Received: 3 August 2016 | Accepted: 9 September 2016

DOI: 10.1111/jpi.12367

ORIGINAL ARTICLE

WILEY Journal of Pineal Research Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Identification, transcriptional and functional analysis of heat-shock protein 90s in banana (*Musa acuminata* L.) highlight their novel role in melatonin-mediated plant response to Fusarium wilt

Yunxie Wei[1†] | Wei Hu[2†] | Qiannan Wang[1†] | Hongqiu Zeng[1] | Xiaolin Li[1] | Yu Yan[1] | Russel J. Reiter[3] | Chaozu He[1] | Haitao Shi[1]

[1]Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresources, College of Agriculture, Hainan University, Haikou, China

[2]Key Laboratory of Biology and Genetic Resources of Tropical Crops, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan Province, China

[3]Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, TX, USA

Correspondence
Haitao Shi and Chaozu He, Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresources, College of Agriculture, Hainan University, Haikou, China.
Emails: haitaoshi@wbgcas.cn; haitaoshi@hainu.edu.cn and czhe@hainu.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31570249; the Startup Funding and the Scientific Research Foundation of Hainan University, Grant/Award Number: kyqd1531

Abstract

As one popular fresh fruit, banana (*Musa acuminata*) is cultivated in the world's subtropical and tropical areas. In recent years, pathogen *Fusarium oxysporum* f. sp. cubense (*Foc*) has been widely and rapidly spread to banana cultivated areas, causing substantial yield loss. However, the molecular mechanism of banana response to *Foc* remains unclear, and functional identification of disease-related genes is also very limited. In this study, nine 90 kDa heat-shock proteins (HSP90s) were genomewide identified. Moreover, the expression profile of them in different organs, developmental stages, and in response to abiotic and fungal pathogen *Foc* were systematically analyzed. Notably, we found that the transcripts of 9 *MaHSP90s* were commonly regulated by melatonin (*N*-acetyl-5-methoxytryptamine) and *Foc* infection. Further studies showed that exogenous application of melatonin improved banana resistance to Fusarium wilt, but the effect was lost when cotreated with HSP90 inhibitor (geldanamycin, GDA). Moreover, melatonin and GDA had opposite effect on auxin level in response to *Foc4*, while melatonin and GDA cotreated plants had no significant effect, suggesting the involvement of *MaHSP90s* in the cross talk of melatonin and auxin in response to fungal infection. Taken together, this study demonstrated that *MaHSP90s* are essential for melatonin-mediated plant response to Fusarium wilt, which extends our understanding the putative roles of *MaHSP90s* as well as melatonin in the biological control of banana Fusarium wilt.

KEYWORDS

90-kDa heat-shock protein, banana (*Musa acuminata*), expression profile, fungal pathogen *Fusarium oxysporum* f. sp. cubense, melatonin

1 | INTRODUCTION

As a popular fresh fruit, banana (*Musa acuminata*) is widely cultivated in the world's subtropical and tropical areas.[1-5] So far, considering different objections such as yield, storage, favor, or resistance, many banana varieties have been screened and cultivated in the world. For example, BaXi jiao (*Musa acuminata* L. AAA group cv. Cavendish, BX) is widely cultivated in China because of its high production and long-term storage, and Fen jiao (*Musa* ABB Pisang Awak, FJ) is popular due to its good flavor and abiotic stress resistance.[6,7] Since 1960, virulent pathogen *Fusarium oxysporum* f. sp. cubense

[†]These authors contributed equally to this work.

Race 1 (*Foc1*) and *Foc4* have been widely and rapidly spread to banana cultivate areas, causing substantial yield loss in many countries, especially in Australia and Asia.[8-13] Additionally, because banana is asexually propagated using suckers from the rhizomes, the soil-grown pathogens are not easily to be removed, thus making the panama disease (a fungal disease of bananas producing yellowing and wiltint of the leaves) more and more serious and becoming a worldwide problem of banana industry.[14-20]

Ninety kDa heat-shock protein (HSP90) is one type of the highly conserved molecular chaperone, with three conserved domains. The N-terminal ATPase domain, the middle domain, and the C-terminal domain are responsible for HSP90 protein inhibition, conformation, and dimerization, respectively.[21,22] HSP90, as molecular chaperone, is involved in the growth, development, and immune response in animals through regulating and maintaining the conformation of multiple proteins.[23,24] The in vivo roles of *HSP90* are characterized in several model plants, such as *Arabidopsis*, rice, and wheat.[25-32] Using knockout/knockdown mutants and specific inhibitor treatment, it is found that *HSP90s* are widely involved in seedling, leaf, hypocotyls and reproductive development,[22,28,29,33-35] abiotic stress response, including heat, drought, salt,[36-40] as well as *Resistant* (*R*) gene (which is encoding immune receptor)-mediated immune responses.[25-29,31,32,37]

With the enlargement of the domestic and international market, there is a need for worldwide banana production. To better develop sustainable banana industry, it is necessary to characterize disease-related genes and investigate their underlying molecular mechanism.[15-18] Based on the public available of a double-haploid banana (*Musa acuminata* L. AA) genome sequence,[41] many research groups start to identify differentially expressed genes in response to *Foc* infection.[11-13,19] However, the molecular mechanism of banana response to *Foc* remains unclear, and functional identification of disease-related genes is also very limited.

