Postharvest Biology and Technology 156 (2019) 110933

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Bamboo shoot-lignification delay by melatonin during low temperature storage

Changtao Li[a,1], Jinwei Suo[a,b,1], Lingling Xuan[a], Mingzhu Ding[a], Hui Zhang[a], Lili Song[a,b,*], Yeqing Ying[a,**]

[a] State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, 311300, Zhejiang Province, People's Republic of China
[b] Sino-Australia Plant Cell Wall Research Centre, School of Forestry and Biotechnology, Zhejiang A&F University, Lin'an, 311300, Zhejiang Province, People's Republic of China

ARTICLE INFO

Keywords:
Melatonin
Lignification
Bamboo shoots
Low temperature
Storage

ABSTRACT

Bamboo shoots may become lignified after harvest and even deteriorate rapidly under low-temperature storage. Melatonin reportedly delays plant senescence. This study was designed to investigate the effect of melatonin on texture quality index, lignin formation, scavenging-enzyme activity, and transcription-factor expression in Moso bamboo (*Phyllostachys edulis*) shoots stored at 4 °C for 12 d. Application of melatonin effectively retarded shoot lignification, as indicated by a reduction in the rate of hardening and yellowing, as well as reduced lignin and cellulose contents. Furthermore, melatonin treatment inhibited phenylalanine ammonia-lyase and peroxidase activities significantly, while enhancing superoxide dismutase, catalase, and ascorbate peroxidase activities at different storage stages. Additionally, transcription factors of *SND2*, *KNAT7*, *MYB20* and *MYB85* from the NAC and MYB families were upregulated during postharvest storage of bamboo shoots, and melatonin treatment significantly inhibited their expression. These results suggest that the delaying effect of postharvest bamboo shoot lignification by melatonin treatment was mainly attributed to decreased activity of lignin biosynthesis-related enzymes and induced activation of antioxidant enzyme activity. Moreover, exogenous melatonin may be involved in the transcriptional regulation of the lignification process of bamboo shoots.

1. Introduction

In recent years, young bamboo shoot has become increasingly popular around the world, as it is considered a health caring vegetable because of its rich dietary fiber and distinctive flavor (Kleinhenz et al., 2000). More than 1250 edible bamboo species have been reported worldwide (Satya et al., 2010). Usually, the young shoots, which are the tender stalks emerging from the nodes of the (pseudo-) rhizome of bamboo plants, are enveloped in protective, non-edible leaf sheaths, with the edible part consisting of rapidly growing meristematic tissue (Kleinhenz et al., 2000). Commercially, the fresh bamboo shoot is consumed in the local market due to the rapid lignification and hardening after harvest. Low temperature effectively extends the shelf-life and preserves the quality of fruits and vegetables at postharvest (Cao et al., 2012; Luo et al., 2012b). However, Luo et al. (2008) reported that lignin and cellulose–and consequently hardness–of bamboo shoots increased rapidly when stored at 2 °C. Therefore, there is a need to explore more effective techniques for bamboo shoot storage.

In most fruits and vegetables, lignification is attributed to secondary cell wall (SCW) formation, following upregulation of cellulose, hemicellulose, and lignin biosynthesis-related genes (Zhang et al., 2018). Further, the biosynthetic pathways associated with SCW formation are highly regulated at the transcriptional level (Zhong and Ye, 2015); thus, much attention has been paid to a network of transcription factors (TFs) regulating SCW biosynthesis. These TFs are mainly divided into two categories: those in the MYB family and those in the NAC family (Lipsick, 1996; Stracke et al., 2001). NAC TFs act as "master switches" and regulate downstream levels of transcription factors to initiate SCW synthesis. On the other hand, MYB TFs play a central role in the transcriptional regulation of the deposition of plant SCW materials and have been reported to function as a link between upstream NAC TFs and downstream structural genes (Hussey et al., 2013; Nakano et al., 2015).

* Corresponding author at: State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, 311300, Zhejiang Province, People's Republic of China.
** Corresponding author.
E-mail addresses: lilisong@zafu.edu.cn (L. Song), yeqing@zafu.edu.cn (Y. Ying).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.postharvbio.2019.110933
Received 30 October 2018; Received in revised form 11 June 2019; Accepted 12 June 2019
Available online 18 June 2019
0925-5214/

AtMYB58, AtMYB63, AtMYB46, AtMYB83, and AtMYB103 are involved in the process of SWC synthesis in Arabidopsis (Hussey et al., 2011; Guo et al., 2017). In contrast, AtMYB52, AtMYB54, and AtMYB85 reportedly reduce cell wall thickness (Zhong et al., 2008). Candidate genes involved in the deposition of SCW materials through the regulation of lignin synthesis have been found mainly in *Betula platyphylla* and *Sorghum bicolor* (Scully et al., 2016; Guo et al., 2017). However, TFs related to lignification of bamboo shoots after harvest have not been identified.

Melatonin (MT) is a natural indoleamine thought to be widely distributed in the plant kingdom, and has been identified in different plant organs, including roots, stems, leaves, flowers, fruits, and seeds (Reiter et al., 2015). The remarkable role of MT is attributed to its antioxidant activity and protection of plants against internal and environmental oxidative stresses (Reiter et al., 2015; Nawaz et al., 2016). For example, it has been reported that MT resulted in a delay of dark-induced senescence and decreased chlorophyll degradation in barley leaves (Arnao and Hernández-Ruiz, 2009). Wang et al. (2012) found that MT treatment upregulated the expression of genes encoding enzymes involved in the cycle of ascorbic acid and glutathione, thereby reducing reactive oxygen species (ROS) production and delaying senescence of detached apple leaves. Gao et al. (2016) reported that MT treatment delayed senescence of peach fruit by activating antioxidant enzymes and preserving membrane integrity. However, there is little information on the effect of MT on lignification, which is closely related to the senescence of bamboo shoots during storage.

Moso bamboo (*Phyllostachys edulis*) shoots belong to the mostly covered genus in China and is an important crop species with high amount of shoots produced (Yang and Xu, 1998). To determine the effects of MT on Moso bamboo shoots during low temperature storage, the present study was designed to study (1) effect of MT on lignin and cellulose contents, as well as activity of lignin biosynthetic enzymes, phenylalanine ammonia-lyase (PAL) and peroxidase (POD); (2) the mechanism underlying the regulation of lignification by MT. Our findings have increased our understanding of the role of MT in postharvest lignification of bamboo shoots, and provide a sound basis for the use of MT for the preservation of other lignified fruits and vegetables.

