboo shoot during storage

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A R T I C L E I N F O

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A B S T R A C T

Melatonin, a crucial bioactive molecule, involved in several physiological processes in plants. This study investigated the effects of melatonin (MT) treatment on lignification, including firmness, lignin, lignified-enzyme activities, the expression patterns of genes encoding corresponding enzymes and transcription factors in water bamboo shoot during storage for 8 days. MT treatment decreased the firmness and content of lignin. It inhibited the degradation of total phenols and ascorbic acid and delayed the lignin biosynthesis, via reducing the activities of phenylalanine ammonia-lyse cinnamyl alcohol dehydrogenase and peroxidase, as well as lignin biosynthesis-related genes expression levels. Transcription factors of *ZlNAC1, ZlNAC2, ZlNAC3* and *ZlNAC4* from NAC family and *ZlMYB1* and *ZlMYB2* from MYB family were increased in water bamboo shoot after harvest and MT-treated markedly reduced their expression. Therefore, our findings supply a fundamental understanding of MT treatment suppression of lignification and establish a foundation for further research on transcriptional regulation.

1. Introduction

Water bamboo shoot (*Zizania latifolia* (Griseb.) Stapf), commonly known as the 'Jiaobai' in China, is a fascicular and perennial aquatic plant that grows in water, which is a commercially cultivated edible vegetable in Asia (Ye, He, Zhang, Wang, & Wang, 2020). The swollen stem is an edible part with dietary fiber. Water bamboo shoots rapidly worsen (especially lignification and texture toughing) during storage, which reduced the commercial quality and market value. Therefore, repressing lignin biosynthesis is very important to maintain the fresh quality during storage.

The accumulation of lignin produces lignification, which affects the texture, taste and nutrition of fruit and vegetables during storage. There are some researches on transcriptional regulation mechanisms of lignin synthesis in some plants (Xu et al., 2014). Lignin is a complicated substance that constitutes a crucial part of secondary cell walls in higher plants, frequently associated with tissue firmness (Toscano, Ferrante, Leonardi, & Romano, 2018). Lignin consists of three monomers, p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) (Xu et al., 2015). The three monomers are biosynthesized through the phenylpropanoid metabolism, subsequently polymerized to the lignin compound (Xu et al., 2014). In kiwifruit, lignin content is reduced by inhibited activities of PAL, CAD, and POD, as well as the expression levels of coordinated genes (Li et al., 2017). The above three enzymes are important in changing lignin biosynthesis (Jin et al., 2021). Except for enzymes and their coding genes, transcription factors respond to lignin biosynthesis, and transcriptionally regulate lignin biosynthesis-related genes (Zhao & Dixon, 2011). It has been widely reported that NAC and MYB families are considered crucial in changing the expression of genes encoding lignified-related enzymes (Xu et al., 2015). *MYB46*, *MYB83*, as 'master switches', could manipulate secondary cell biosynthetic genes, including transcription factors (Zhong & Ye, 2012). *EjNAC1* activated *EjPAL1* and *Ej4CL1* genes promoters in loquat lignin biosynthesis (Xu et al., 2015). *EjMYB1* causes loquat lignification via activating promoters of lignin biosynthesis genes (Xu et al., 2014). Some lignin biosynthesis-related genes, including *CAD* and 4-hydroxycinnamate: CoA ligase genes, are directly regulated by *AtMYB4* in *Arabidopsis* (Zhao & Dixon, 2011). The NAC and MYB family, influencing the transcriptional level, participate in regulating the metabolism in secondary wall formation and lignification (Taylor-Teeples et al., 2015).

Melatonin (MT), or *N*-acetyl-5-methoxytryptamine, a derivative of tryptophan, is an important indoleamine hormone that ubiquitously

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exists in organisms (Gao et al., 2016). In horticultural crops, melatonin is considered a novel preservative agent that may remarkably delay senescence. Exogenous MT reportedly retards senescence and quality deterioration in sweet cherries (Wang, Zhang, Yang, & Zhao, 2019). MT has been thoroughly explored to extend postharvest vegetable post-harvest life and to maintain quality attributes. Melatonin significantly improves the peach quality during storage, thereby delaying weight loss, keeping firmness and some qualities (Gao et al., 2016). 1 mM MT application can maintain postharvest quality by enhancing the accumulation of phenols substances and improving antioxidant ability in kiwifruit during storage (Wang et al., 2019). Furthermore, Li et al. (2019) demonstrated that MT treatment reduced the cellulose and lignin content of bamboo shoots after harvest and increases the expression of transcription factors from the NAC and MYB families. MT effectively retarded lignification in loquat fruit mainly attributed to lower key enzyme activities (PAL, 4CL, CAD and POD) (Wang et al., 2021). Some methods have been applied to inhibit lignification, such as radio frequency treatments (Ye et al., 2020), Nitric oxide alleviates lignification (Qi et al., 2020). Little data is acquirable about the result of MT application on the lignification in water bamboo shoot during storage. Therefore, this research aimed to clarify the effect of MT on lignification in water bamboo shoots after harvest. We examined the influence of MT on lignin contents, activities of phenylalanine ammonia-lyse (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) and their genes expression, as well as transcription factors from the *NAC* and *MYB* families. Studying lignification after harvest of water bamboo shoots can provide further insight into the mechanisms by which changes in lignification occur.

2. Materials and methods

2.1. Materials and treatments

Water bamboo shoots "Longjiao II"were harvested at the commercial maturity stage from Dongjia Jiaobai farm in Tongxiang, Zhejiang province of China. Water bamboo shoots were transported to the laboratory after harvest. They were chosen based on uniform shape and appearance and free from damage. The water bamboo shoots were randomly grouped into 2 lots with three replicates: the control (distilled water) and MT (Sangon Biotech Co., Ltd., Shanghai, China) at 0.5 mmol L^{-1} for 20 min. The optimal concentration of MT (0.5 mmol L^{-1}) was chosen based on a small-scale test. Then vegetables were air-dried and stored at 20 °C and 85 ± 5 % relative humidity RH for up to eight days.