To investigate the in vivo role of *HSP90s* and possible utilization for genetic breeding in banana, 9 *MaHSP90s* were characterized by genomewide identification and expression analyses during development and stress response in this study. Notably, the relationship between melatonin and *MaHSP90s* in banana resistance to Fusarium wilt was also revealed.

2 | METHODS

2.1 | Plant materials and growth conditions

Two widely cultivated banana varieties (BX and FJ) were used in this study. Five-leaf stage banana seedlings that are from Banana Tissue Culture Center (Danzhou, Institute of Banana and Plantains, Chinese Academy of Tropical Agricultural Sciences) were grown in soil with Hoagland's solution in the greenhouse. The greenhouse was controlled at 28°C and 12-hour light/26°C and 12-hour dark cycles, with the irradiance of 120-150 μmol quanta m^{-2} s^{-1}.

2.2 | Genomewide identification and systematic analysis of *MaHSP90s*

The predicted *MaHSP90s* were first searched in *Musa acuminata v1* (Banana) Phytozome database v10.3, and further verified using NCBI's conserved domain database (CDD) (http://www.ncbi.nlm.nih.gov/cdd)[42] and Pfam database (http://pfam.xfam.org).[43] After confirmation, the sequences, the locus name, and chromosome location of *MaHSP90s* were downloaded from Phytozome database v10.3 and ProtParam software (http://web.expasy.org/protparam).

Based on the sequences of 9 *MaHSP90s*, 8 *AtHSP90s,* and 9 *OsHSP90s* from Phytozome database v10.3, the neighbor-joining phylogenetic trees were constructed using Clustalx 1.83 and MEGA5.05 softwares.[44] Gene structure (upstream, exon, intron, and downstream) and conserved motifs of *MaHSP90s* were analyzed using Gene Structure Display Server (GSDS) v2.0 (http://gsds.cbi.pku.edu.cn/index.php)[45] and Multiple Em for Motif Elicitation (MEME) v4.11.0 (http://meme-suite.org/tools/meme), respectively, according to the manufacturer's protocol.

2.3 | RNA isolation, reverse transcript, and quantitative real-time PCR

Total RNA was extracted with RNAprep Pure Plant Kit (TIANGEN, DP441, Beijing, China), followed by the elimination of contaminating DNA using RNase-free DNase (NEB, M0303S, USA), according to the manufacturer's instruction. After quantification using NANODROP 2000 (Thermo Scientific, Waltham, MA, USA), the extracted RNA was used for the synthesis of first-strand cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, K1622). Quantitative real-time PCR was then performed using the diluted cDNA and TransStart Tip Green qPCR SuperMix (TransGen Biotech, AQ141, Beijing, China) in LightCycler® 96 Real-Time PCR System (Roche, Basel, Switzerland), according to the manufacturer's instruction. The primers used were listed in Table S1.

2.4 | Expression profile analysis of *MaHSP90s*

The transcriptomic data were obtained from published data in Hu et al.[6,7] and Li et al.[12] For this assay, plant samples were collected from leaves and roots at five-leaf stage and fruits at 0, 20, and 80 days after flowering (DAF), and at 8 and 14 days

postharvest (DPH) (BX variety), or at 3 and 6 days DPH (FJ variety). For abiotic stress treatments, five-leaf stage banana seedlings were treated by 4°C for 22 hours, 200 mmol L^{-1} Mannitol for 7 days, and 300 mmol L^{-1} NaCl for 7 days. For fungal pathogen of *Foc1* and *Foc4* treatments, sterile tissue cultivated roots were inoculated by control (water), *Foc1,* or *Foc4* for 3 hours, 27 hours, and 51 hours.

2.5 | Hierarchical cluster analysis of gene expression

The gene expression data were first normalized to relative value. Thereafter, the hierarchical cluster analysis was performed using CLUSTER software (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm)[46] according to the manufacturer's protocol. Then, the underlying heatmap was constructed and downloaded using Java Treeview software (http://jtreeview.sourceforge.net).[47]

2.6 | Chemical treatment

For the gene expression assay, five-leaf stage banana roots were pretreated by control (mock) and 100 μmol L^{-1} melatonin for 0, 1, 3, and 6 hours. For the assays of physiological parameters, five-leaf stage banana roots were pretreated by control (mock), 100 μmol L^{-1} melatonin, 10 μmol L^{-1} geldanamycin (GDA), 100 μmol L^{-1} melatonin plus 10 μmol L^{-1} GDA for 2 days, thereafter were inoculated by *Foc4* for indicated time points.

2.7 | Determination of electrolyte leakage and chlorophyll

The relative electrolyte leakage (EL) was calculated as the ratio of initial conductivity (C_i) to the maximum conductivity (C_{max}) of plant leaves, which were assayed by the conductivity meter (Yueping-DDS-307, Shanghai, China), according to the manufacturer's protocols. Chlorophyll was extracted from leaves using 80% (v/v) acetone, and the supernatant was determined by examining the absorbance at 645 nm and 663 nm.

2.8 | Quantification of endogenous melatonin and plant hormones

The melatonin and plant hormone (salicylic acid [SA], jasmonic acid [JA], ethylene [ETH] and indole-3-acetic acid [IAA]) levels in plant extract were quantified using Plant melatonin enzyme-linked immunosorbent assay (EIASA) Kit, Plant SA ELISA Kit, Plant JA ELISA Kit, Plant ET ELISA Kit, Plant IAA ELISA Kit (Jianglai Biotechnology, Shanghai, China), respectively, according to the manufacturer's protocols.