2. Materials and methods

2.1. Plant materials, treatment, and storage

Moso bamboo shoots (*Phyllostachys edulis*) were carefully harvested from a plantation in Lin'an, Zhejiang province of China (29°56′–30°23′N; 118°51′–119°52′E) in April 2017, where shoots emerged from approximately 5 cm above ground height were used for commercially maturity and cut with a spade from the rhizome. And then the shoots were packed carefully in fiberboard cartons, transferred to the laboratory within 4 h, and precooled at 8–10 °C overnight. The uniform-sized and lack of blemishes shoots (basal diameter 10 cm and 30 cm in height) were removed 1.0–1.5 cm from the bottom and placed in low-density polyethylene (LDPE) bags (34.5 × 20.5 cm). Three shoots were placed in each bag (not sealed) and 3 bags per treatment were used. There were three replicates per treatment. Shoot bases were dipped in 1.0 mM MT during 1 min, cool air-dried, and stored at 4 °C, under 80–85% relative humidity for 12 d. Samples were dipped in distilled water as the control treatment. Preliminary experiments showed that 1.0 mM MT delayed shoot senescence most effectively. Samples were collected at 3-day intervals during low temperature storage to measure shoot firmness, brightness, and yellowness, as well as lignin and cellulose contents. Samples for enzyme assays and PCR analysis were frozen in liquid nitrogen and stored at −80 °C. For different analyses three replicates were performed and samples of each biological replicate was pooled from three individual shoots in each bag.

2.2. Measurement of shoot firmness and surface color

Firmness was measured by a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, UK) fitted with a 2-mm diameter cylindrical probe which penetrated the shoots to a depth of 15 mm at a rate of 1 mm s^{-1}. Measurements were taken on opposite sides of each middle shoot after non-edible leaf sheaths was removed, results were expressed in 'N'. Surface color was evaluated using a Minolta colorimeter (CR-10, Konica Minolta, Japan). Measurements of brightness and yellowness were conducted on opposite sides around the middle part of each shoot, and were designated as 'L' and 'b', respectively.

2.3. Determination of lignin and cellulose contents

Bamboo shoots in each treatment was cut into small pieces and mixed, then dried in the oven. Dried pieces of bamboo shoots were ground into powder and passed through a 40-mesh sieve. Approximately 3 g of shoot powder were successively extracted during 6 h at 92 °C in a Soxhlet extractor with a benzene/ethanol (2:1, v/v) mixture, extracts was oven-dried and final residues were used for subsequent lignin and cellulose analysis. Lignin content was determined using the method described by Ju et al. (1993). Briefly, residues were mixed with 12 M H_2SO_4 and hydrolyzed for 4 h at room temperature, then diluted with distilled water to a final concentration of 1 M H_2SO_4 and heated for 1 h at 100 °C. The solution was cooled and filtered through a Buchner funnel under vacuum. Then the filters were air-dried to constant weight at 60 °C for lignin measurement. For cellulose content determination, the residues were mixed with 25 mL of a nitric acid-ethanol liquid mixture and heated at 100 °C for 1 h with continuous shaking. The solution was then transferred into a Buchner funnel, the remaining solution was washed successively with the nitric acid-ethanol liquid mixture and water. Finally, the mixed solution was vacuum filtered and oven-dried at 105 ± 2 °C. Lignin (cellulose) content (%) = $(m_1\text{-}m_2)/m_0 \times 100\%$, m_1 represents the mass of lignin (cellulose) and funnel after drying to a constant weight, m_2 represents the mass of the empty funnel, and m_0 represents the mass of the bamboo shoot sample. Three independent replicates were conducted for each treatment.

2.4. Enzyme activity

All enzyme extraction procedures were conducted at 4 °C. For PAL assay, approximately 1 g of each frozen sample was homogenized in 5 mL of 0.2 M borate buffer (pH 8.8) consisting of 0.1 g of polyvinylpyrrolidone (PVPP) and 5 mM β-mercaptoethanol. The homogenates were then centrifuged at 4 °C for 15 min at 10,000 *g*. PAL activity was assayed by incubating 0.4 mL of the supernatant with 1.6 mL of 0.2 M borate buffer (pH 8.0) containing 20 mM L-phenylalanine for 30 min, at 30 °C. One unit of PAL activity was defined as the amount of enzyme that required to increase the absorbance of 0.01 at 290 nm $g^{-1}\,min^{-1}$.

For the measurement of POD, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities, 0.5 g of frozen samples were homogenized in 5 mL of 0.2 M phosphate buffer (pH 7.8). After centrifugation of the homogenate at 4 °C for 10 min at 8000 *g*, the supernatant was collected and used for enzyme assays. POD and CAT activities were measured as previously described (Kochba et al., 1977; Dhindsa et al., 1981). SOD and APX activities were assayed according to the method of Tang et al. (2015). Their activities were subsequently expressed as units $g^{-1}\,min^{-1}$. Three independent replicates were conducted for each treatment. All enzyme activities are expressed on a fresh weight basis.

2.5. Total RNA extraction, reverse transcription, and quantitative real-time (qRT)-PCR analysis

Total RNA was isolated using the E.Z.N.A.® total RNA kit (Omega, USA), and cDNA synthesis was performed using PrimeScript™ RT reagent kit (TaKaRa, Dalian, China) according to the protocols of the manufacturer. qRT-PCR analysis was performed using SYBR® Premix Ex TaqTM II (TaKaRa, Dalian, China) on a CFX96 Touch Real-Time PCR System (Bio-Rad, California, USA). The program was set as follows: 95 °C for 5 min; followed by 40 cycles at 95 °C for 40 s, then at 55 °C for 30 s, and at 72 °C for 30 s. Specific primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA) (Table S1). The relative expression level of each gene was normalized to actin and calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All samples were tested in at least three biological and technical replicates.

2.6. Statistical analysis

Results shown are means ± standard deviation (SD) of at least three replicates. Statistical analysis was performed by one-way ANOVA using IBM SPSS Statistics 25 (SPSS Inc., Chicago, IL) and Student's *t* test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Bamboo-shoot sensory quality

The quality of bamboo shoots declined after a 12-d storage at 4 °C, compared with samples before cold storage. Degrees of yellowness and firmness increased, while brightness decreased progressively (Figs. 1 and 2, $P < 0.05$). However, melatonin treatment resulted in shoots with lower degrees of firmness and yellowness, higher degree of brightness than control shoots (Fig. 2). On day 12, the differences in firmness and brightness between control and MT-treated shoots stored at 4 °C were significant.

3.2. Lignin and cellulose content

Increase in lignin and cellulose content in control shoots reached 118% and 24%, respectively, after the 12-d cold storage period (Fig. 3A), whereas both polymers were significantly reduced in MT-treated shoots (Fig. 3B, $P < 0.05$). Additionally, there was a positive correlation between firmness and lignin content (Fig. 4A), and between firmness and cellulose content (Fig. 4B) in control and MT-treated Moso bamboo shoots during storage at 4 °C.

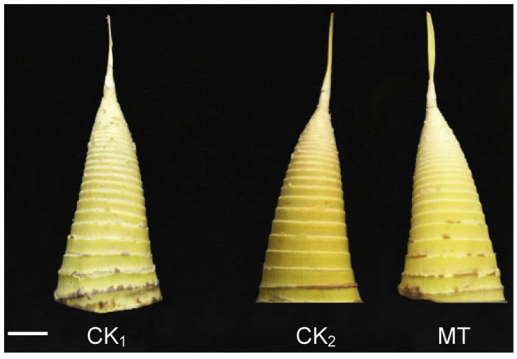

Fig. 1. Effect of melatonin (MT) treatment on the flesh qualities of bamboo shoots during storage at 4 °C. CK_1, storage for 0 d; CK_2, storage at 4 °C for 12 d; MT, melatonin treatment for 12 d during storage at 4 °C; Bar = 3.825 cm.