2.2. Determination of firmness, lignin, total phenols and ascorbic acid content

Firmness was evaluated using a texture analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK) as described by Miao, Wang, Zhang, & Jiang (2011) with some modification. A cylindrical probe diameter of 2×10^{-3} m, 5 mm near the skin of shoot at a speed of 0.5 mm s^{-1}, then recorded the data. Firmness (N) was examined in the middle of the shoots.

The lignin content was measured by kit (Solarbio Science & Technology Co., Ltd., Beijing, China). The samples were dried to constant weight at 80 °C, crushed, passed through a 30–50 mesh sieve, and 5 mg was weighed into a 1.5 mL EP tube. The lignin content is expressed as mg/g fresh weight. According to the instructions provided by the manufacturer. Three replicates for each measurement were utilized.

The total phenols content was determined as described by Silva et al. (2020) with small changes. 1 mL aliquots of the extracts were transferred to 10 mL flasks containing 5 mL distilled water, and 1 mL of 25% sodium carbonate, to which 1 mL Folin–Ciocalteu reagent was added, and the volume was added with distilled water. After 0.5 h, the absorbance was measured at 760 nm. Quantification was detected by using gallic acid as a standard.

The ascorbic acid content was determined according to the method of Jiang et al. (2022). Briefly, 1 g of shoot powder was added to 5 mL of 5% (v/v) trichloroacetic acid (TCA), centrifuged for 15 min at 12,000 × g. And the reaction mixture consisted of 0.1 mL of supernatant, 1 mL of ethanol, 1.9 mL of TCA, 0.5 mL of 0.5% phosphoric acid–ethanol, 1 mL of 0.5% O-phenanthroline-ethanol, 5 mL of 0.03% $FeCl_3$-ethanol. The mixture was kept at 30 °C for 1 h. Subsequently, the mix was used in the analysis. Ascorbic acid content was calculated by measuring absorbance at OD534 nm.

2.3. Wiesner staining

Lignification was observed via Wiesner staining using the method of Li et al. (2017). The flesh sample was cut to 2 mm thickness. And a few drops of 37% concentrated HCl were added, then the phloroglucinol reagent for 5 min. Subsequently, lignified tissues showed pink.

2.4. Determination of activities of PAL, CAD and POD

For PAL, was measured as described in Cheng and Breen (1991) with slight changes. 5.0 g sample added in 5 mL extracted solution (50 mM sodium borate buffer, pH 6.8, 4 mM EDTA and 10 mM β-mercaptoethanol). The solution was mixed with 0.1 M sodium borate buffer (pH 8.8), 4 mM L-phenylalanine and 0.5 mL enzyme extract then kept at 40 °C for 60 min and stopped by increasing hydrochloric acid. PAL activity was defined as a change in OD333. POD activity was extracted and detected according to Zeng, Luo, Xie, & Feng (2015). 5 g samples were crushed in 20 mL of 0.05 mM sodium phosphate buffer (pH 7.8) at 4 °C for 10 min, and the supernatant was collected as the crude containing 1% (w/v) PVPP. After centrifugation at 12,000 × g, the supernatant was used for the test. In a final volume of 3.0 mL, the assay mixture contained 50 mM sodium phosphate buffer (pH 7.0), 12 mM H_2O_2, 7 mM guaiacol, and 0.1 mL of enzyme solution. POD activity was defined as a 0.001 change in OD470 per g fresh weight each minute. CAD activity was measured according to the methods of Pan, Xu, Guo, Zhang, and Li (2020). 10g samples were added in 8 mL extracted solution (200 mM Tris–HCl buffer, pH 7.5). After centrifugation at 5000 × g, the supernatant was used for the test. The reaction mixture contained 2.5 mL sodium phosphate buffer (pH 6.5), 2.0 mL of 3 mM $NADP^+$, 2.0 mL of 3.2 mM *trans*-cinnamic acid and 1.0 mL of extract. CAD activity was defined as a change of 0.01 in OD340 per g per min and expressed as U g-1 fresh weight.

2.5. Relative transcript level analysis

Total RNA was extracted from water bamboo shoot flesh, using a plant RNA kit (Omega Bio-tek, Norcross, GA, USA). The RNA samples were determined according to the ratio of OD260/OD280 to quantify total RNA using a BioSpec-nano spectrophotometry (Shimadzu, Tokyo, Japan). Subsequently, RNA integrity was verified through electrophoresis which was implemented on 1.2% agarose gel. The cDNA was obtained by the manufacturer's instructions of the RevertAID master mix (Thermo Fisher Scientific, Waltham, MA, USA). And the cDNA was diluted five times and was prepared for gene expression analysis as a template. RT-PCR analysis was performed using Aceq qPCR (TaKaRa, Dalian, China) on an ABI steponeplus Real-Time PCR System (Thermo Fisher Scientific, Wilmington, USA). The program consisted of a denaturation step at 95 °C for 5 min; 95 °C for 40 s with 40 cycles and 55 °C, and at 72 °C. Some primer sets for *ZlPAL1*, *ZlPAL2, ZlPAL3, ZlPAL4, ZlCAD1, ZlCAD2* were chosen from Chu et al. (2020), other primers were designed with PrimerPremier 5.0 (Table S1). The expression of each gene was normalized to *Actin* and calculated.