3 | RESULTS

3.1 | Genomewide identification of *MaHSP90s*

After initial BLAST analysis and further verification using CDD[42] and Pfam database,[43] 9 *MaHSP90s* were successfully identified from the banana genome. Totally, Arabidopsis, rice, and banana have 7, 9, and 9 *HSP90s*, respectively (Figure 1; Table S2). The unrooted neighbor-Joining tree was constructed to investigate the evolutionary relationship among *MaHSP90s*, *AtHSP90s,* and *OsHSP90s*. Generally, *MaHSP90s* have closer relationship with *OsHDP90s* than *AtHSP90s* (Figure 1), which is consistent with the current understanding of plants evolutionary history.

To investigate the structural features of 9 *MaHSP90s*, GSDS v2.0[45] was used to analysis upstream (5′ UTR)/downstream (3′ UTR) and intron/exon structures. The *MaHSP90s* in the same subfamilies displayed the similar structure (Figure 2A), indicating the relationship between evolution and gene structure. *MaHSP90.1*, *2*, *3*, *4*, *5*, *6* have 4-6 introns, while *MaHSP90.7*, *8*, *9* show 19, 18, 18 introns, respectively (Figure 2A). Additionally, MEME v4.11.0 was used to reveal the similar motifs of

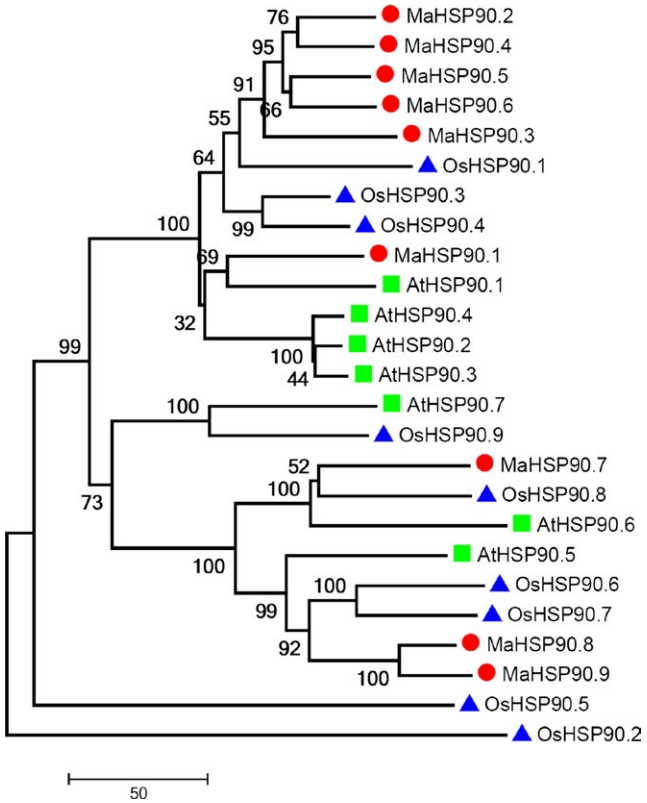


FIGURE 1 The neighbor-joining phylogenetic trees of *MaHSP90s*, *AtHSP90s,* and *OsHSP90s*. *AtHSP90s*, *OsHSP90s,* and *MaHSP90s* were indicated in green, blue, and red, respectively

the 9 *MaHSP90s*. Consistently, two subfamilies (one is *MaHSP90.1*, *2*, *3*, *4*, *5*, *6*, and the other one is *MaHSP90.7*, *8*, *9*) exhibited more conserved motifs (Figures 2B and S1), suggesting the link between evolution and conserved motifs.

3.2 | The expression profiles of *MaHSP90s*

To get more clues on the roles of *MaHSP90s* in banana growth and development, their expression patterns in two widely cultivated varieties (BX and FJ) were analyzed