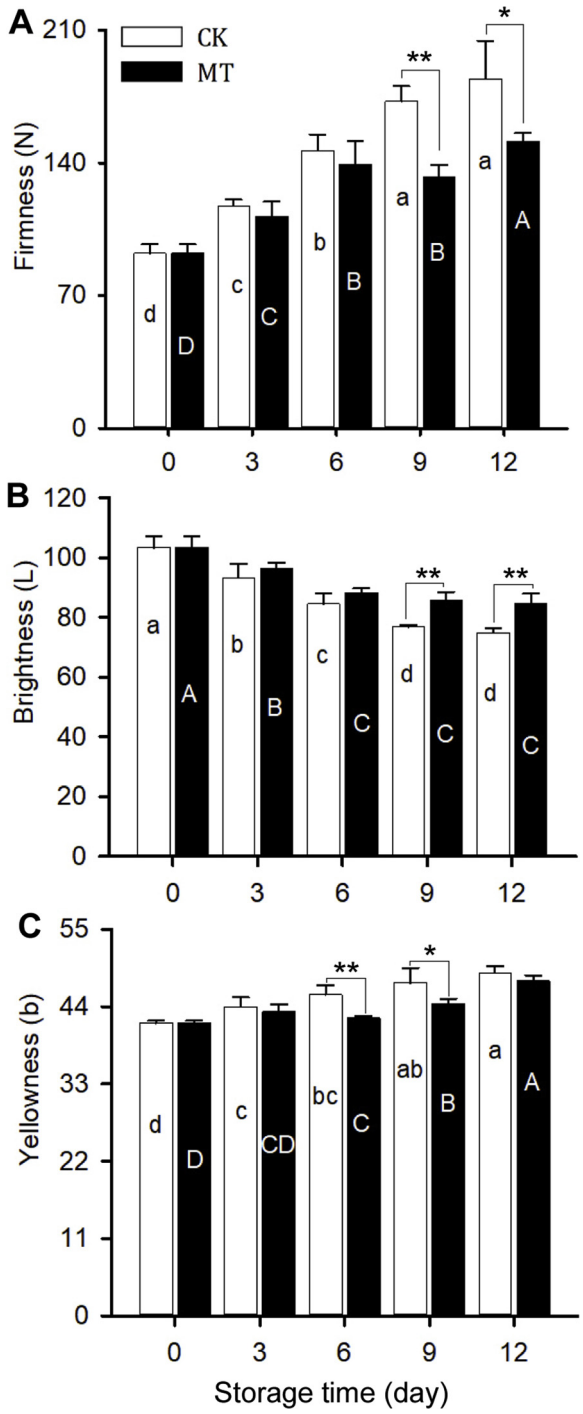

Fig. 2. Effect of melatonin (MT) treatment on firmness (A), brightness (B), and yellowness (C) of bamboo shoots during storage at 4 °C. The values were presented as means ± SD ($n \geq 3$). Different letters indicated significant differences ($p < 0.05$) among various storage days in control bamboo shoots (lowercase letters) and melatonin-treated bamboo shoots (uppercase letters). Asterisks indicate significant differences between CK and MT treatment within the same storage time (*$P < 0.05$, **$P < 0.01$). CK, control; MT, melatonin treatment.

3.3. PAL and POD activity

PAL and POD activities in bamboo shoots increased and reached maximum values on day 9 during storage at 4 °C, then were followed by a gradual decrease after 12 d (Fig. 5). On the other hand, MT treatment inhibited PAL and POD activities significantly by 24% and 14%,

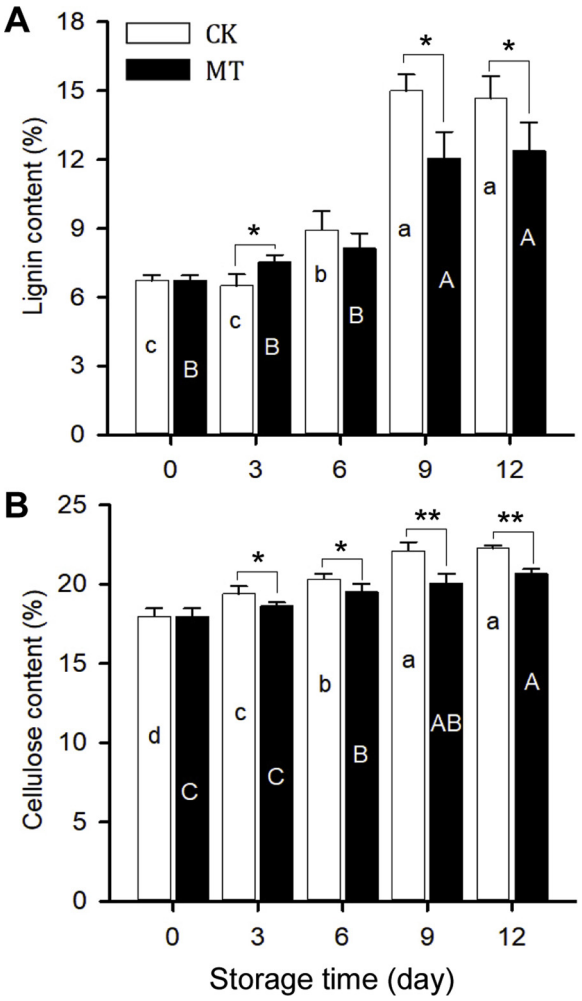

Fig. 3. Effect of melatonin (MT) treatment on lignin (A) and cellulose (B) contents of bamboo shoots during storage at 4 °C. The values were presented as means ± SD (n ≥ 3). Different letters indicated significant differences ($p < 0.05$) among various storage days in control bamboo shoots (lowercase letters) and melatonin-treated bamboo shoots (uppercase letters). Asterisks indicate significant differences between CK and MT treatment within the same storage time (*P < 0.05, **P < 0.01). CK, control; MT, melatonin treatment.

respectively, on day 9, compared with controls (Fig. 5; P < 0.05).

3.4. SOD, CAT and APX activity

As shown in Fig. 6, compared to the shoots before storage, the activities of SOD, CAT, and APX during storage at 4 °C increased gradually, and reached maximum values on day 9, with 38%, 54% and 86%, increments respectively. Such rise was subsequently followed by a slow decrease after 12 d (Fig. 6). Conversely, MT treatment resulted in all three activities at levels similar to controls, i.e., they gradually increased over the first 9 d but then slowly decreased after 12 d at 4 °C. Interestingly, exogenous MT application significantly enhanced SOD activity during the first 9 d and those of CAT and APX during the whole cold storage period, compared to control shoots (Fig. 6).

