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Exogenous Melatonin Enhances Cold Resistance by Improving Antioxidant Defense and Cold-Responsive Genes' Expression in Banana

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Abstract: Accumulated evidence has revealed the mitigation effects of exogenous melatonin on cold stress in plants. In this study, to investigate the defensive roles of exogenous melatonin in banana under cold stress, we researched the influences of exogenous melatonin on the chlorophyll fluorescence parameters, antioxidant defense indexes and expression levels of cold-responsive genes in cold-stressed 'Brazil' banana seedlings. Results showed that 100 μM of exogenous melatonin achieved the best cold-resistance-promoting effect in banana. Exogenous melatonin treatment significantly increased the electron transfer rate, light harvesting efficiency, total antioxidant capacity, catalase and superoxidase activities and proline and soluble sugar contents and significantly reduced the accumulations of malondialdehyde, superoxide anion and hydrogen peroxide in the leaves of cold-stressed banana. In addition, under cold stress, melatonin significantly induced the expression of low-temperature-responsive genes, such as *MaChiI1*, *MaCSD1C*, *MaWhy1*, *MaKIN10*, *MaADA1* and *MaHOS1*. It was concluded that the application of exogenous melatonin enhanced antioxidant defense and induced the expression of cold-responsive genes, thereby improving the cold resistance of banana. Our study will provide a basis for the application of exogenous melatonin in improving plant cold resistance.

Keywords: melatonin; banana; cold resistance; antioxidant defense; cold-responsive genes

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1. Introduction

Cold stress greatly influences the growth and development of plants and has caused great economic losses of many horticultural plants. It can influence the cell membrane structure and permeability, destroy the intracellular homeostasis in plant cells and affect the photosynthetic rate of plants [1,2]. Under cold stress, a large amount of reactive oxygen species (ROS), such as superoxide anion free radicals, hydrogen peroxide (H_2O_2), and so on, accumulates [3], which leads to lipid peroxidation and enzyme activity inactivation in plants [4]. To ameliorate these damages caused by stress-induced excessive ROS accumulation, several antioxidant enzymes, including superoxidase (SOD), catalyzing the dismutation of superoxide anion (O^{2-}) into H_2O_2 and oxygen (O_2); catalase (CAT), catalyzing the decomposition of H_2O_2 into H_2O and O_2, and some other antioxidant enzymes, are activated [5]. In addition, to maintain normal intracellular homeostasis during and after cold stress, plants also elevate the accumulation of osmoregulatory substances, such as free proline and soluble sugars [6].

Many methods have been tried and applied by scientists and farmers to improve the cold resistance of plants. Recently, melatonin (N-acetyl-5-methoxytryptamine), a new type

of phytohormone [7], has been found to have the ability of improving plant cold stress resistance by directly or indirectly eliminating ROS, increasing the activities of antioxidant enzymes, enhancing the antioxidant activity of other antioxidants and altering the electron permeability to increase the oxidative phosphorylation level in the mitochondria, and so on [8,9]. Exogenous melatonin application increased the activities of antioxidant enzymes under cold stress and enhanced the cold resistance of bermudagrass (*Cynodon dactylon*) [10]. Turk et al. [8] found that exogenous melatonin treatment increased the accumulation of osmoprotectants, such as carbohydrates and free proline, in wheat (*Triticum aestivum*) seedlings. Li et al. [11] reported that exogenous melatonin reduced the ROS level in camellia (*Camellia sinensis* (L.) O. Kuntze) and increased the activities of antioxidant enzymes such as POD, CAT and SOD.

Banana (*Musa* spp.), a monocotyledonous perennial herbaceous plant belonging to the *Musa* genus of the Musaceae family, is one of the most important economic fruit crops with a huge commercial export in many tropical and subtropical countries [12–14]. Cultivated banana plants are generally sensitive to low temperature, and their growth is greatly inhibited when the temperature is below 10 °C [14]. However, the banana planting areas in China are mainly located in the northern subtropical areas, where banana plants often suffered greatly from cold stress in winter and early spring [13]. In 2016, the total production of bananas in China decreased by more than 3 million tons compared with that of 2015 due to biotic and abiotic stresses including cold [15,16]. Therefore, improving the cold resistance of banana is very necessary for the healthy development of the banana industry [17–19].

Melatonin has been successfully applied in the control of fruit ripening and anthracnose disease of banana [20,21]. Given the cold resistance enhancement ability of melatonin, in this study, we tested and investigated the defensive roles of exogenous melatonin in banana under cold stress. First, we compared the effects of different concentrations (0, 50, 100, 150 and 200 μM) of melatonin solutions on the cold resistance of 'Brazil' banana. Then, to uncover the physiobiochemical mechanism of the cold-resistance-promoting effect of exogenous melatonin, we measured the chlorophyll fluorescence parameters; activities of SOD, CAT, peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR); contents of malondialdehyde (MDA), proline, O^{2-} and hydrogen peroxide (H_2O_2) and the total antioxidant capacity in the leaves of melatonin pre-treated and non-treated control banana seedlings before and after 4 °C treatment. Additionally, the molecular mechanism underlying this effect was explored by comparing the expression of six banana cold-responsive genes in cold-stressed melatonin pre-treated and non-treated control banana seedlings [13,19]. The results obtained in our study will be helpful in clarifying the mechanism of melatonin-improved cold resistance in banana and will provide a basis for the application of melatonin in improving plant cold resistance.