2.6. Statistical analyses

All statistical analyses were performed by one-way ANOVA using

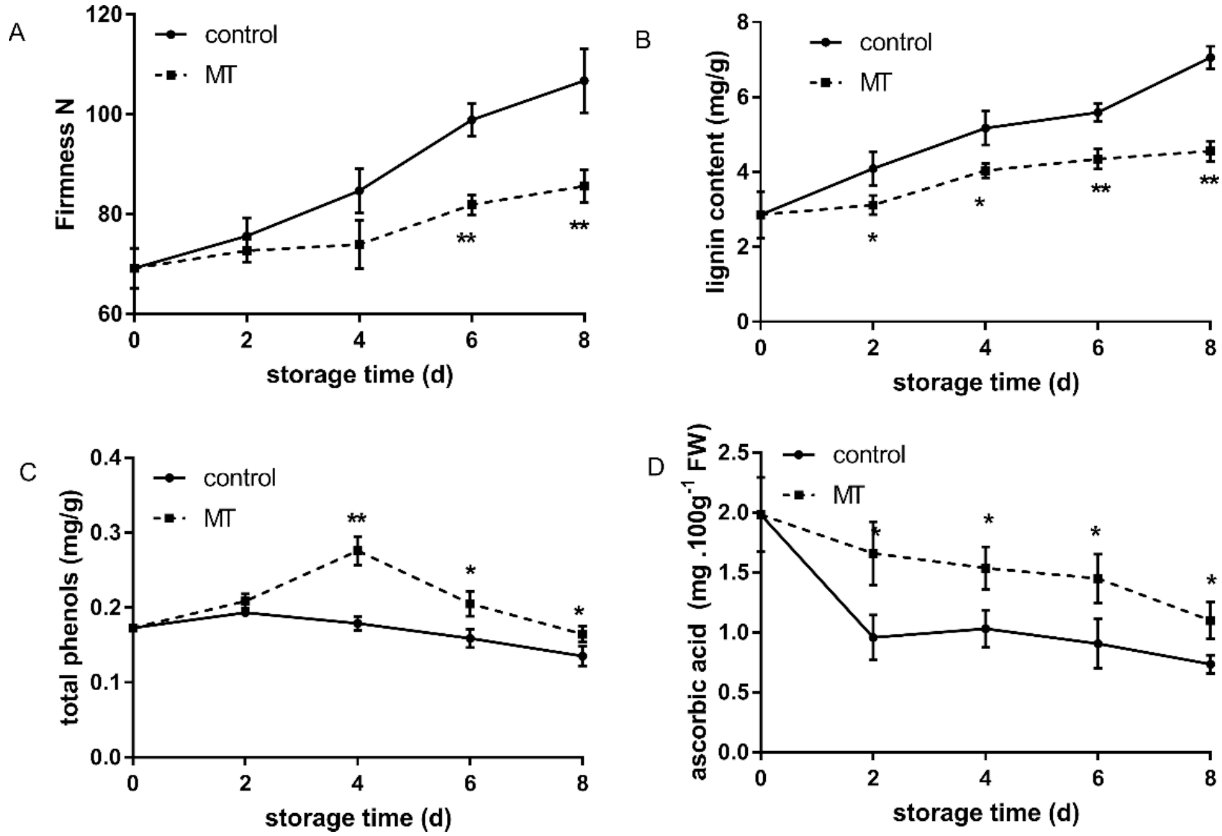

Fig. 1. Effect of MT treatment on the content of firmness (A), lignin content (B), total phenols (C), ascorbic acid (D) in water bamboo shoot. Statistical significance was determined using Student's *t*-test: ** $P \leq 0.01$, * $P \leq 0.05$.

SPSS 20.0 (SPSS Inc., USA) and Student's test at an obvious level of p-value < 0.05. Correlation analysis was elucidated between the several traits during the entire storage using Pearson's correlation coefficient. The correlation analysis was illustrated using Hiplot software (Shanghai Tengyun Biotech Co., Ltd, Shanghai, China).

3. Results and discussion

3.1. Changes in firmness and lignin content

Harvested fruit and vegetables used firmness as an indicator to estimate physical properties. As with the variational trend of the firmness, both control and MT treatment increased after harvest (Fig. 1A). In the present study, when postharvest water bamboo shoots stored at 20 °C and continued to increase firmness. However, MT treatment delayed the increase in firmness of water bamboo shoot. (Fig. 1A). The firmness decreased in the MT-treated compared to the control until day 8; when the firmness of the MT-treated was 20.56% lower than ($P < 0.01$) that of the control group at the end of storage. Firmness measurements are an integration of several textural properties, and in this case, lignin is only part of that number. Lignin has been shown to participate in cross-linking between the lignin polymers and cell wall polysaccharides and proteins (Dangcham, Bowen, Ferguson, & Ketsa, 2008). These may result in strong lignin complexes and thus an increase in firmness of water bamboo shoot. This finding indicates that firmness could be the reason for lignin deposition. The firmness increase was inhibited by MT treatment after harvest. In agreement with the previous report on bamboo shoots during storage (Zeng et al., 2015), the firmness increase was inhibited after 0.5 kGy gamma radiation.

Edibility is seriously affected by lignified tissues. MT-treated shoots exhibited lower lignin content during storage (Fig. 1B, $P < 0.05$). All the samples exhibited increased lignin content values during storage, compared to day 0. Along with an increase in storage time, the control had a faster increase rate than the MT treatment, showing significant differences. After the 8 d storage, lignin accumulation in control and MT-treatment increased to 125% and 31.8% respectively. A study by Qi et al. (2020) has demonstrated that the lignin content increased in water bamboo shoot after harvest. The lignification and thickening of the cell wall may be caused by the increase of cellulose and lignin content (Zhang et al., 2020). Moreover, MT inhibited the lignification via reducing lignin content in bamboo shoots after harvest (Li et al., 2019). In the present results, MT treatment was highly effective in retarding lignification, compared to the control of water bamboo shoot.