3.5. Gene expression of PALs, PODs and several transcription factors (TFs)

Expression levels of genes encoding PAL, POD and several transcription factors involved in the regulation of SCW biosynthesis were evaluated in Moso bamboo shoots. The expression level of *PAL1-4* showed similar pattern during 4 °C storage, which were upregulated within the 9 d after storage and slightly downregulated at the 12 d

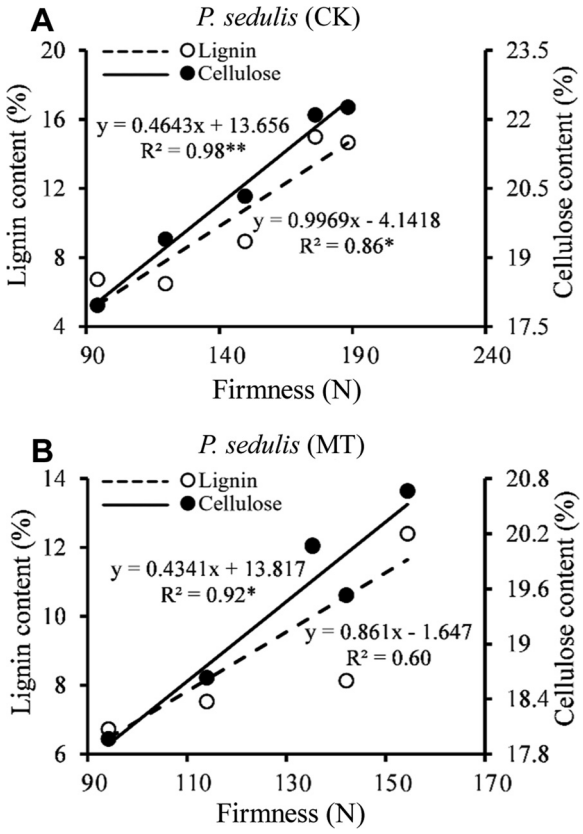

Fig. 4. Correlation analysis between firmness and lignin and cellulose contents in bamboo shoots with (A) or without (B) melatonin treatment during storage at 4 °C (*P < 0.05, **P < 0.01). CK, control; MT, melatonin treatment.

storage (Fig. S1). The expression of *POD1* and *POD3* were increased within 6 d after storage and then decreased, and the expression of *POD2* was inhibited obviously during storage at 4 °C (Fig. S1). Expression of *VND7*, *SND2*, *MYB63*, *MYB85*, and *MYB20* were induced in both control and MT-treated bamboo shoots (Fig. 7), while *NST1*, *MYB42*, and *MYB43* were downregulated during storage at 4 °C (Fig. 7). Transcript abundance of *KNAT7* increased significantly in control shoots and remained stable in MT-treated shoots during storage (Fig. 7). Furthermore, gene expression of *NST1*, *SND2*, *KNAT7*, *MYB63* and *MYB85* were significantly inhibited in MT-treated bamboo shoots, compared to controls (Fig. 7). Correlation analysis between these TFs expression levels and lignin and cellulose contents showed that the expression pattern of *MYB20* was highly consistent with both lignin and cellulose contents, while MT-treatment caused the correlation coefficient to decrease slightly (Table 1). Similarly, *SND2* and *KNAT7* also showed a positive correlation with lignin and cellulose contents (Table 1). However, the expressions of *MYB42*, *MYB43* and *NST1* were negatively correlated with the extent of lignification of the shoots during low temperature storage (Table 1). Correlation analysis between *TFs*, *PAL* and *POD* gene expression showed that *PAL2*, *PAL3* and *PAL4* were positively correlated with the expression of *MYB63*, *MYB85* and *SND2* (Table 2). The expression of *POD1* and *POD2* were negatively correlated with *MYB20* and *KNAST7*, while the expression of *POD3* were positively correlated with *MYB42* (Table 2).

4. Discussion

Bamboo shoots are excellent food materials with high nutrient content, but their quality is difficult to preserve, even under low temperature (Luo et al., 2012a). Senescence and deterioration of postharvest bamboo shoots is a process involving flesh lignification, which

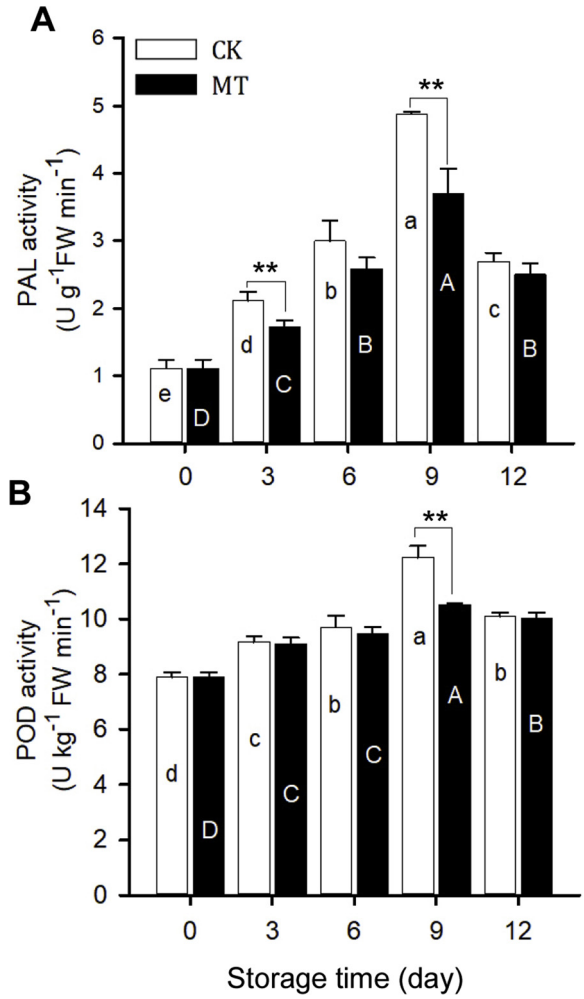

Fig. 5. Effect of melatonin (MT) treatment on PAL (A) and POD (B) activities of bamboo shoots during storage at 4 ℃. The values were presented as means ± SD (n ≥ 3). Different letters indicated significant differences ($p < 0.05$) among various storage days in control bamboo shoots (lowercase letters) and melatonin-treated bamboo shoots (uppercase letters). Asterisks indicate significant differences between CK and MT treatment within the same storage time (*P < 0.05, **P < 0.01). CK, control; MT, melatonin treatment; PAL, phenylalanine ammonia-lyase; POD, peroxidase.