2. Materials and Methods

2.1. Plant Materials and Cold Treatment

The 'Brazil' banana seedlings used in this study were provided by the Chinese Academy of Tropical Agricultural Sciences. Unique and healthy banana seedlings were selected and cultivated in plastic pots containing nutrient-rich soil in a growth chamber (GXZ-280C, Ningbo, China) at 28 °C.

To determine the optimal concentration of melatonin solution to be applied to banana seedlings, we first compared the influences of melatonin solutions of different concentrations (0, 50, 100, 150 and 200 μM) on banana cold resistance. According to the method described by Yang et al. [22], melatonin solution was sprayed onto banana leaves until water dropping every 24 h and, in total, three times. Then, banana seedlings were transferred into a growth chamber (GXZ-280C, Ningbo, China) at 4 °C, light intensity of about 1500 ± 200 lx, photoperiod of 16/8 h (day/night) and 80% relative humidity. For each treatment, at least five banana seedlings were used. At 12 h post treatment, phenotypes of banana seedlings were observed and compared. Then, a melatonin solution of the optimal

concentration was used for further experiments. The four-leaf-stage banana seedlings were first divided into two groups: one group was treated with 100 μM melatonin (M group) and the other group was treated with distilled water as control group (CK group). Then, the banana seedlings of each group were separately further divided into two groups: one group cultured in a 4 °C growth chamber for 24 h and the other in a 28 °C growth chamber (the 4 °C low-temperature-treated CK and M seedlings are hereinafter named T and MT, respectively).

2.2. Measurement of Chlorophyll Fluorescence Parameters

After cold treatment, the phenotypes of banana seedlings from each group were observed and photographed. Chlorophyll fluorescence parameters of the second leaves of banana seedlings in the CK, M, T and MT groups were measured using a portable chlorophyll fluorometer (Pocket PEA, Norfolk, UK) [13,23]. For each group, the chlorophyll fluorescence parameters were measured nine times.

2.3. Measurement of O^{2-} and H_2O_2 Contents, Total Antioxidant Capacity and Antioxidant Enzyme Activities

The second leaves of seedlings from each group were harvested, quick-frozen in liquid nitrogen and stored in a −80 °C freezer for later studies. After grinding leaf samples in liquid nitrogen with a mortar and pestle, 0.1 g of banana leaf fine powder together with 1 mL of extracting solution was added into an EP tube and centrifuged at 8000× *g* for 10 min at 4 °C, and the supernatant was collected and used for measurement of the O^{2-} and H_2O_2 contents using specific kits. The total antioxidant capacity (T-AOC) and activities of SOD, CAT, peroxidase (POD), glutathione reductase (GR) and ascorbate peroxidase (APX) were determined on the infinite M200 Pro microplate reader (Tecan, Grödig, Austria) by absorbance at 593, 560, 240, 470, 412 and 290 nm using specific kits, respectively. All the kits used here were produced by Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China), and for all these indexes, three replications were utilized.

2.4. Measurement of MDA, Proline and Soluble-Sugar Contents

The contents of malondialdehyde (MDA), proline (PRO) and soluble sugar (SS) in leaves of banana seedlings from different groups were determined according to the method of Li et al. [13]. The MDA content was calculated based on the absorbance values at 532 and 600 nm, and the PRO and SS contents were calculated using absorbance value at 550 and 620 nm, respectively. For the measurements of MDA, PRO and SS contents, three independent replications were conducted.

2.5. Expression Analysis of Cold-Responsive Genes Using Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from banana leaves using an RNAprep Pure Plant Kit (TIANGEN, Beijing, China), and cDNA used for qRT-PCR was synthesized using PrimerScript™ RT Reagent Kit (Perfect Real Time) (Takara, Shiga, Japan). qRT-PCR analysis of six cold-responsive banana genes, including *MaChiI1*, *MaCSDS1*, *MaADA1*, *MaHOS1*, *MaWhy1* and *MaKIN10*) [13], were performed on the LightCycler480 instrument (Roche) using the same primer pairs and amplification programs described by Li et al. [13]. The relative expression levels of these six genes among the four groups were calculated using the $2^{-\Delta\Delta CT}$ method with *MaCAC* as the internal reference gene [24,25]. For qRT-PCR, three biological replications were performed.

2.6. Statistical Analysis

Excel 2010 and SPSS 25.0 were used for the data analysis. All the data are shown as average ± standard deviation (SD) of with nine replications for chlorophyll fluorescence parameters and three replications for other indexes. Difference significance analysis was

performed by Duncan's multiple-range test at 5% significance level using SPSS 25.0, and GraphPad Prism 6.0 was used for figure drawing.

3. Results

3.1. Effects of Different Concentrations of Melatonin on the Cold Resistance of Banana Seedlings

After a 4 °C low-temperature treatment for 12 h, obvious cold-induced symptom differences were observed among the banana seedlings pre-treated with 0, 50, 100, 150 and 200 μM melatonin solution (Figure 1). Leaf wilting and drooping symptoms appeared in banana seedling pre-treated with 0 μM (Figure 1A) and 50 μM (Figure 1B) melatonin. For the banana seedlings pre-treated with 150 μM melatonin (Figure 1D), the second leaf drooped and curled, and the first leaf slightly curled. Banana seedlings pre-treated with 200 μM melatonin showed a slight leaf drooping symptom, which was much milder than that in the banana seedlings pre-treated with 0, 50 and 150 μM melatonin (Figure 1E). The banana seedlings treated with 100 μM melatonin (Figure 1C), however, showed almost no cold-induced symptom. Thus, 100 μM was determined as the optimal concentration and was used for further studies.