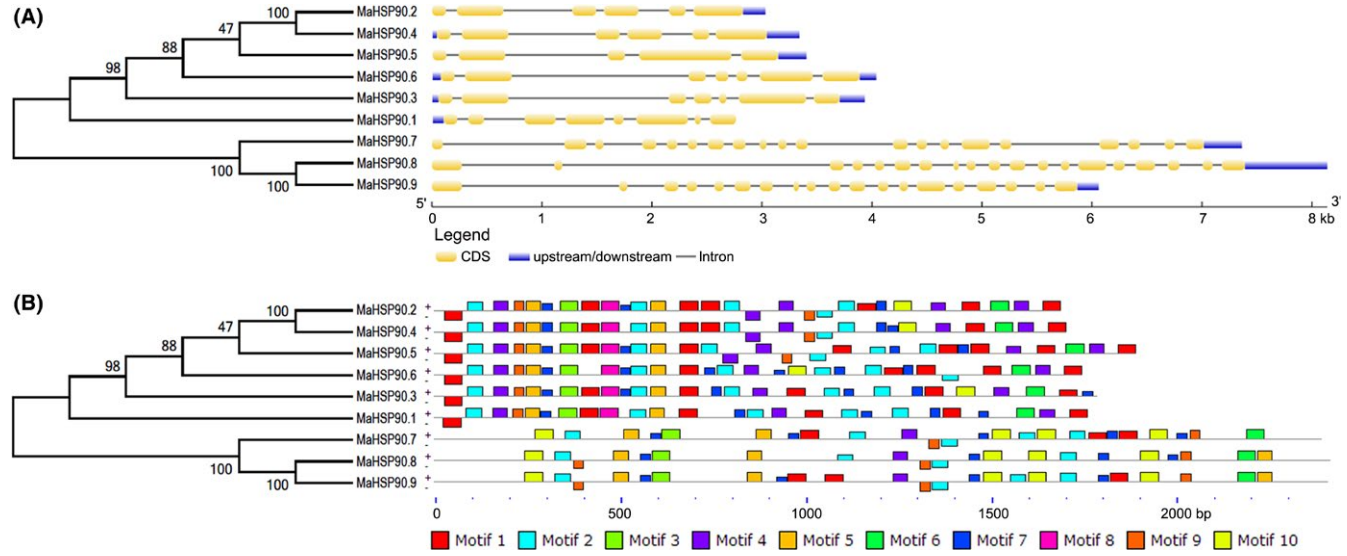


FIGURE 2 The relationship among phylogenetic link, gene structure, and conserved motifs of *MaHSP90s*. A, The link between phylogenetic relation and gene structure of *MaHSP90s*. B, The link between phylogenetic relation and conserved motifs of *MaHSP90s*. The 10 conserved motifs were indicated by different colored boxes and shown in Fig. S1

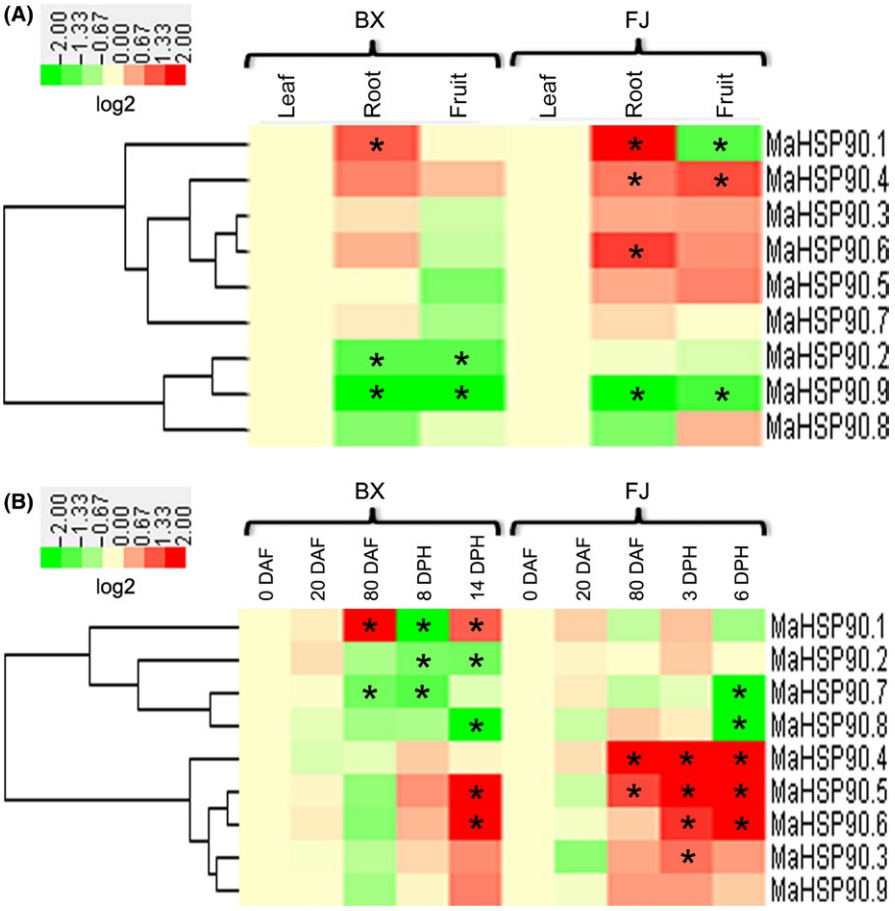


FIGURE 3 The expression profiles of *MaHSP90s* in different organ and developmental stages. A, The expression profiles of *MaHSP90s* in five-leaf stage leaves, roots, fruits of 80 DAF in BX and FJ varieties. B, The expression profiles of *MaHSP90s* in fruits of 0, 20, 80 DAF, 8 and 14 DPH (BX) or 3 and 6 DPH (FJ). Asterisk symbol (*) indicates fold change >2 in comparison with leaves or 0 DAF fruits

based on transcriptomic analysis.[6,7] For BX variety, *MaHSP90.1* transcripts exhibited higher levels in roots, while *MaHSP90.2* and *MaHSP90.9* transcripts showed lower levels in roots and fruits (Figure 3A,B). Moreover, *MaHSP90.1* displayed higher transcripts accumulation in the later fruit developmental and ripening stages; *MaHSP90.5* and *MaHSP90.6* showed higher transcripts accumulation only in the later fruit ripening stage; and *MaHSP90.2*, *7*, *8* exhibited lower transcripts in fruit ripening stages (Figure 3A,B). For FJ variety, *MaHSP90.4* transcript exhibited higher levels in roots and fruits, while *MaHSP90.9* transcripts showed lower levels in roots and fruits (Figure 3A,B). Additionally, *MaHSP90.7* and *MaHSP90.8* displayed lower transcript accumulation in the later fruit ripening stage, while *MaHSP90.4*, *5*, *6* exhibited higher transcripts in fruit ripening stages (Figure 3A,B).