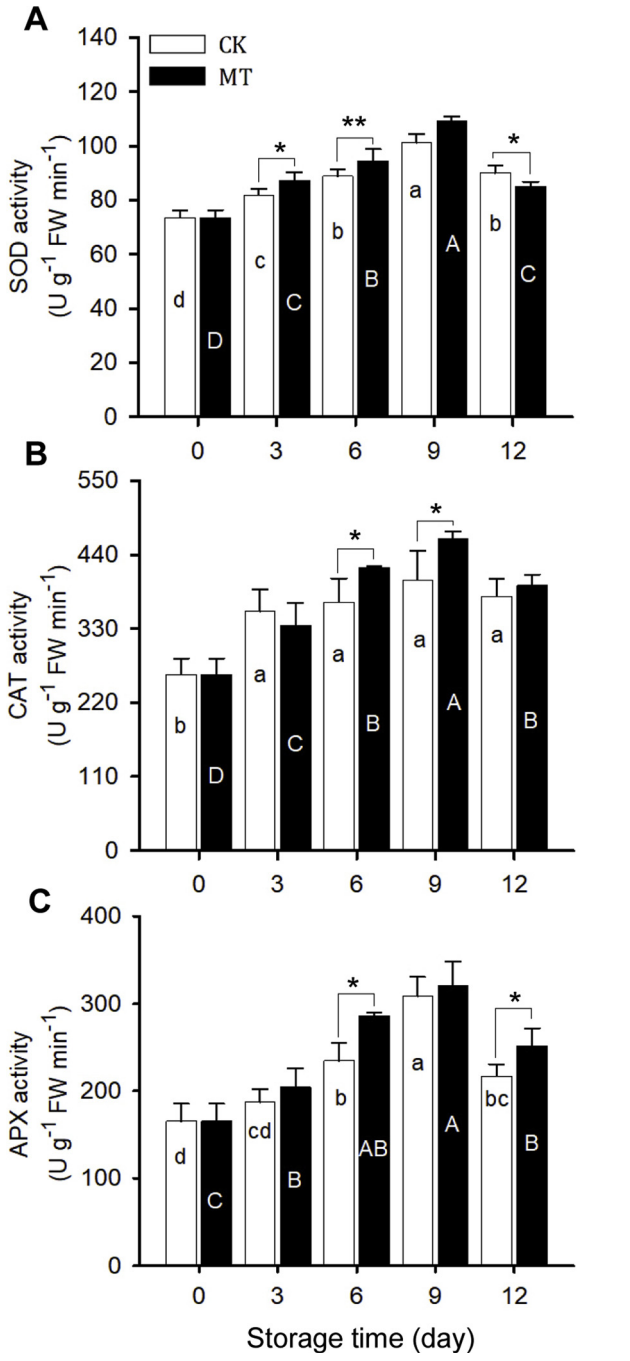

Fig. 6. Effect of melatonin treatment on SOD (A), CAT (B) and APX (C) activities of bamboo shoots during storage at 4 ℃. The values were presented as means ± SD (n ≥ 3). Different letters indicated significant differences ($p < 0.05$) among various storage days in control bamboo shoots (lowercase letters) and melatonin-treated bamboo shoots (uppercase letters). Asterisks indicate significant differences between CK and MT treatment within the same storage time (*P < 0.05, **P < 0.01). CK, control; MT, melatonin treatment; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

is usually accompanied by oxidative stress (Luo et al., 2008; Song et al., 2013). Melatonin has been recently reported to delay postharvest senescence of fruits by regulating antioxidant enzyme activities, gene expression, and membrane integrity (Gao et al., 2016; Liu et al., 2018). In the present study, we first investigated the effects of melatonin on lignification of bamboo shoots during cold storage, then gained a deeper insight into physiological and molecular mechanism of MT regulation of bamboo shoot-lignification, and provided an effective and practical method to preserve the quality of bamboo shoots after harvested.

4.1. Exogenous MT treatment delayed the lignification of postharvest bamboo shoots during low temperature storage

The lignification of post-harvest bamboo shoots is characterized by an unusual increase in firmness and rigidity of the shoot (Luo et al., 2008). In the present study, the firmness and yellowness increased gradually, while brightness decreased with the time under cold storage (Figs. 1 and 2), suggesting the occurrence of lignification of harvested bamboo shoots under low temperature conditions. Consistent with this result, lignin and cellulose content also increased significantly during cold storage, and showed positive correlations with firmness (Figs. 3 and 4). Similarly, an increase in lignification in bamboo shoots has been found under cold storage conditions by Luo et al. (2008, 2012a, 2012b) and Chen et al. (2013). To our knowledge, this is the first report on the delay of lignification of bamboo shoots by MT treatment, concomitant with decreased firmnessand yellowness, and increased brightness, as well as lower lignin and cellulose contents in MT-treated bamboo shoots (Figs. 2 and 3).