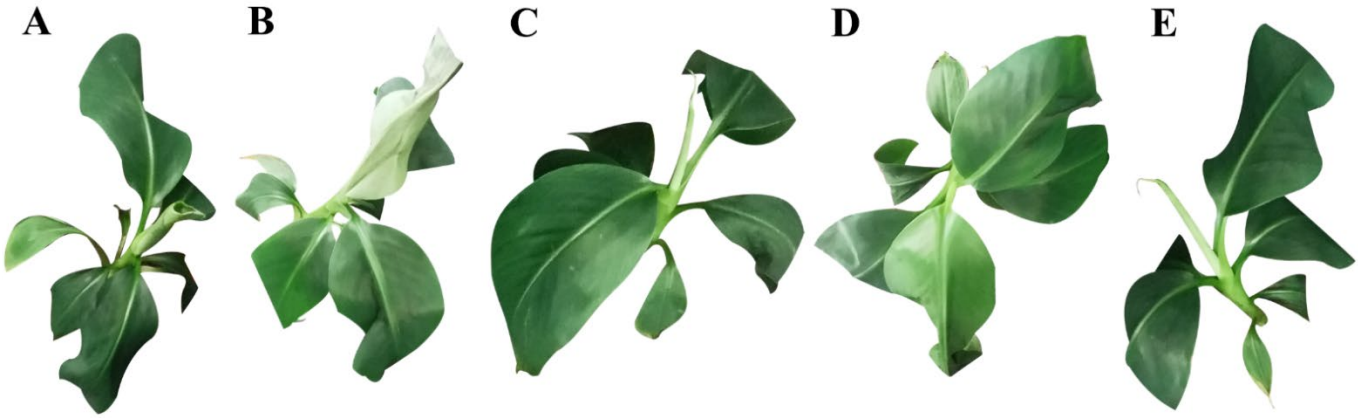

Figure 1. Effects of different concentrations of melatonin on the cold resistance of banana. (**A**–**E**) Typical phenotype for banana seedlings pre-treated with 0, 50, 100, 150 and 200 μM melatonin at 12 h post 4 °C low-temperature treatment, respectively.

3.2. Melatonin Treatment Alleviates the Symptoms Caused by Cold Stress in Banana

We further compared the phenotypes of melatonin pre-treated and non-treated banana seedlings before and after cold treatment. No obvious phenotype difference was found between CK and M. At 24 h post treatment, however, the cold-induced injuries in banana seedlings between T and MT differed obviously (Figure 2). All the leaves in the T group displayed water-stained spots and wilted and drooped. The MT plants also showed water-stained spots, leaf curling and drooping symptoms, but the symptoms were obviously milder than those in the T group (Figure 2). This result indicated that the application of exogenous melatonin alleviated the damages caused by cold stress in banana seedlings.

3.3. Effects of Melatonin on the Chlorophyll Fluorescence Parameters of Banana

To reveal the underlying mechanism of the melatonin-improved cold resistance of banana, we determined the chlorophyll florescence parameters of banana seedlings from the CK, M, T and MT groups (Figure 3). Results showed that the F_v/F_m and F_v/F_o values of the T group were lower than those of the CK group, but the differences were not significant. The F_v/F_m and F_v/F_o values of the MT group were similar to those of the CK group (Figure 3A,B). PI abs and ETo/CSm values of T group were significantly higher than those of CK. The PI abs, ABS/CSm, TRo/CSm and ETo/CSm values of MT group were all the highest among the four groups and significantly higher than those of CK, amounting to 1.31-, 1.05-, 1.07- and 1.13-fold that of CK, respectively. Moreover, the ABS/CSm, TRo/CSm

and ETo/CSm values of MT group were also significantly higher than in the T group, i.e., 1.04-, 1.07- and 1.06-fold that of the T group, respectively (Figure 3C–F).