3.2. Changes in total phenols and ascorbic acid content

Polyphenols were generally regarded as precursors for lignin biosynthesis (Gong, Chen, Li, & Liu, 2018). Fig. 1C showed that the total level of phenolic compounds of the water bamboo shoot stored remarkably ($P < 0.05$) rise on the first 4d, then descend ($P < 0.05$) at the end of storage. Notably, a similar upward trend was found in total phenols contents and the CAD activity in the water bamboo shoots. Total phenols in water bamboo shoots increased first, then decreased in both treatments. Our results showed that MT had a better influence on the maintenance of total phenols during storage. 0.5 kGy Gamma radiation treatment could increase total phenols content in bamboo shoots after harvest (Zeng et al., 2015). Phenolics play a vital role in the precursor of lignin synthesis. The decrease in total phenols content is accompanied by the increase in firmness and lignin content, which is consistent with that oxidation of phenolic substances can promote the synthesis of lignin (Pan et al., 2020). Total phenols of vegetables including bamboo shoots, asparagus spears have also been reported to decrease during storage (Zeng et al., 2015; Toscano et al., 2018). In our study, compared to control water bamboo shoot, we observed that MT treatment lower the

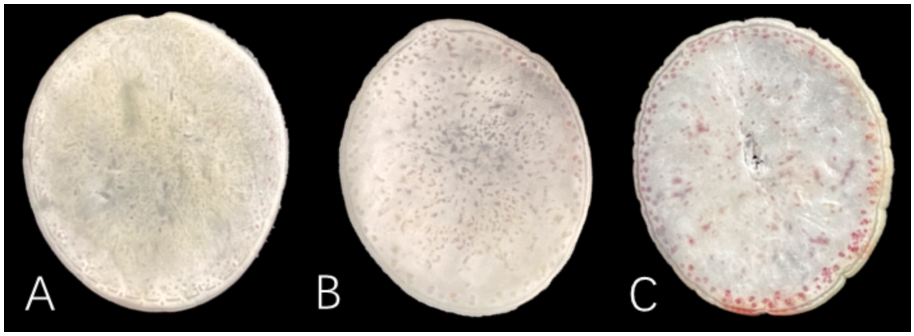

Fig. 2. Cross sections staining and observation of lignified cells. 0 day (A); 8 days after storage with MT treatment (B); 8 days after storage (control) (C).

decreases of phenols content and increases of lignin content the decrease in total phenol content during storage. These results were in agreement with EAWP treatment delayed phenolic content reduction, which indicated the inhibition of conversion of phenolics to lignin (Lwin, Srilaong, Boonyaritthongchai, Wongs-Aree, & Pongprasert, 2020). High total phenolic content in EAWP-treated spears reduced the biosynthesis of lignin during storage. The accumulation of lignin and the disruption of vacuoles by chilling injury in areca nut were associated with the loss of phenolics (Toor and Savage, 2005). The high content of total phenols in water bamboo shoots maybe inhibit the activity of PAL, CAD and POD enzymes during storage, and reduce the biosynthesis of lignin.

Phenolic and ascorbic acid have antioxidant activities and play important roles in plant stress resistance . As shown in Fig. 1D, the contents of ascorbic acid (AA) diminished in both treatments of water bamboo shoots after harvest. In the controls, AA content decreased about 60% lower than the initial values. MT treatment induced a lower rate of decrement in ascorbic acid. There were huge changes ($P < 0.05$) in total phenols content in water bamboo shoot during storage, and the differences were more noticeable. Therefore, MT-treated samples showed markedly ($P < 0.05$) higher levels than controls during the storage. Gao et al. (2016) had the same result and showed that MT inhibited the decline of reducing ascorbic acid content in peaches.

3.3. Identification of lignified tissues

To look into a possible physiological mechanism underlying lignification, crosswise sections from water bamboo shoots harvest were analyzed using Wiesner staining for lignin identification. Stained hand-cut slices showed lignified tissues in pink. Li et al. (2017) revealed that the 4-O-linked hydroxycinnamyl aldehyde structures could be the result of the reaction. In fresh water bamboo shoot, the pink spot is little (Fig. 2A). With the storage extending, we found that 8 days after storage displayed obvious pink in the vascular (Fig. 2C). In this study, histochemical staining showed that lignin content significantly increased over time. And the MT treatment inhibited lignin deposits, exhibiting little pink spots on transection (Fig. 2B). The results showed MT treatment inhibited the deposition of lignin cells compared with the control. In kiwifruit, the Weisner reagent displayed lignification in the flesh tissues (Suo et al., 2018).

3.4. Changes in activities of PAL, CAD and POD

PAL and CAD catalyze lignification of plant tissues. The monolignols synthesized through these effects polymerize with the participation of POD to form lignin macromolecules (Barros, Serk, Granlund, & Pesquet, 2015). PAL has a significant effect on lignin deposition (Cai, Xu, Li, Ferguson, & Chen, 2006). A few biosynthetic enzymes in the phenylpropanoid pathway have been reported to be involved in loquat lignification, for instance, PAL and CAD activities were associated with senescence-related lignification in loquat fruit (Cai et al., 2006). The three enzymes participate in the progress of lignification, whichinfluences lignin metabolism in plants.

PAL activity exhibited the same type in MT treated and control groups (Fig. 3A). PAL activity of water bamboo shoot at both treatments increased throughout the storage time. A quick augment in PAL activity of samples was shown with 8 days in control, with an increase of 84.3%. In both groups, the activities of POD increased gradually over the entire storage time, with faster increases in the control, being about approximately 80.2% increase within 8 days, and MT treatment reducing the rates of increase, within 27.4% increase (Fig. 3C). The CAD activity exhibited a slightly increasing tendency during the first 4 days and then decreased in the control (Fig. 3B). The MT treatment also delayed the peak at 6 days, then was followed by a little change.