Generally, abiotic stress such as cold, osmotic, and salt stresses had slight effects on the transcripts of *MaHSP90s* in the transcriptomic analysis.[6,7] *MaHSP90.1* transcript was downregulated by osmotic stress in BX, but was upregulated by cold and salt stresses in FJ (Figure 4). *MaHSP90.4* transcript was only downregulated by osmotic stress in BX, while *MaHSP90.8* transcript was upregulated by osmotic stress in both BX and FJ varieties (Figure 4).

In comparison with abiotic stress, fungal pathogen of *Foc1* and *Foc4* treatments commonly regulated the transcripts of 7 *MaHSP90s* (Figure 5). Among them, *MaHSP90.1* transcript was commonly downregulated by *Foc1* and *Foc4* infection, *MaHSP90.8* transcript was downregulated by *Foc4* infection, while *MaHSP90.7* and *MaHSP90.9* transcripts were upregulated by *Foc1* and *Foc4* infection, respectively (Figure 5). Unlike the above genes, *MaHSP90.1*, *5*, *6* showed complex expression patterns in response to *Foc* infection (Figure 5). After Foc1 infection, the transcript levels of *MaHSP90.1*, *5*, *6* were upregulated at 3 hours postinfection (hpi), but were downregulated at 27 or 51 hpi (Figure 5). At 3 hpi of *Foc4*, *MaHSP90.1,* and *MaHSP90.6,* transcripts were upregulated; at 27 hpi of *Foc4*, *MaHSP90.5,* and *MaHSP90.6,* transcripts were downregulated; at 51 hpi of *Foc4*, *MaHSP90.1,* and *MaHSP90.5,* transcripts were upregulated and downregulated, respectively (Figure 5).

3.3 | The common regulation of *MaHSP90s* in response to melatonin

Interestingly, we found that the transcripts of all *MaHSP90s* were commonly regulated by melatonin, one widely known

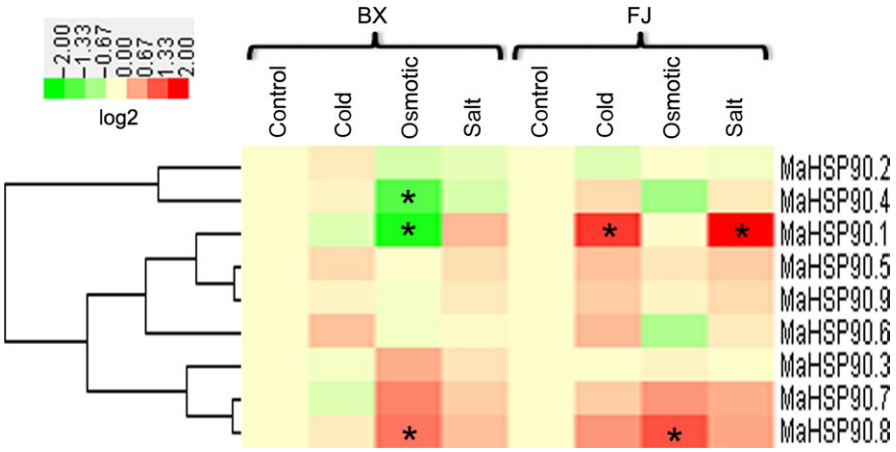


FIGURE 4 The expression profiles of *MaHSP90s* in response to 4°C for 22 h, 200 mmol L^{-1} Mannitol for 7 d, and 300 mmol L^{-1} NaCl for 7 d. Asterisk symbol (*) indicates fold change >2 in comparison with control treatment

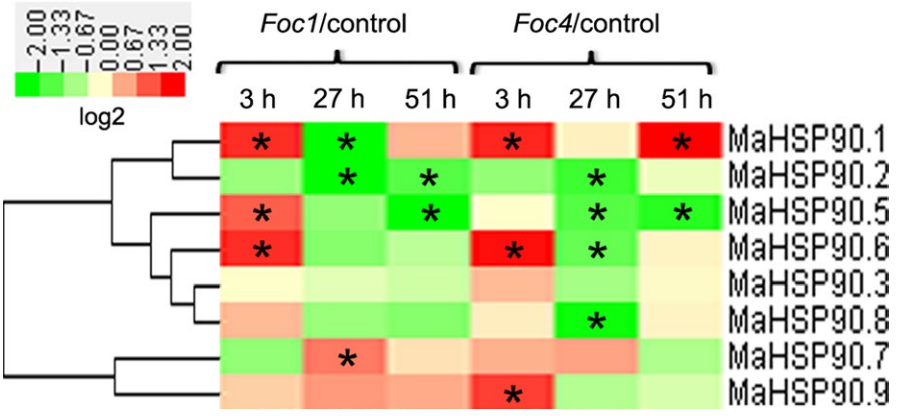


FIGURE 5 The expression profiles of *MaHSP90s* in response to fungal pathogen of *Foc1* and *Foc4* treatments. Asterisk symbol (*) indicates fold change >2 in comparison with control treatment