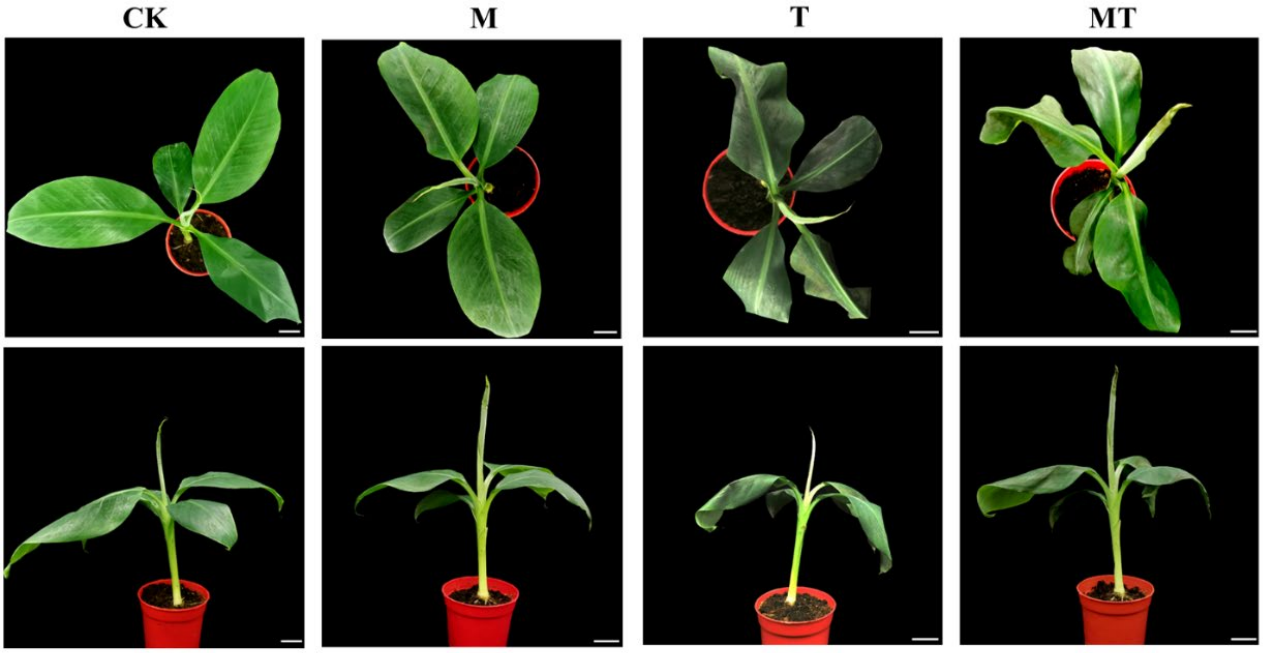

Figure 2. Typical phenotypes of 100 μM melatonin pre-treated and non-treated banana seedlings before and after 4 °C low-temperature treatment for 24 h. CK: control; M: melatonin treatment; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment (bar = 4 cm).

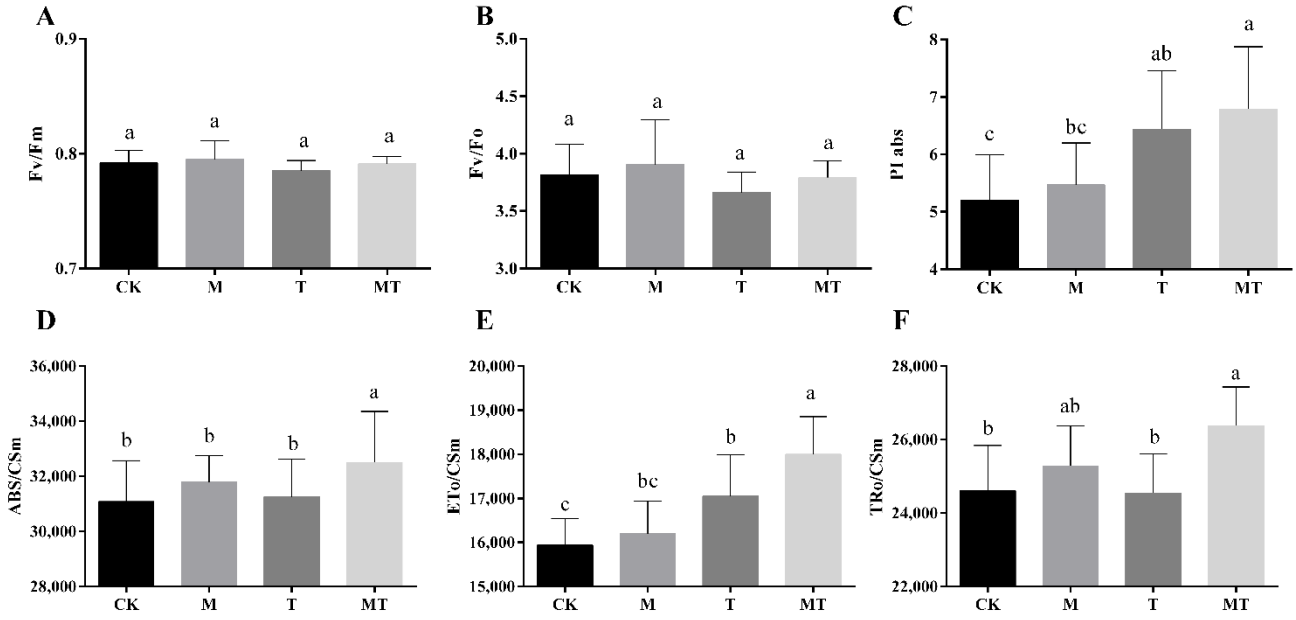

Figure 3. Changes of chlorophyll fluorescence parameters of melatonin pre-treated and non-treated 'Brazil' banana before and after cold stress. (**A**) F_v/F_m: the maximum photochemical efficiency PS II of photosystem II; (**B**) F_v/F_o: the potential photosynthetic efficiency of PS II; (**C**) PI abs: light energy absorption performance index; (**D**) ABS/CSm: absorbed light energy per unit area at the end; (**E**) TRo/CSm: captured light energy per unit area at the end; (**F**) ETo/CSm: electron transfer quantum yield per unit area at the end. Different letters above bars indicate a significant difference among samples determined using Duncan's test at the $p < 0.05$ level. All the data are displayed as mean ± standard deviation for nine replications. CK: control; M: melatonin treatment; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment.

3.4. Influences of Melatonin on the O^{2-} and H_2O_2 Contents and Total Antioxidant Capacity (T-AOC) in Banana Leaves

Results showed that the O^{2-} content of M was similar to that of CK, the O^{2-} content of T was higher than in CK, and the O^{2-} content of MT was found to be the lowest, accounting for only about 88.55% of that of CK (Figure 4). The H_2O_2 content of T was found to be the highest and was significantly higher than that in the other three groups. The H_2O_2 contents of CK, M and MT showed no significant difference. The H_2O_2 content of T was about 1.04-fold that of CK. The T-AOC of the four groups followed the order: MT > T > M > CK, and the T-AOC of MT was found to be significantly higher than that of CK, accounting for about 1.14-fold that of CK.