In comparison with the control, MT-treated obviously restrained the activities of PAL, CAD, POD. The differences in the treatments for all three enzymes were remarkable ($P < 0.05$). The increases in PAL, CAD, POD activities and lignin content of the different treatments showed a consistent trend. Collectively, these data indicate that MT treatment restrains lignin deposits via diminishing the PAL, CAD and POD activities of lignified-enzymes in the samples. Li et al. (2019) supposed a similar conclusion, finding that the delayed activities of PAL, CAD and POD lead to lignin deposition or lignification decrease in bamboo shoot. Besides, 0.5 kGy gamma radiation reduces the activities of PAL, POD and CAD, thereby delaying the accumulation of lignin in bamboo shoots

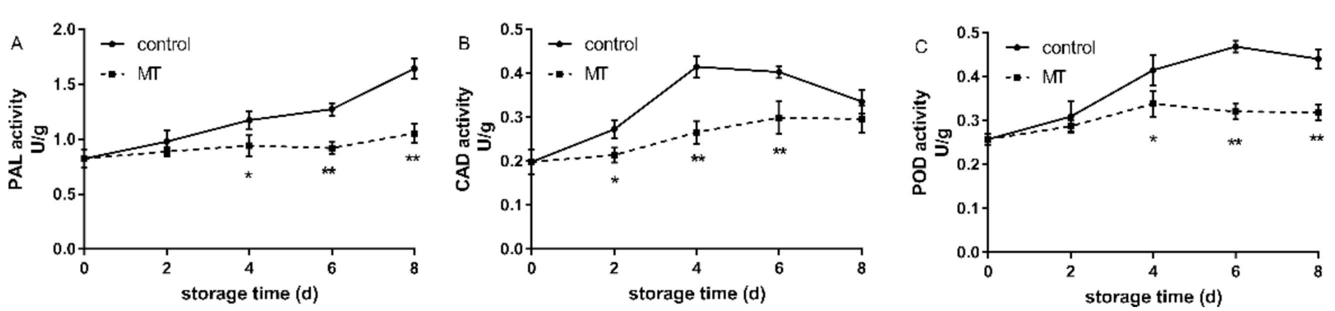

Fig. 3. The changes in the PAL (A), CAD (B) and POD (C) activities of water bamboo shoots treated with MT. Statistical significance was determined using Student's *t*-test: ** $P \le 0.01$, * $P \le 0.05$.

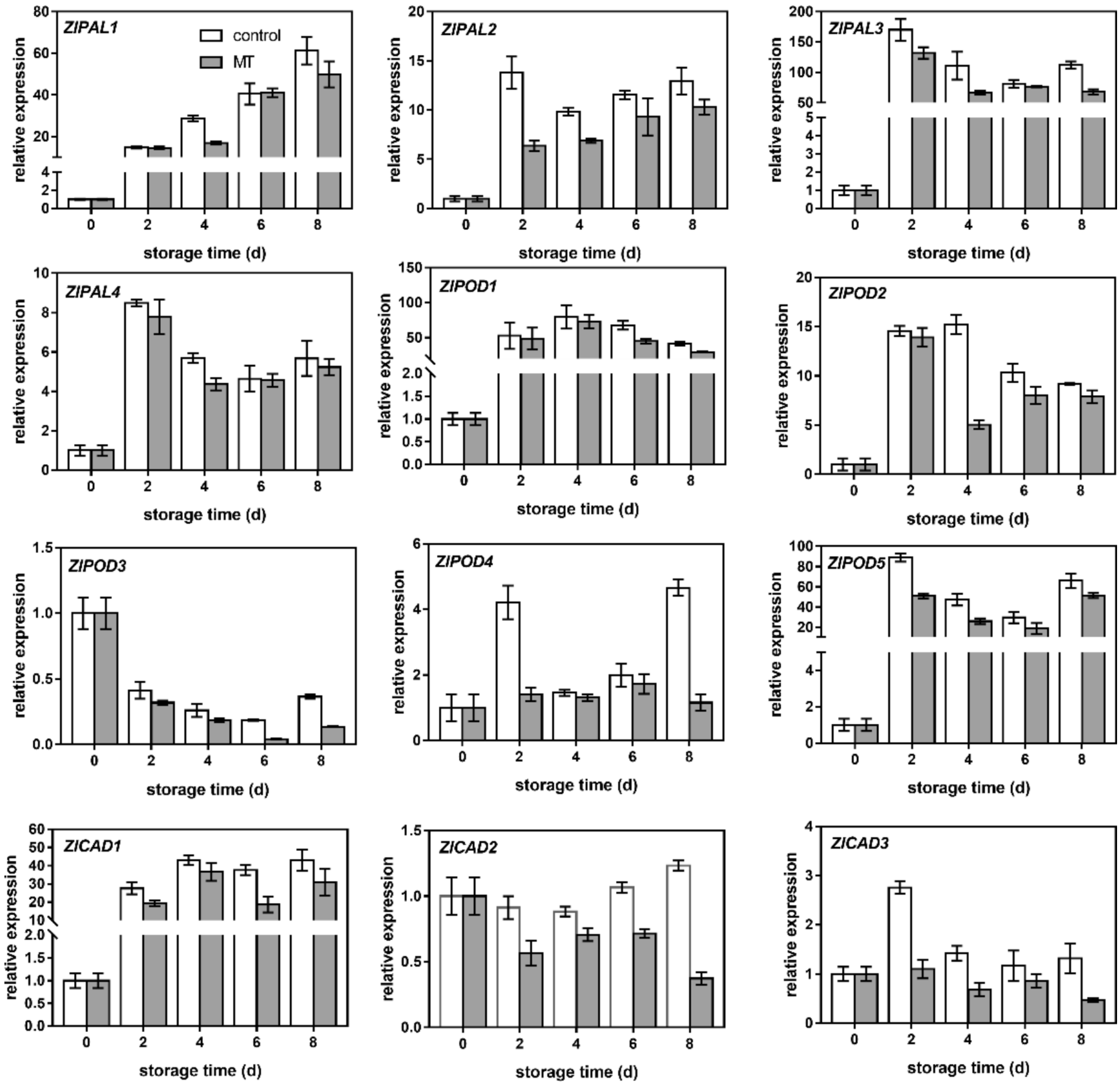

Fig. 4. Effect of MT treatment on the expression of lignified-enzyme activities.