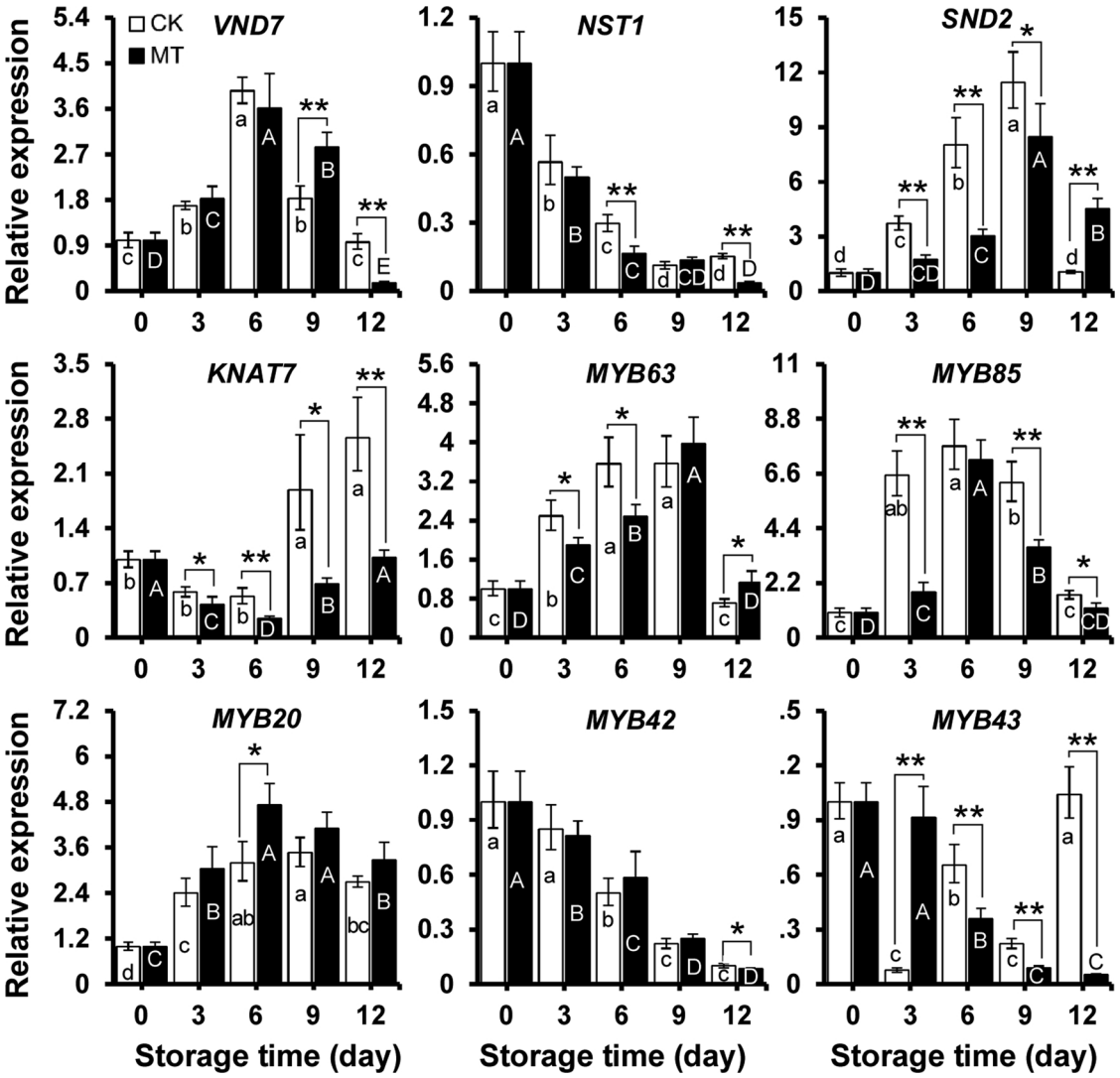

Fig. 7. Effect of melatonin (MT) treatment on the gene expression patterns of transcription factors in bamboo shoots during storage at 4 °C. The values were presented as means ± SD (n ≥ 3). Different letters indicated significant differences ($p < 0.05$) among various storage days in control bamboo shoots (lowercase letters) and melatonin-treated bamboo shoots (uppercase letters). Asterisks indicate significant differences between CK and MT treatment within the same storage time (*$P < 0.05$, **$P < 0.01$). CK, control; MT, melatonin treatment.

The lignification of plant tissues includes the polymerization of monolignols catalyzed by PAL and POD; PAL catalyzes the formation of cinnamic acid derivatives, while POD involved in the subsequent polymerization to form the lignin polymer units (Boudet, 2000). Exogenous MT treatment significantly inhibited PAL activity during low temperature storage, and slightly suppressed POD activity in comparison to control shoots (Fig. 5), indicating that exogenous MT delayed shoot-flesh lignification through the regulation of PAL and POD activities during cold storage. Similar results were found in post-harvest peach and strawberry, in which cases MT treatment affected PAL and POD activities and effectively delayed fruit senescence (Gao et al., 2018; Liu et al., 2018).

4.2. Exogenous MT treatment enhanced the antioxidant potential of post-harvest bamboo shoots during low temperature storage

It is well known that lignification of post-harvest fruits and vegetables is closely associated with overproduction of ROS that leads to oxidative damage to the cell membrane and biomolecules (Liu and Jiang, 2006; Jiang et al., 2010). Antioxidant enzymes, such as SOD, CAT, and APX, represent the primary line for ROS scavenging (Mittler, 2002). Higher activities of these enzymes have been reported to be an

Table 1
Correlation analysis between transcription factors expression, lignin and cellulose contents in control and MT-treated bamboo shoots during low temperature storage.

Treatment	Value	*MYB20*	*MYB42*	*MYB43*	*MYB63*	*MYB85*	*SND2*	*NST1*	*VND7*	*KNAT7*
Control	Lignin	0.60*	−0.92**	0.07	−0.28	−0.08	0.34	−0.80**	−0.16	0.80**
	Cellulose	0.76**	−0.92**	−0.08	−0.06	0.18	0.42	−0.91**	0.05	0.70**
Melatonin	Lignin	0.48	−0.89**	−0.86**	0.41	−0.03	0.85**	−0.74**	−0.12	0.35
	Cellulose	0.60*	−0.90**	−0.90**	0.29	0.19	0.64*	−0.86**	−0.01	0.06

*Significant at the $p < 0.05$ and ** highly significant at the $p < 0.01$.

essential mechanism involved in alleviation of oxidative stress and delay of senescence in many horticultural crops (Xia et al., 2016; Pal and Kar, 2018). In the present study, induced activities of SOD, CAT, and APX in bamboo shoots seem to have contributed to ROS scavenging during low temperature storage (Fig. 6). More importantly, MT treatment significantly enhanced SOD, CAT, and APX activities during cold storage (Fig. 6). These results indicate that exogenous MT treatment may prevent ROS accumulation by the activation of antioxidant enzymes during flesh lignification of bamboo shoots, which might be also part of the mechanisms implicated in the delay of lignification of postharvest bamboo shoots. This is consistent with the results from tomato and peach fruits, in which case, exogenous MT treatment significantly enhanced the activities of antioxidant enzymes SOD, CAT and APX (Gao et al., 2016; Ding et al., 2017).

4.3. Effects of MT treatment on transcriptional regulation of bamboo shoot-flesh lignification during low temperature storage

Lignification of postharvest bamboo shoots is regulated by a complex transcriptional network encompassing NAC and MYB TFs families (Zhong and Ye, 2015; Zhang et al., 2018). Among them, NAC domain containing TFs act as top-level master switches that directly activate cell wall biosynthetic genes and a series of downstream MYB TFs (such as, MYB20, MYB42, MYB43, MYB46, MYB58, MYB63, and MYB83, among others) (Zhou et al., 2014). In turn, these MYB TFs regulate the expression of genes involved in lignin, cellulose, and xylan biosynthesis (Zhong et al., 2007, 2008). Currently, these TFs have been widely investigated in many model and non-model pants (Hussey et al., 2011; Zhao et al., 2014; Zhong and Ye, 2015). Additionally, NAC and MYB TFs were identified in bamboo shoots of *P. edulis* and shown higher expression levels during the process of bamboo shoot lignification (Zhang et al., 2018). This is consistent with the up-regulation of *VND7*, *SND2*, *MYB63*, *MYB85* and *MYB20* in Moso bamboo shoots within 9 d during low temperature storage (Fig. 7, Table 1), which indicated a positive regulation role of these TFs in bamboo shoot-lignification after harvested. Similarly, simultaneous mutations of *SND1*, *NST1*, and *NST2* in Arabidopsis exhibit a complete loss of SCW thickening (Zhong et al., 2007). Knockout *OsSND2* rice mutants show decreased cellulose content and inhibited expression of several SCW biosynthesis-related genes (Ye et al., 2018). In addition, decrease in abundance of transcripts of *MYB42* in the present study (Fig. 7, Table 1) may enhance shoot lignification, as MYB42 is supposed to be the transcriptional repressor that negatively regulates SCW biosynthesis in Arabidopsis (Sonbol et al., 2009). More importantly, exogenous MT treatment inhibited the relative expression level of these TFs (such as, *NST1*, *SND2*, *KNAT7*, *MYB63*, *MYB85*, and *MYB43*) compared to the control group (Fig. 7); this observation suggested that MT is also involved in the transcriptional regulation of harvested bamboo shoot-lignification, and thus may be used to delay the process during low temperature storage. In the present study, the involvement of these TFs in the lignification of Moso bamboo shoots might be through the gene expression regulation of several lignin and cellulose biosynthetic enzymes, such as PAL and POD (Fig. S1). Expression level of *PALs* and *PODs* showed positive correlations with the expression of *SND2*, *MYB85*, *MYB63*, *MYB43* and *MYB42* in Moso bamboo shoots during low temperature storage (Table S2). Similarly, it has been reported that expression of Arabidopsis *PAL1* was up-regulated in *KNAT7* knockdown mutants (Li et al., 2012). Moreover, BplMYB46 could regulate the gene expression of *PAL* and *POD* in transgenic tobacco, and genome-wide analysis also demonstrated that Arabidopsis *PAL1* and *PAL4* may be the direct targets of AtMYB46 (Zhong and Ye, 2012; Guo et al., 2018).