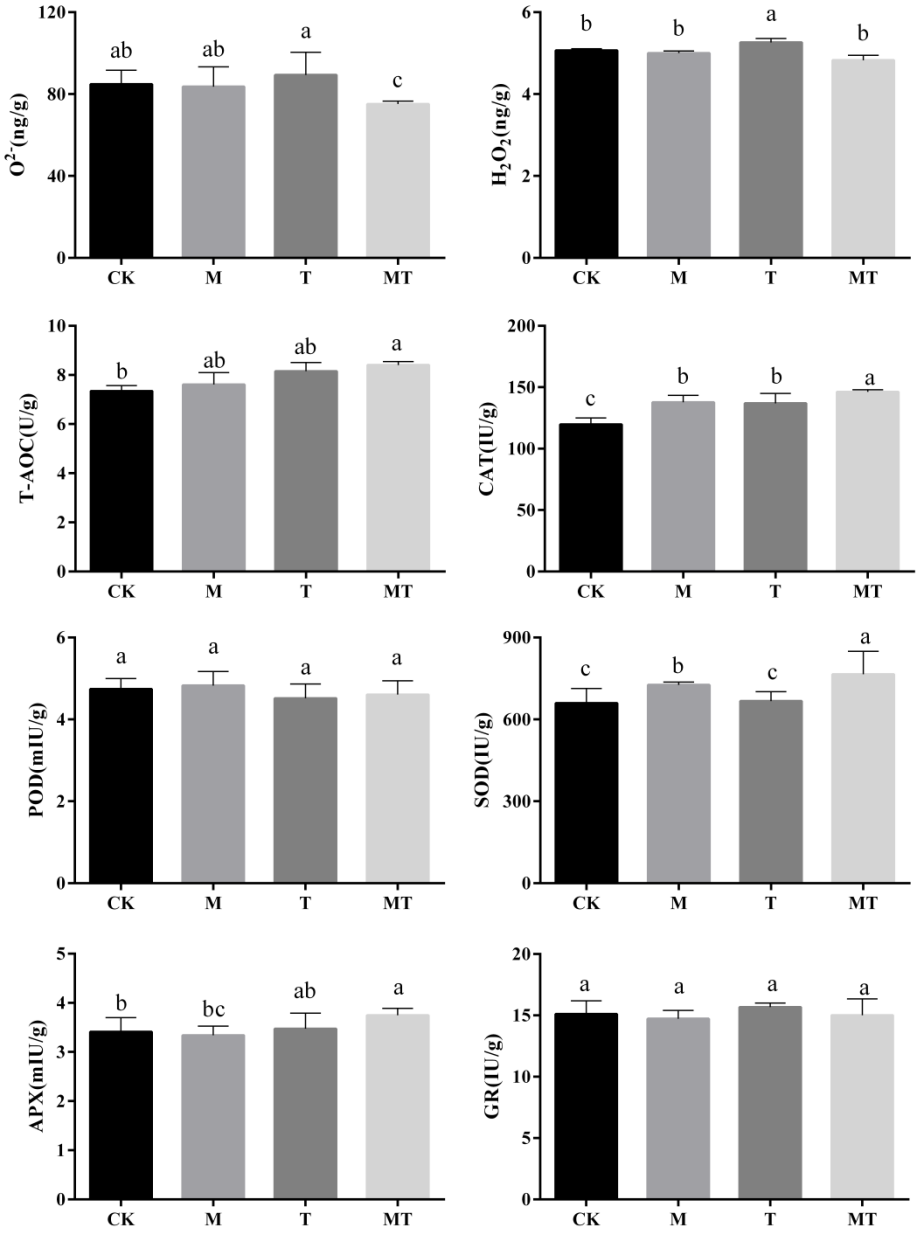

Figure 4. Influences of melatonin and cold stress on the O^{2-} and H_2O_2 contents, total antioxidant capacity (T-AOC) and antioxidant enzyme activities in banana leaves. Different letters above bars indicate significant differences among samples determined using Duncan's test at the $p < 0.05$ level. All values are displayed as mean ± standard deviation for three replications at the $p < 0.05$ level. CK: control; M: melatonin treatment; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment.

3.5. Influences of Melatonin on the Antioxidant Enzyme Activities in Banana Leaves

Both melatonin and cold treatments significantly induced the activity of CAT in banana leaves. Moreover, the CAT activities in MT, T and M were all significantly higher than that in CK, accounting for 1.22-, 1.14- and 1.15-fold that of CK, respectively. Melatonin treatment significantly induced the SOD activity in banana leaves. Furthermore, the SOD activities in MT and M were found to be significantly higher than that in CK (accounting for 1.16- and 1.10-fold of CK, respectively). Melatonin treatment also significantly induced the APX activity in banana leaves under cold stress, and the APX activity in MT was found to be significantly higher than that in CK, accounting for about 1.10−fold that of CK. Moreover, no significant POD and GR activity differences were identified among the four groups.

3.6. Effects of Melatonin on Osmoregulatory Substance Accumulations

The MDA content of T was significantly higher than that of CK (about 1.08-fold that of CK). However, the MDA content in M was found to be significantly lower than that of CK (Figure 5). This suggested that cold stress significantly induced the accumulation of MDA, but the application of exogenous melatonin suppressed its accumulation. Consistently, the MDA content in MT was similar to that in CK, suggesting that melatonin pre-treatment suppressed the cold-induced MDA accumulation in banana leaves.