(Zeng et al., 2015). Taken together, MT-tread could efficiently inhibit the deposition of lignin in water bamboo shoots by controlling enzyme activities.

3.5. Gene expression of PALs, CADs and PODs

The relative lignin content of water bamboo shoots exhibited a gradually increasing tendency after harvest, and the lignin biosynthesis-related genes displayed different expression patterns. Expression levels of enzymes genes that participated in the control of lignin metabolism were examined in water bamboo shoot. Activities of

enzymes including PAL, CAD, 4CL and POD accompanied by their gene expression collectively regulate the synthesis and accumulation of lignin (Zheng et al., 2020) The expression of *ZlPAL2/3/4* displayed the highest value at 2 d, and then descended steadily, and exposed no significant difference. MT treatment significantly restrained *ZlPAL2* expression in the middle-later time and showed a 2-fold difference with control at 2d. The *ZlPAL1* expression was increased during the whole storage period in both groups. Expression of *ZlPAL1/2/3/4* increased at the control and this was significantly inhibited by MT treatment (Fig. 4). Most strikingly, the expression of *ZlPAL3* in 2 days was upregulated 170-fold and 132-fold in control and MT treatment, respectively, compared with that at 0 day. The overexpression of *ZlPAL3* results in an uprising in the lignin content, which identified the important role in the lignin biosynthesis. Similarly, under the control and MT treatment, POD expression increased gradually during storage, with obvious changes from the control group. In water bamboo shoot, the control group increased peak *ZlPOD2, ZlPOD4, ZlPOD5,* expression levels to about 3.1 fold,s 4.1 fold,1.8 fold, higher than those of the MT treatment at the same stage during the whole storage. MT restrained *ZlPOD2* and *ZlPOD4* expression markedly during initial storage. *ZlPOD3* gene expression kept at an advanced state and rapidly increased the peak value at 2 d. Both groups displayed the same expression characters of *ZlCAD1/2/3* during the storage period; MT treatment strikingly reduced the CAD expression peak, producing various with the control at other stages after harvest. During storage for 8d, MT slightly decreased *ZlCAD1* expression levels in water bamboo shoot, keeping an upward trend from 2 to 8 d, which showed significant changes. *ZlCAD3* expression decreased steadily MT treatment, consistent with the control, verifying obvious modification. Activities expression levels of MT-treated shoots were obviously lower than those of controls during the whole storage. In line with Xu et al. (2019), *EjCAD5* has a corresponding impact on chilling-induced lignin biosynthesis in loquat fruit. The upward expressions level of *PAL, CAD and POD* genes were consistent with the changes in the corresponding enzyme activities, which were suggested that the PAL, CAD and POD regulation was possible at transcriptional level. The transcript levels of

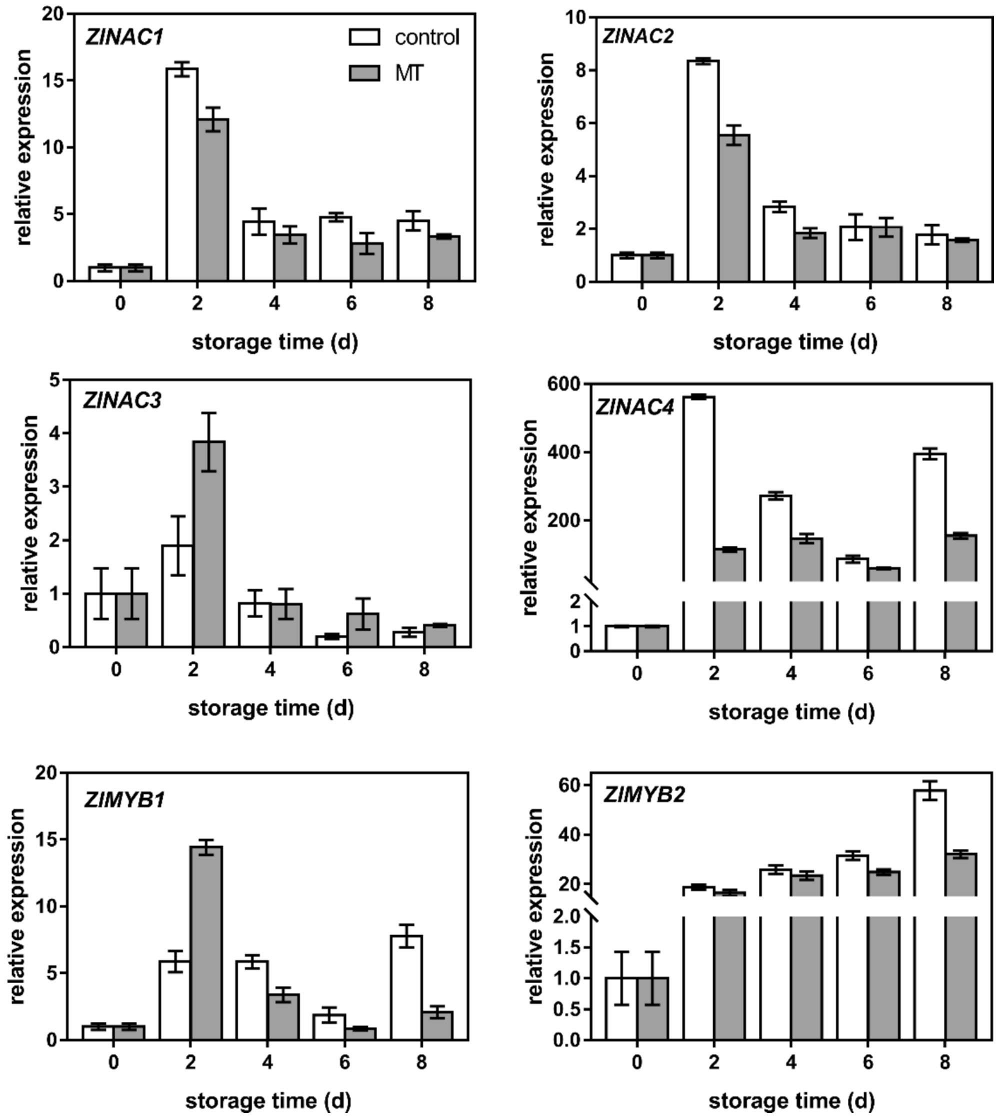

Fig. 5. Effect of MT treatment on the relative expression level of transcription factors.