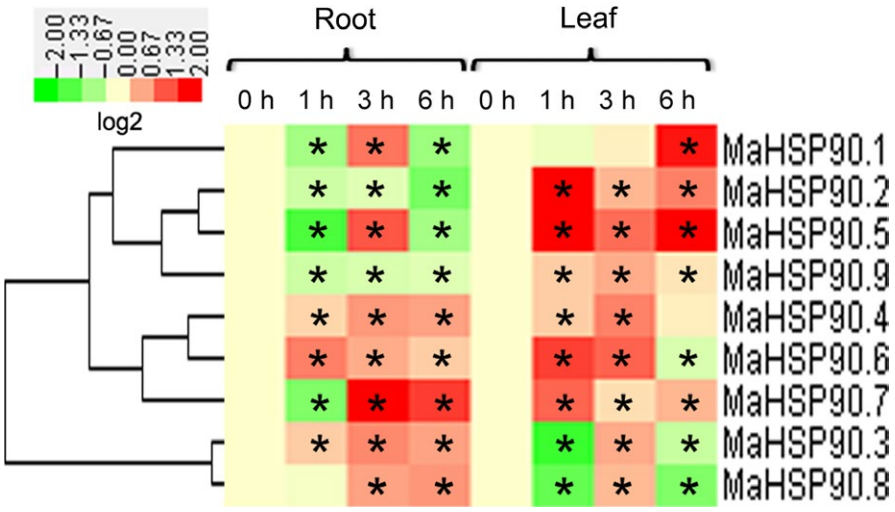


FIGURE 6 The expression profiles of *MaHSP90s* in response to exogenous melatonin treatment. For the assay, five-leaf stage banana leaves and roots were treated by control and 100 μmol L^{-1} melatonin for 0, 1, 3, and 6 h. Asterisk symbol (*) indicates a significant difference in comparison with control treatment

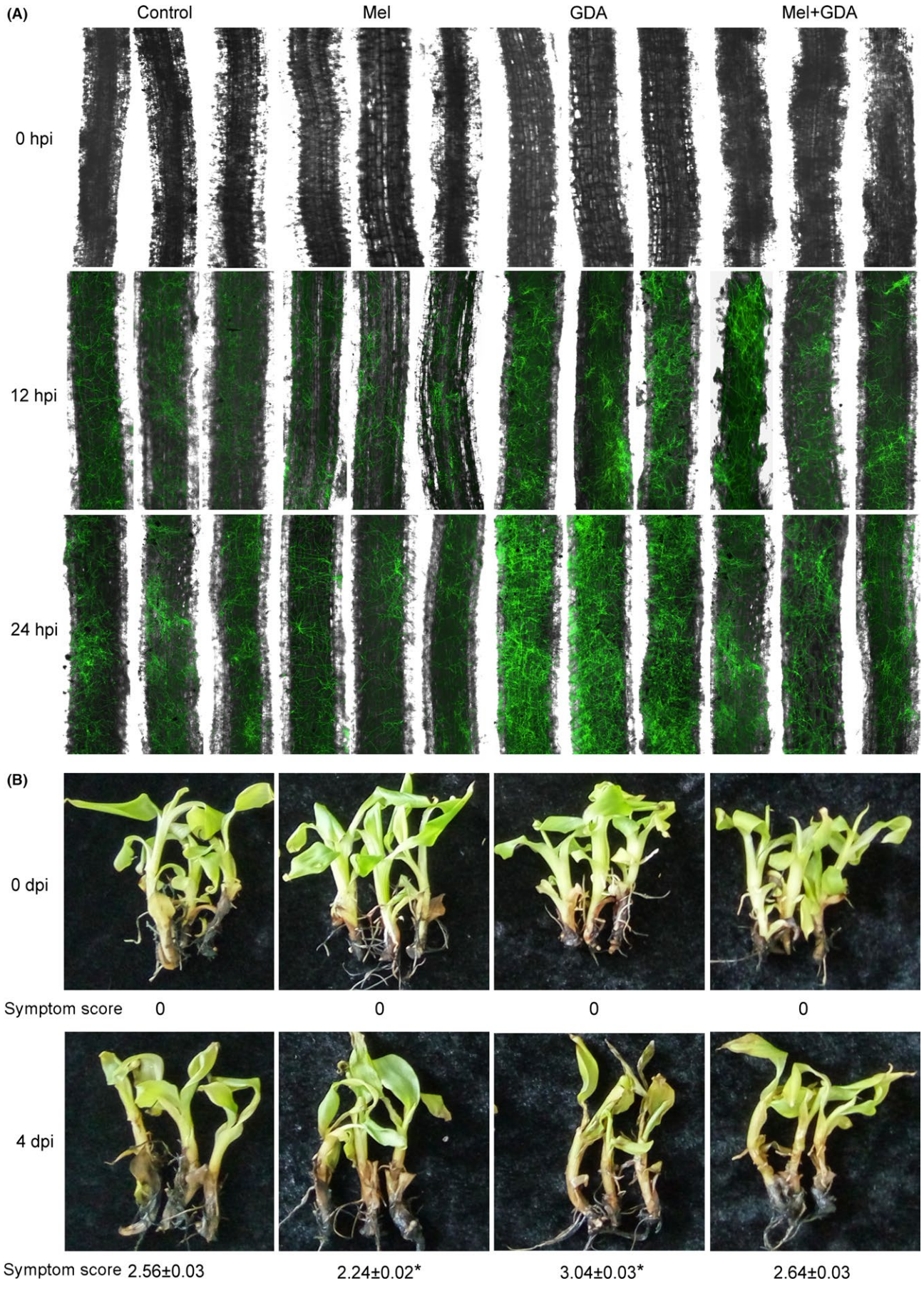
molecule and possible secondary messenger in plant stress responses. Overall, most of the *MaHSP90s* transcripts were significantly upregulated after melatonin treatment in banana roots and leaves (Figure 6). Together with the common regulation of *MaHSP90s* by various stresses and widely involvement of melatonin in stress resistance, we concluded the possible involvement of *MaHSP90s* in melatonin-mediated disease resistance.