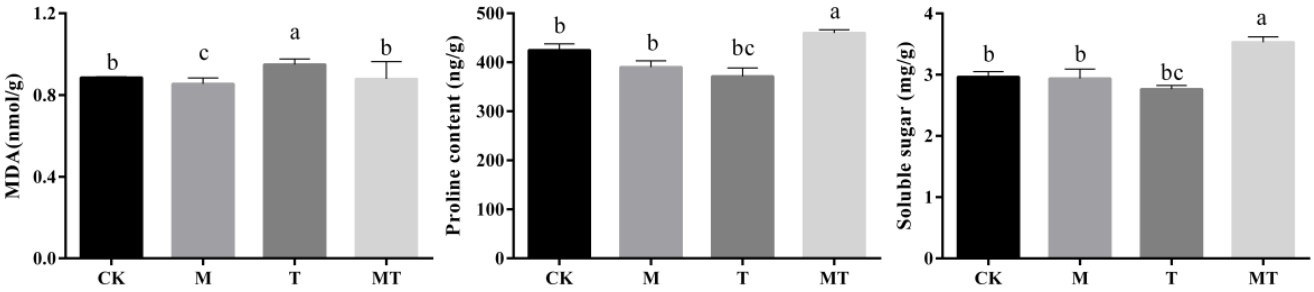

Figure 5. Influences of cold stress and melatonin treatments on the accumulations of osmoregulatory substances (MDA, proline and soluble sugar) in banana. Different letters above bars indicate a significant difference among samples at the $p < 0.05$ level. All the data are displayed as mean ± standard deviation for three replications. CK: control; M: melatonin treatment; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment.

The PRO and SS contents in the leaves of the four groups both followed the order: MT> CK > M> T, and their contents in MT were both significantly higher than in CK (accounting for 1.08- and 1.19-fold that of CK, respectively). These indicated that cold stress could suppress the accumulations of two osmoregulatory substances, but the application of exogenous melatonin significantly induced their accumulations under cold stress, thereby improving the cold resistance of banana.

3.7. Influences of Melatonin on the Expression of Cold-Responsive Genes in Banana

To reveal the possible molecular mechanism of the melatonin-enhanced cold resistance in banana, we studied and compared the expression of six known banana cold-responsive genes in the four groups (Figure 6). Results showed that the relative expression of *MaChiI1*, *MaCSDS1* and *MaADA1* all followed the order MT > T > M > CK, suggesting that these genes could be induced by both melatonin and cold stress, and melatonin pre-treatment further enhanced their upregulation under cold stress (Figure 6A–C). Noteworthily, the expression of *MaChiI1* in T and MT was significantly higher than that in CK, accounting for 57.92- and 83.94-fold that of CK, respectively (Figure 6A). The expression of *MaHOS1* was also significantly induced by melatonin, and its expression levels in M and MT were found to be significantly higher than that in CK, accounting for about 1.21- and 1.31-fold that of CK, respectively (Figure 6D). However, its expression in T was similar to that in CK. Melatonin also significantly induced the expression of *MaWhy1*, and its expression level in

M was about 1.30-fold that of CK. Its expression levels in T and MT were also higher than that in CK, but no significant difference was found (Figure 6E). Moreover, cold treatment significantly downregulated the expression of *MaKIN10*, whose expression level in T was only about 56.62% of CK (Figure 6F).