5. Conclusion

The results reported herein suggest that exogenous MT treatment effectively delayed the lignification of bamboo shoots, as indicated by a significant decrease in the degrees of firmness and yellowness, and increase in brightness, the lower lignin and cellulose contents, as well as decreased PAL and POD activities recorded in our experiments. At the same time, MT treatment enhanced the antioxidant potential of postharvest bamboo shoots by enhancing activities of antioxidant enzymes. Moreover, exogenous MT was also involved in the NAC and MYB TFs-dependent transcriptional regulation of SCW biosynthesis and deposition process. This study suggests that exogenous application of MT may be effectively used as a practical method to prolong postharvest life and preserve the market quality of Moso bamboo shoots.

Acknowledgement

This work was supported by the Zhejiang Provincial Natural Science Foundation of China (grant no. LR15C160001).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2019.110933.

References

Arnao, M.B., Hernández-Ruiz, J., 2009. Chemical stress by different agents affects the melatonin content of barley roots. J. Pineal Res. 46, 295–299. https://doi.org/10.1111/j.1600-079X.2008.00660.x.

Boudet, A.M., 2000. Lignins and lignification: selected issues. Plant Physiol. Biochem. 38, 81–96. https://doi.org/10.1016/S0981-9428(00)00166-2.

Cao, S., Cai, Y., Yang, Z., Zheng, Y., 2012. MeJA induces chilling tolerance in loquat fruit by regulating proline and gamma-aminobutyric acid contents. Food Chem. 133, 1466–1470. https://doi.org/10.1016/j.foodchem.2012.02.035.

Chen, H., Ling, J., Wu, F., Zhang, L., Sun, Z., Yang, H., 2013. Effect of hypobaric storage on flesh lignification, active oxygen metabolism and related enzyme activities in bamboo shoots. LWT - Food Sci. Technol. 51, 190–195. https://doi.org/10.1016/j.lwt.2012.09.031.

Dhindsa, R.S., Pulmb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32, 93–101.

Ding, F., Liu, B., Zhang, S., 2017. Exogenous melatonin ameliorates cold-induced damage in tomato plants. Sci. Hortic. 219, 264–271. https://doi.org/10.1016/j.scienta.2017.03.029.

Gao, H., Lu, Z., Yang, Y., Wang, D., Yang, T., Cao, M., Cao, W., 2018. Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism. Food Chem. 245, 659–666. https://doi.org/10.1016/j.foodchem.2017.10.008.

Gao, H., Zhang, Z.K., Chai, H.K., Cheng, N., Yang, Y., Wang, D.N., Yang, T., Cao, W., 2016. Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. Postharvest Biol. Technol. 118, 103–110. https://doi.org/10.1016/j.postharvbio.2016.03.006.

Guo, H., Wang, L., Yang, C., Zhang, Y., Zhang, C., Wang, C., 2018. Identification of novel *cis*-elements bound by BplMYB46 involved in abiotic stress responses and secondary wall deposition. J. Integr. Plant Biol. 60, 1000–1014. https://doi.org/10.1111/jipb.12671.

Guo, H., Wang, Y., Wang, L., Hu, P., Wang, Y., Jia, Y., Zhang, C., Zhang, Y., Zhang, Y., Wang, C., Yang, C., 2017. Expression of the MYB transcription factor gene *BplMYB46* affects abiotic stress tolerance and secondary cell wall deposition in *Betula platyphylla*. Plant Biotechnol. J. 15, 107–121. https://doi.org/10.1111/pbi.12595.

Hussey, S.G., Mizrachi, E., Creux, N.M., Myburg, A.A., 2013. Navigating the transcriptional roadmap regulating plant secondary cell wall deposition. Front. Plant Sci. 29, 325. https://doi.org/10.3389/fpls.2013.00325.

Hussey, S.G., Mizrachi, E., Spokevicius, A.V., Bossinger, G., Berger, D.K., Myburg, A.A., 2011. *SND2*, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in *Arabidopsis* fibres and increases fibre cell area in *Eucalyptus*. BMC Plant Biol. 11, 173. http://dol.org/10.1186/1471-2229-11-173.

Jiang, T.J., Jahangir, M.M., Wang, Q.S., Ying, T.J., 2010. Accumulation of lignin and malondialdehyde in relation to quality changes of button mushrooms (*Agaricus bisporus*) stored in modified atmosphere. Food Sci. Technol. Int. 16, 217–224. https://doi.org/10.1177/1082013209353840.

Ju, Z., Liu, C., Yuan, Y., Dai, H., Yan, S., Xu, J., 1993. Regulation of phenolics synthesis and their effects on fruit quality in laiyang pear. Chin. Agric. Sci. 26, 44–48 (in Chinese).

Kleinhenz, V., Gosbee, M., Elsmore, S., Lyall, T.W., Blackburn, K., Harrower, K., Midmore, D.J., 2000. Storage methods for extending shelf life of fresh, edible bamboo shoots [*Bambusa oldhamii* (Munro)]. Postharvest Biol. Technol. 19, 253–271. https://doi.org/10.1016/S0925-5214(00)00094-6.

Kochba, J., Lavee, S., Spiegel-roy, P., 1977. Differences in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines.