ZlPAL, ZlPOD, ZlCAD gradually increased and coincided with the increasing lignin accumulation during storage. These results were highly in accordance with previous reports indicating improved the PAL, CAD, POD activities and corresponding gene expressions were consistent with higher content of firmness and lignin in pummelo (Chen, Nie, Wan, Gan, & Chen, 2021) and orange (Wu et al., 2020), bamboo shoot (Li et al., 2019). In three celery cultivars, the expression level of AgCAD and AgPAL gene was positively correlated with the lignin content (Ding et al., 2020). 20 mg/L abscisic acid treatment on kiwifruit suppressed levels of corresponding genes expression, including AcPAL, AcCAD and AcPOD (Jin et al., 2021). In summary, MT can effectively inhibit lignin deposition and may influence lignin biosynthesis-related genes expression levels. MT delays the progress of lignification by decreasing the expression of enzyme genes in water bamboo shoots.

3.6. The relative expression of NAC and MYB transcription factors

Transcription factors, such as NAC and MYB involved in lignin synthesis regulation and control the expression of lignin biosynthesis-related genes (Xu et al., 2015; Jia et al., 2018). At present, NAC and MYB families have already been studied in vegetables and fruits, for instance, loquat (Ge et al., 2017), bamboo shoots (Li et al., 2019). The NAC domain containing TFs acts as a top-level master switch, directly activating cell wall biosynthesis genes and a series of downstream MYB TFs. These MYB transcription factors regulate the expression of genes involved in lignin, biosynthesis (Li et al., 2019). We researched the expression pattern of *NAC* and *MYB* family transcriptional factors, gene expression of *ZlNAC1, ZlNAC2, ZlNAC4* were dramatically decreased in the MT treatment group, compared to the others. The NAC family factors showed the highest relative expression level at 2 day, and then decreased. In loquat fruit, *EjNAC3* is an immediate activator of lignin deposition by controlling *EjCAD*-like (Ge et al., 2017). The transcript levels for each gene were significantly depressed by MT-treated. The relative level of *ZlNAC1* at 2 d was 15.85 and 12.09 in MT and control, and this difference was significant at 8d (Fig. 5). *ZlNAC4* transcripts also increased, but this was finished two days later, and MT was effective in inhibiting the transcriptional expression. Furthermore, the data indicated that *ZlMYB1* and *ZlMYB2* exhibited various expressions in water bamboo shoot at various patterns. *ZlMYB1* expression was markedly increased in MT treatment at 2 day reaching a maximum that was 2.48 times higher than control, after then expression levels continued to descend, with a lower level to controls by the end of the storage. In contrast, *ZlMYB2* violently rose expression at 2 day, and then the expression levels increased slowly in two groups during storage. *EjMYB8* and *EjMYB9* regulate low-temperature induced lignification of loquat fruit (Wang et al., 2016). NAC and MYB transcription factors regulated