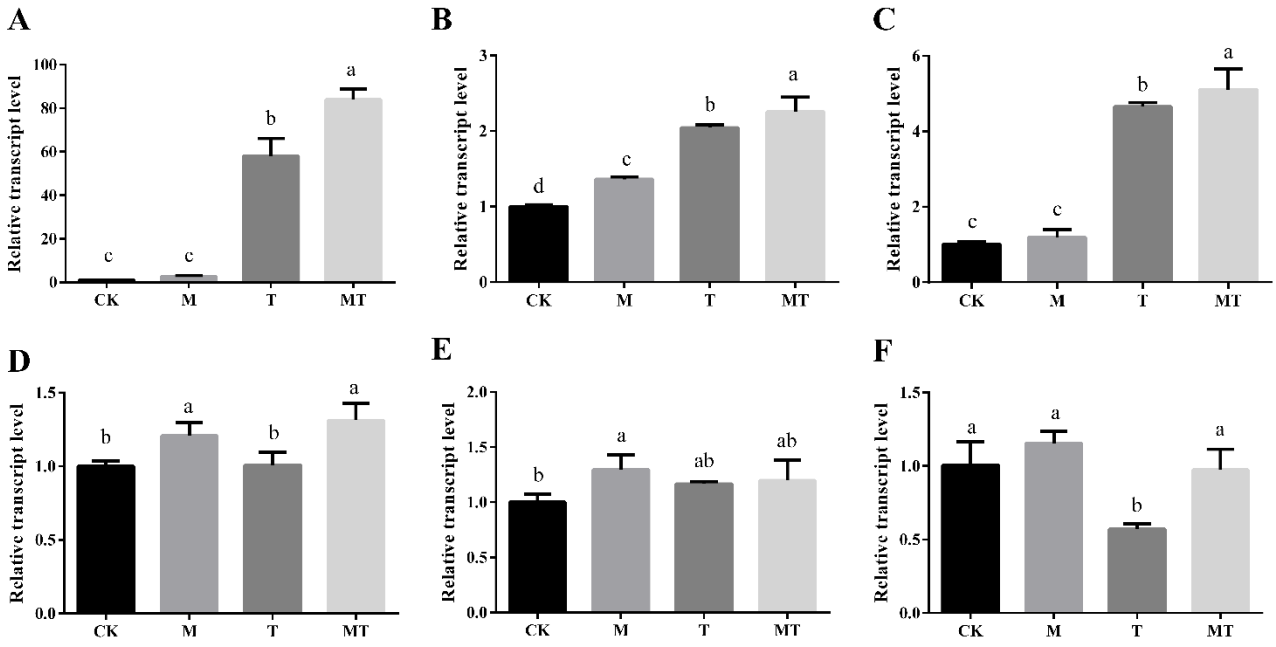

Figure 6. Expression patterns of cold-responsive genes in banana leaves from different groups. (**A**–**F**) *MaChiI1*, *MaCSDS1*, *MaADA1*, *MaHOS1*, *MaWhy1* and *MaKIN10* genes [13], respectively. Different letters above bars indicate a significant difference among samples determined using Duncan's test at the $p < 0.05$ level. All the data are displayed as mean ± standard deviation for three replications. CK: control; M: melatonin treatment; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment.

4. Discussion

The role of melatonin in improving plant cold resistance has been reported in many plants [22,26]. In tomato [22] and strawberry [27], the concentration of 100 μM had been identified as the optimal melatonin concentration that could show the best photosynthesis ability or cold-tolerance-promoting effects. Consistently, in our present study, we also found that 100 μM melatonin solution treatment showed the best cold-resistance-improving effect in banana. By using melatonin of this concentration, we investigated the influences of exogenous melatonin on physiochemical and molecular parameters in cold-stressed banana seedlings. The results obtained in this study are listed subsequently.

4.1. Application of Exogenous Melatonin Enhanced the Photosynthesis Ability of Banana under Cold Stress

Chlorophyll fluorescence parameters can reflect well the cold resistance of plants [28]. Studies have shown that F_v/F_m is the most intuitive indicator for the evaluation of plant photosynthesis ability. Under stress conditions, this parameter will be greatly suppressed [29,30]. In this study, we found that cold stress reduced the F_v/F_m value of banana, while the F_v/F_m value of the MT group was higher than that of the T group, indicating that melatonin reduced the inhibition of cold stress on banana photosynthesis ability. Moreover, the PI abs and ETo/CSm values of the T group were significantly higher than those of the CK, which was similar to the results obtained in cold-stressed bermudagrass [10]. Yang et al. [22] reported that 100 μM of melatonin increased the electron transfer rate and quantum yield of PSI and PSII photochemistry of tomato under cold stress. In our present study, we also found that the ABS/CSm, TRo/CSm and ETo/CSm values of the MT group

were significantly higher than those of the T group, indicating that melatonin promoted the absorption of light energy and the electron transfer efficiency of banana under cold stress.

4.2. Exogenous Melatonin Improves Banana Cold Resistance by Enhancing the Antioxidant Defense of Banana

Melatonin acts as an antioxidant to mitigate ROS damage to plants [31]. It functions by direct scavenging of ROS [32] or by producing N^1-acetyl-N^2-formyl-5-methoxykynuramine (AMFK, which has much higher antioxidant activity than melatonin) [33] or by regulating antioxidant enzyme activities [34,35]. Cold stress can lead to the extensive accumulation of O^{2-} and H_2O_2 in plants [36,37]. In our study, we also found that the accumulation of O^{2-} and H_2O_2 was significantly induced by low temperature [13]. The contents of O^{2-} and H_2O_2 in the MT group were significantly lower than those in the T group, which also indicated that melatonin reduced the accumulation of ROS in banana under cold stress.

The ROS accumulation leads to an increase in the activities of plant antioxidant enzymes [38]. Li et al. [11] found that application of exogenous melatonin reduced the ROS level; increased the activities of CAT and SOD and induced the expression of *CsSOD*, *CsPOD*, *CsCAT* and *CsAPX* genes in camellia under cold stress. Hu et al. [10] found that exogenous melatonin enhanced the antioxidant enzyme activities of bermudagrass under a low-temperature environment. Consistent with these studies, in the present study, the activities of CAT, SOD and APX in banana leaves were found to be upregulated by cold treatment. Notably, the CAT and SOD activities in the MT group were both found to be the highest, indicating that melatonin treatment improved the CAT and SOD activities in banana under cold stress. In plants, CAT and SOD function in the catalytic decomposition of hydrogen peroxide and superoxide anion, respectively [39]. Consistently, we found that the contents of O^{2-} and H_2O_2 in the MT group were both the lowest. Moreover, the T-AOC of the MT group was the highest among the four groups, indicating that melatonin can improve the cold resistance of banana by increasing the activities of antioxidant enzymes and by enhancing the antioxidant capacity [19,31].

MDA content reflects the stability of the cell membrane [40]. Under cold stress, the MDA content in plant leaves is usually upregulated [37]. In camellia [11] and cucumber [40], applications of exogenous melatonin were proved to have the ability of reducing the MDA accumulations caused by cold stress. In this study, the content of MDA in the T group was the highest, and the MDA content in the MT group was only 92.45% of that in the T group. Moreover, exogenous melatonin increased the contents of proline and soluble sugar in banana leaves under cold stress, which is consistent with the results of Turk et al. [8]. All these results indicated that melatonin is capable of maintaining the stability of plant cell membranes after cold stress by increasing the content of osmoregulatory substances.