Plant Cell Physiol. 18, 463–467.
Li, E., Bhargava, A., Qiang, W., Friedmann, M.C., Forneris, N., Savidge, R.A., Johnson, L.A., Mansfield, S.D., Ellis, B.E., Douglas, C.J., 2012. The Class II *KNOX* gene *KNAT7* negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. New Phytol. 194, 102–115. https://doi.org/10.1111/j.1469-8137.2011.04016.x.
Lipsick, J.S., 1996. One billion years of MYB. Oncogene 13, 223–235.
Liu, C., Zheng, H., Sheng, K., Liu, W., Zheng, L., 2018. Effects of melatonin treatment on the postharvest quality of strawberry fruit. Postharvest Biol. Technol. 139, 47–55. https://doi.org/10.1016/j.postharvbio.2018.01.016.
Liu, Z.Y., Jiang, W.B., 2006. Lignin deposition and effect of postharvest treatment on lignification of green asparagus (*Asparagus officinalis* L.). Plant Growth Regul. 48, 187–193. https://doi.org/10.1007/s10725-005-6112-z.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Luo, Z., Wu, X., Xie, Y., Chen, C., 2012a. Alleviation of chilling injury and browning of postharvest bamboo shoot by salicylic acid treatment. Food Chem. 131, 456–461. https://doi.org/10.1016/j.foodchem.2011.09.007.
Luo, Z., Feng, S., Pang, J., Mao, L., Shou, H., Xie, J., 2012b. Effect of heat treatment on lignification of postharvest bamboo shoots (*Phyllostachys praecox* f. prevernalis.). Food Chem. 135, 2182–2187. https://doi.org/10.1016/j.foodchem.2012.07.087.
Luo, Z.S., Xu, X.L., Yan, B.F., 2008. Accumulation of lignin and involvement of enzymes in bamboo shoot during storage. Eur. Food Res. Technol. 226, 635–640. https://doi.org/10.1007/s00217-007-0595-y.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9.
Nakano, Y., Yamaguchi, M., Endo, H., Rejab, N.A., Ohtani, M., 2015. NAC-MYB-based transcriptional regulation of secondary cell wall biosynthesis in land plants. Front. Plant Sci. 5, 288. https://doi.org/10.3389/fpls.2015.00288.
Nawaz, M.A., Huang, Y., Bie, Z., Ahmed, W., Reiter, R.J., Niu, M., Hameed, S., 2016. Melatonin: current status and future perspectives in plant science. Front. Plant Sci. 6, 1230. https://doi.org/10.3389/fpls.2015.01230.
Pal, L., Kar, R.K., 2018. Role of reactive oxygen species in cotyledon senescence during early seedling stage of mung bean [*Vigna radiata* (L.) Wilczek]. J. Plant Growth Regul. 1–10. https://doi.org/10.1007/s00344-018-9845-4.
Reiter, R.J., Tan, D.X., Zhou, Z., Cruz, M.H., Fuentes-Broto, L., Galano, A., 2015. Phytomelatonin: assisting plants to survive and thrive. Molecules 20, 7396–7437. https://doi.org/10.3390/molecules20047396.
Satya, S., Bal, L.M., Singhal, P., Naik, S.N., 2010. Bamboo shoot processing: food quality and safety aspect (a review). Trends Food Sci. Tech. 21, 181–189. https://doi.org/10.1016/j.tifs.2009.11.002.
Scully, E.D., Gries, T., Sarath, G., Palmer, N.A., Baird, L., Serapiglia, M.J., Dien, B.S., Boateng, A.A., Ge, Z., Funnell-Harris, D.L., Twigg, P., Clemente, T.E., Sattler, S.E., 2016. Overexpression of *SbMYB60* impacts phenylpropanoid biosynthesis and alters secondary cell wall composition in *Sorghum bicolor*. Plant J. 85, 378–395. https://doi.org/10.1111/tpj.13112.
Sonbol, F.M., Fornalé, S., Capellades, M., Encina, A., Touriño, S., Torres, J.L., Rovira, P., Ruel, K., Puigdomènech, P., Rigau, J., Caparrós-Ruiz, D., 2009. The maize ZmMYB42 represses the phenylpropanoid pathway and affects the cell wall structure, composition and degradability in Arabidopsis thaliana. Plant Mol. Biol. 70, 283. https://doi.org/10.1007/s11103-009-9473-2.
Song, L., Chen, H., Gao, H., et al., 2013. Combined modified atmosphere packaging and low temperature storage delay lignification and improve the defense response of minimally processed water bamboo shoot. Chem Cent J. 7, 147. https://doi.org/10.1186/1752-153X.
Stracke, R., Werber, M., Weisshaar, B., 2001. The R2R3-MYB gene family in Arabidopsis thaliana. Curr. Opin. Plant Biol. 4, 447–456. https://doi.org/10.1016/S1369-5266(00)00199-0.
Tang, H., Hu, Y.Y., Yu, W.W., Song, L.L., Wu, J.S., 2015. Growth, photosynthetic and physiological responses of *Torreya grandis* seedlings to varied light environments. Trees 29, 1011–1022. https://doi.org/10.1007/s00468-015-1180-9.
Wang, P., Yin, L., Liang, D., Li, C., Ma, F., Yue, Z., 2012. Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. J. Pineal Res. 53, 11–20. https://doi.org/10.1111/j.1600-079X.2011.00966.x.
Xia, Y., Chen, T., Qin, G., Li, B., Tian, S., 2016. Synergistic action of antioxidative systems contributes to the alleviation of senescence in kiwifruit. Postharvest Biol. Technol. 111, 15–24. https://doi.org/10.1016/j.postharvbio.2015.07.026.
Yang, Y.M., Xu, J.R., 1998. Bamboo resources and their utilization in China. Liu, W., Hui, C., Wang, F., Wang, M., Liu, G. (Eds.), Proceedings of the Workshop Bamboo Conservation, Diversity, Ecogeography, Germplasm, Resource Utilization and Taxonomy 10–17.
Ye, Y., Wu, K., Chen, J., Liu, Q., Wu, Y., Liu, B., Fu, X., 2018. OsSND2, a NAC family transcription factor, is involved in secondary cell wall biosynthesis through regulating MYBs expression in rice. Rice 11, 36. https://doi.org/10.1186/s12284-018-0228-z.
Zhang, H., Ying, Y., Wang, J., Zhao, X., Zeng, W., Beahan, C., He, J., Chen, X., Bacic, A., Song, L., Wu, A., 2018. Transcriptome analysis provides insights into xylogenesis formation in Moso bamboo (*Phyllostachys edulis*) shoot. Sci. Rep. 8, 3951. https://doi.org/10.1038/s41598-018-21766-3.
Zhao, Y., Sun, J., Xu, P., Zhang, R., Li, L., 2014. Intron-mediated alternative splicing of wood-associated NAC transcription factor1b regulates cell wall thickening during fiber development in *Populus* species. Plant Physiol. 164, 765–776. https://doi.org/10.1104/pp.113.231134.
Zhong, R., Lee, C., Zhou, J., McCarthy, R.L., Ye, Z.H., 2008. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell 20, 2763–2782. https://doi.org/10.1105/tpc.108.061325.
Zhong, R., Richardson, E.A., Ye, Z.H., 2007. Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta 225, 1603–1611. https://doi.org/10.1007/s00425-007-0498-y.
Zhong, R., Ye, Z.H., 2012. MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription factors and secondary wall biosynthetic genes. Plant Cell Physiol. 53, 368–380. https://doi.org/10.1093/pcp/pcr185.
Zhong, R., Ye, Z.H., 2015. Secondary cell walls: biosynthesis, patterned deposition and transcriptional regulation. Plant Cell Physiol. 56, 195–214. https://doi.org/10.1093/pcp/pcu140.
Zhou, J., Zhong, R., Ye, Z.H., 2014. Arabidopsis NAC domain proteins, VND1 to VND5, are transcriptional regulators of secondary wall biosynthesis in vessels. PLoS One 9, e105726. https://doi.org/10.1371/journal.pone.0105726.