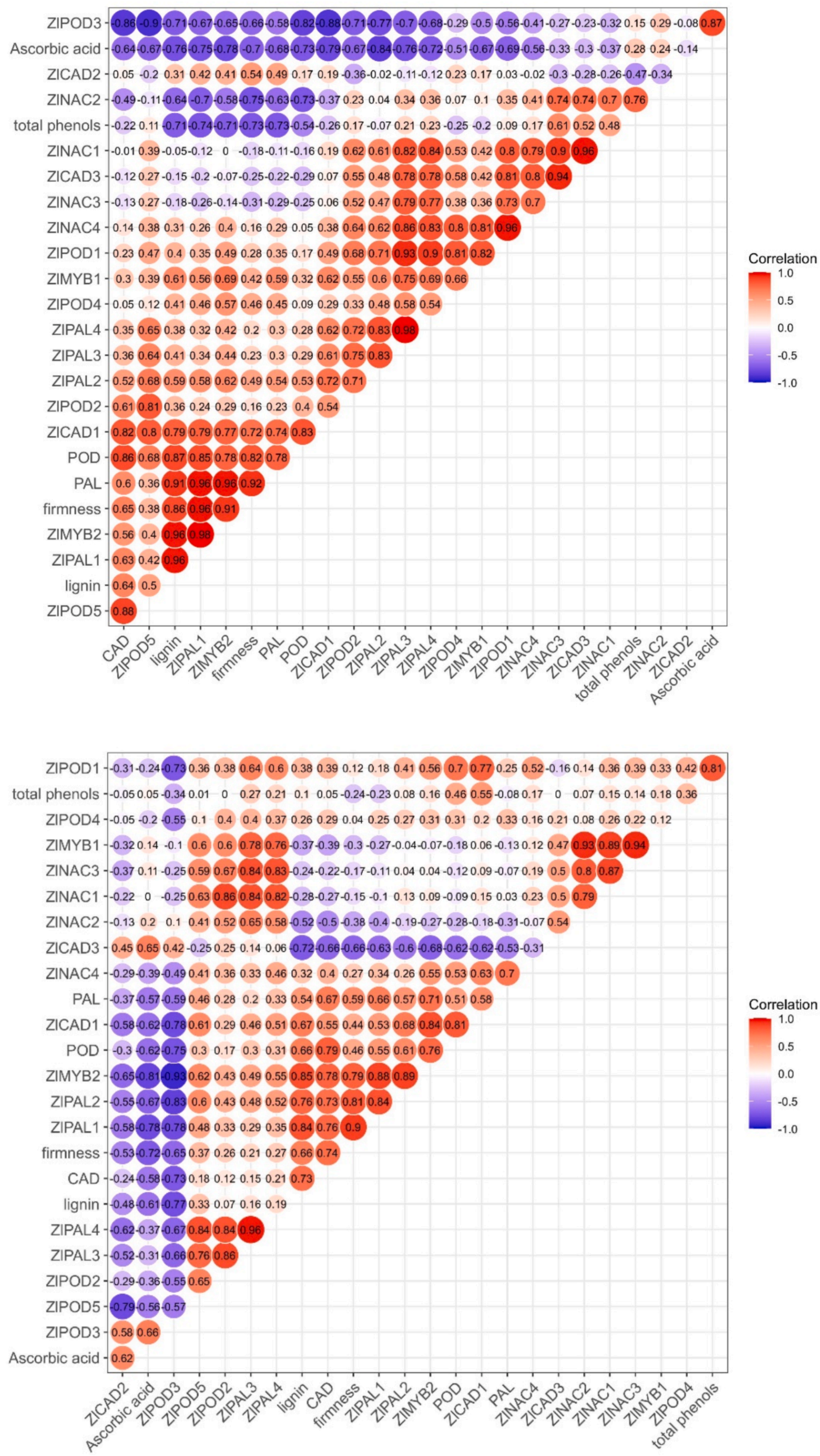

Fig. 6. Correlation analysis between the measured parameters in control (A) and melatonin (MT) treatment (B) at 20 °C for 8 d. Positive effects are exhibited in red and negative effects in blue.

the lignification of bamboo shoots, and exhibited up-regulated expression patterns under the accumulation of lignin in bamboo shoots (Zhang et al., 2020). It is similar to the upward expression level of *ZlNAC1, ZlNAC2, ZlNAC3, ZlNAC4, ZlMYB1 and ZlMYB2* in water bamboo shoots within 8 d during storage. More strikingly, MT-treated reduced the gene expression pattern of the TFs compared to the control group. We concluded that MT maybe participate in the transcriptional regulation of lignin synthesis, and it is probably a useful strategy to restrain the lignification during storage. Similarly, CsMYB330 and CsMYB308 have been implicated in the regulation of lignin metabolism in citrus fruit juice sacs (Jia et al., 2018).

4. Correlation analysis

Pearson's correlation coefficients analysis exhibited positive and negative correlations between several traits in the MT-treatment and control group of water bamboo shoots (Fig. 6). The PAL, CAD and POD activities and the expression of related genes in water bamboo shoot in the control group were significantly higher than those in the MT group. Notably, lignin content had a positive correlation ($P < 0.05$; $P < 0.01$) with firmness, PAL activity, CAD activity, POD activity, *ZlPAL1, ZlCAD1, ZlMYB2* in the control group. Lignin content was most strongly related to PAL activity, *ZlPAL1, ZlMYB2*. These results suggested that PAL activity, *ZlPAL1, ZlMYB2,* were involved in the lignification of water bamboo shoots. Lignin content had a positive relationship ($P < 0.05$; $P < 0.01$) with firmness, PAL activity, CAD activity, POD activity, *ZlPAL1, ZlPAL2, ZlCAD1, ZlMYB2* in MT treatment.

The lignification depends on enzyme activity (Li et al., 2017). Relevance analysis suggests that lignin content correlated significantly with PAL activity, with a correlation coefficient of 0.91. Other studies have discovered similar results (Zuo et al., 2021). PAL is the important enzyme regulating the progress of lignification in plants which is the entry into and a key regulatory step in the phenylpropanoid (Abbate et al., 2018). This result is consistent with the findings of Toscano et al. (2018), which restrain the lignification in asparagus through influencing PAL activity. The *ZlPAL1* expression level was positively correlated with the PAL activities in the water bamboo shoot, indicating that *ZlPAL1* was one of the key structural genes for PAL. There was a positive relationship between *ZlPAL3, ZlPAL4* and the expression levels of *ZlNAC1, ZlNAC2, ZlNAC3, ZlNAC4, ZlMYB1* and *ZlMYB2*.

5. Conclusion

The present study demonstrated that MT treatment significantly delayed the lignin biosynthesis of water bamboo shoot, inhibited the increase in firmness and accumulation of lignin during storage, as well as decreased the activities of PAL, CAD and POD, while maintaining the contents of total phenols and ascorbic acid. The lignin distribution of the water bamboo shoot coincided with lignin accumulation and showed that MT treatment exhibits a low lignin content. What's more, the genes involved in the synthesis of lignin were down-regulated, including *ZlPAL1/2/3/4, ZlCAD1/2/3, ZlPOD1/2/3/4/5*. Besides, MT has the function of regulating lignin biosynthesis with the NAC and MYB TFs participated in. In summary, MT treatment controls lignin accumulation via regulating lignin biosynthesis-related enzyme activities and corresponding gene expression. Our research lays the foundation for transcriptional regulation, and the MT treatment is probably an effective method to maintain the quality of water bamboo shoots.

CRediT authorship contribution statement

Baiqi Yang: Investigation, Formal analysis, Writing – original draft. **Yanchao Han:** Investigation, Formal analysis, Writing – review & editing. **Weijie Wu:** Writing – review & editing. **Xiangjun Fang:** Writing – review & editing. **Hangjun Chen:** Supervision, Project administration, Writing – review & editing. **Haiyan Gao:** Supervision, Project administration, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100254.

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