4.3. Exogenous Melatonin Induces the Expression of Cold-Responsive Genes to Reprogram Banana Cold Responses

Melatonin can improve the cold resistance of plants by upregulating the expression of cold-signaling-related genes [41]. In our study, it was found that low-temperature treatment significantly induced the expression levels of *MaChiI1*, *MaCSD1C*, *MaWhy1*, *MaADA1* and *MaHOS1*. Moreover, the expression levels of these five genes in the M group were all higher than in the CK group, and their expression levels in the MT group were significantly higher than in the T group, indicating that melatonin treatment induced their expression at both normal and cold conditions. Among these genes, *MaChiI1* has been reported to be upregulated by low temperature for dozens of times and to play a key role in banana cold resistance [13]. In this study, *MaChiI1* was found to be significantly upregulated by about 58 times in cold-stressed banana leaves. In the MT group, however, the expression of *MaChiI1* was found to be more than 80 times that of CK, suggesting that exogenous melatonin further enhanced its upregulation under cold stress. The *MaKIN10* gene has been identified as a 4 and 0 °C low-temperature-suppressed banana low-temperature-responsive gene that functions in banana low-temperature responses by regulating sucrose

biosynthesis [17]. Consistently, in this study, we also found that cold stress significantly downregulated the expression of *MaKIN10*, and the SS content in T group was the lowest. Moreover, the expression of *MaKIN10* and the SS content in the MT group were both significantly higher than those in the T group. All these results indicated that melatonin could improve the cold resistance of banana by inducing the expression of cold-responsive genes.

5. Conclusions

In conclusion, melatonin can improve the cold resistance of banana mainly by (1) increasing the photosynthesis efficiency under cold stress; (2) enhancing the antioxidant defense of banana through increasing the activities of antioxidant enzymes such as CAT and SOD; suppressing the accumulations of MDA, O^{2-} and H_2O_2; enhancing the T-AOC and promoting proline and soluble sugar accumulations and (3) inducing the expression of cold-responsive genes (Figure 7).

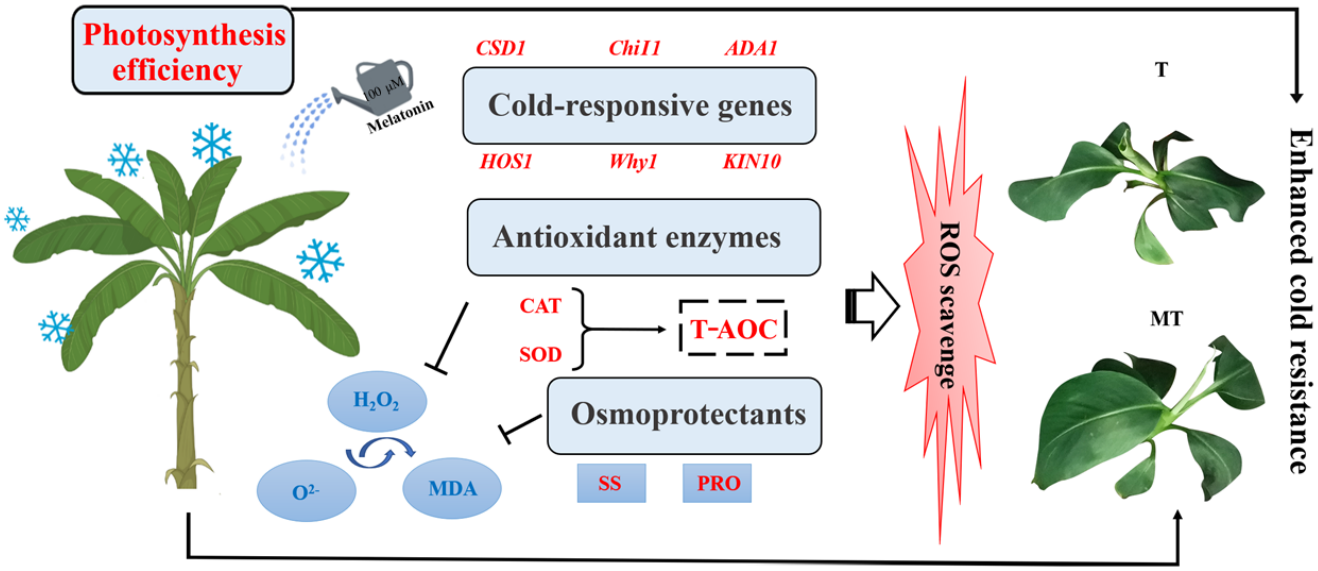

Figure 7. Schematic diagram for the mechanism of melatonin-improved cold resistance of banana. Red and blue font represents upregulation and downregulation under low-temperature treatment, respectively. *CSD1C*: copper/zinc superoxide dismutase 1C; *ChiI1*: chitinase anti-freeze protein I1; *ADA1*: adaptor 1; *HOS1*: high expression of osmotically responsive gene 1; *Why1* transcription factor WHIRLY 1; *KIN10*: SNF1-related protein kinase catalytic subunit alpha 10; CAT: catalase; SOD: superoxide dismutase; T-AOC: total antioxidant capacity; H_2O_2: hydrogen peroxide; O^{2-}: superoxide anion; MDA: malondialdehyde; SS: soluble sugar; PRO: proline; T: low-temperature treatment; MT: combined melatonin and low-temperature treatment.

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