3.4 | The involvement of *MaHSP90s* in melatonin-mediated plant response to Fusarium wilt

To better investigate the phenotypes during fungal infection, the green fluorescent protein (GFP)-tagged *Foc4* strain was used to inoculate five-leaf stage banana roots with 2 days of different pretreatments (control, 100 μmol L^{-1} melatonin, 10 μmol L^{-1} HSP90 inhibitor (GDA), 100 μmol L^{-1} melatonin plus 10 μmol L^{-1} GDA). At 12- and 24-hour postinoculation (hpi), melatonin-pretreated roots and GDA-pretreated roots showed significantly less and more fluorescence in comparison with control, while melatonin and GDA cotreated roots exhibited no significant difference to control (Figure 7A). At 4-day postinoculation (dpi) of *Foc4*, melatonin-treated plants continued to display less symptoms than control, while symptoms on GDA-treated plants were severer (Figure 7B). Consistently, melatonin and GDA cotreated plants exhibited similar symptoms to those of control plants (Figure 7B). The degree of resistance values was further confirmed by the relative EL and chlorophyll levels (Figure 8A,B). Melatonin-treated plants showed lower EL but higher chlorophyll levels. In contrast, GDA-treated plants displayed the opposite results. Melatonin and GDA cotreated plants showed no significant difference to control plants (Figure 8A,B). All the results indicate the essential role of *MaHSP90s* in melatonin-mediated plant response to Fusarium wilt.

To further investigate the possible mechanism, we quantified the effects of 2 days treatments of 100 μmol L^{-1} melatonin and 10 μmol L^{-1} HSP90 inhibitor (GDA) on endogenous levels of melatonin and plant hormones in banana. We found that both melatonin and GDA had significant effects on the accumulation of melatonin and disease-related plant hormones (IAA, SA, JA, and ETH) (Figure 9). Melatonin-treated plants showed lower levels of IAA, SA, JA, and ETH at 0 hpi of *Foc4*, but exhibited higher levels of them at 12 hpi than control (Figure 9). GDA-treated plants displayed lower levels of IAA, SA, JA, and ETH at 0 hpi of *Foc4*, but exhibited higher levels of SA and ETH at 12 hpi than control (Figure 9). Overall, melatonin and GDA cotreated plants had less significant effects on these hormones than melatonin-treated plants and GDA-treated plants (Figure 9). The modulation of melatonin and GDA on various plant hormones suggested that these hormones may be involved in melatonin and *MaHSP90s*-mediated plant response to Fusarium wilt, at least partially.

FIGURE 7 The involvement of *MaHSP90s* in melatonin-mediated disease resistance in response to *Foc4* in banana. A, Micrographs of banana roots at 0, 12, 24 hpi with GFP-tagged *Foc4* strain. B, The typical *Foc4*-infected symptoms and symptom scores after 0 and 4 dpi of *Foc4* in banana. Symptoms were evaluated with a scoring scale for each leave, from 0 to 4 as follows: 0, no symptoms; 1, the leaves turn to be chlorosis slightly; 2, the leaves turn to be chlorosis and yellow; 3, the leaves turn to be yellow and most withering; 4, the total leave withering. For the assay, five-leaf stage banana roots were pretreated by control, 100 μmol L^{-1} melatonin, 10 μmol L^{-1} GDA, 100 μmol L^{-1} melatonin plus 10 μmol L^{-1} GDA for 2 d, thereafter were inoculated by *Foc4*. At least, leaves from 30 plants were used for symptom score assays. Asterisk symbol (*) indicates a significant difference in comparison with control treatment


(A)
Control
Mel
GDA
Mel+GDA
0 hpi
12 hpi
24 hpi
(B)
0 dpi
Symptom score 0 0 0 0
4 dpi
Symptom score 2.56±0.03 2.24±0.02* 3.04±0.03* 2.64±0.03

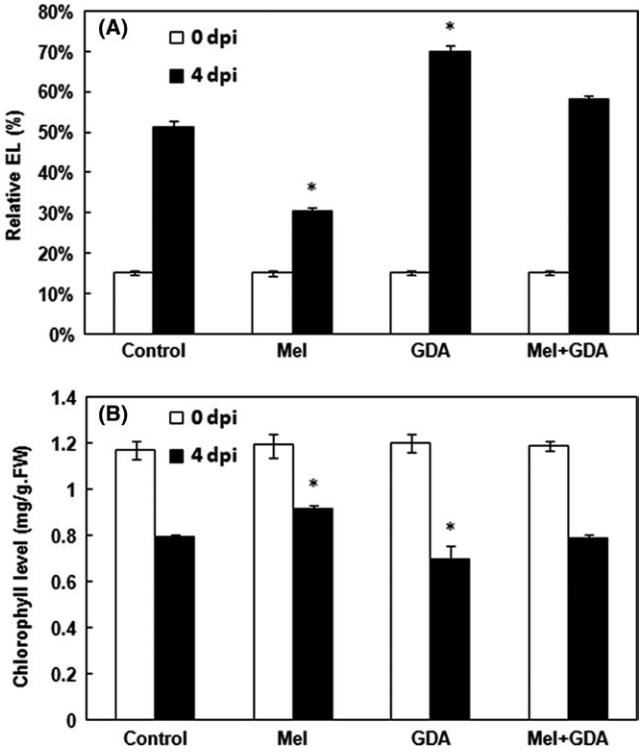


FIGURE 8 The relative EL (A) and chlorophyll level (B) in banana leaves with different treatment. For the assay, five-leaf stage banana roots were pretreated by control, 100 μmol L^{-1} melatonin, 10 μmol L^{-1} GDA, 100 μmol L^{-1} melatonin plus 10 μmol L^{-1} GDA for 2 d, thereafter were inoculated by *Foc4* for 4 d. Asterisk symbol (*) indicates a significant difference in comparison with control treatment

4 | DISCUSSION

As a widely known amine molecule, melatonin (*N*-acetyl-5-methoxytryptamine) is very important in innate immunity in animals.[48-55] To date, several studies have also reported the protective role of melatonin in plant immunity.[56-64] Yin et al.[63] found that melatonin application improved apple resistance to Marssonina apple blotch (*Diplocarpon mali*) by regulating antioxidants, hydrogen peroxide (H_2O_2), and pathogen-related proteins (PRs). Arnao and Hernández-Ruiz[49] found that melatonin pretreatment largely resists fungal infection (*Penicillium* spp.) in *Lupinus albus* seeds, and melatonin limited the growth of *Alternaria* spp. in potato dextrose agar (PDA) plate.

Moreover, our group together with other two independent groups confirmed the protective effect of melatonin treatment on plant disease resistance against bacterial pathogen *Pseudomonas syringe* pv. tomato such as *Pst* DC3000 in *Arabidopsis*, which was also supported by the results in *Nicotiana benthamiana*.[56-62,64] Back's group found that SA and ETH signaling as well as mitogen-activated protein kinase (MAPK) pathways are required for melatonin-mediated defense resistance in *Arabidopsis*.[56-58] Ma's group found that melatonin-regulated carbohydrate metabolism (sucrose, xylose, galactose, and invertase activity including cell wall invertase [CWI] and vacuolar invertase [VI]) as well as melatonin-activated SA responsive genes could contributed to disease resistance against *Pseudomonas syringae* pv. tomato DC3000 infection in *Arabidopsis*.[64] Besides some consistent results, our studies had some novel findings.[59-62] On one hand, we revealed the involvement of sugars and glycerol in melatonin-mediated basal immunity, in SA and nitric oxide (NO) signaling-dependent pathways.[59,60] On the other hand, we demonstrated the diurnal changes of endogenous melatonin and the corresponding changes of *C-repeat-binding factors* (*CBFs*)/*Drought response element Binding 1 factors* (*DREB1s*) expression in the diurnal cycle of plant innate immunity in *Arabidopsis*.[61,62]

Considering the widely involvement of *HSP90s* in plant stress resistance,[25-32] *MaHSP90s* were chosen as candidate genes for possible utilization in genetic breeding. Totally, 9 *MaHSP90s* were characterized through genomewide identification, phylogenetic relationship, gene structure, and conserved motif analyses. Based on the transcriptomic data,[6,7,12] the transcription profiles of 9 *MaHSP90s* in different organs, developmental stages of fruit, as well as in response to abiotic and biotic stress were comprehensively revealed. Compared with the effects of abiotic stress, fungal pathogen of *Foc1* and *Foc4* treatments commonly regulated the transcripts of 7 *MaHSP90s*, indicating their possible role in banana disease resistance. Notably, the transcripts of 9 *MaHSP90s* were commonly regulated by melatonin, suggesting the possible involvement of them in melatonin signaling in banana. In addition, melatonin confers improved disease resistance of banana to *Foc* infection, whereas exogenous application of HSP90 specific inhibitor (GDA) had the converse effect. Interestingly, combined application of melatonin and HSP90 inhibitor nullified the protective role of melatonin on disease resistance. We concluded that *MaHSP90s* may be essential for melatonin in the biological control of banana Fusarium wilt.

Plant hormone, including SA, JA, ETH, and auxin, plays important roles in pathogen-activated defense signaling.[65-70] SA is required for plant basal defense, such as R protein-mediated plant immunity as well as pattern recognition receptor (PRR).[65-67] JA and ET play important roles in plant basal defense especially against fungal infection.[65-67] Auxin is also an important regulator in plant-pathogen interaction. Pathogen, such as *Pseudomonas syringae*, regulated host auxin synthesis, and auxin directly modulated plant disease resistance.[67-70] In recent years, emerging evidence has shown that the cross talks among these hormones are involved in balancing fitness cost and immune responses.[65-67] So far, the direct effects of these hormones on fungal resistance in banana remain unclear. In this study, both melatonin and GDA had significant effects on the accumulation of disease-related plant hormones (IAA, SA, JA, and ETH),

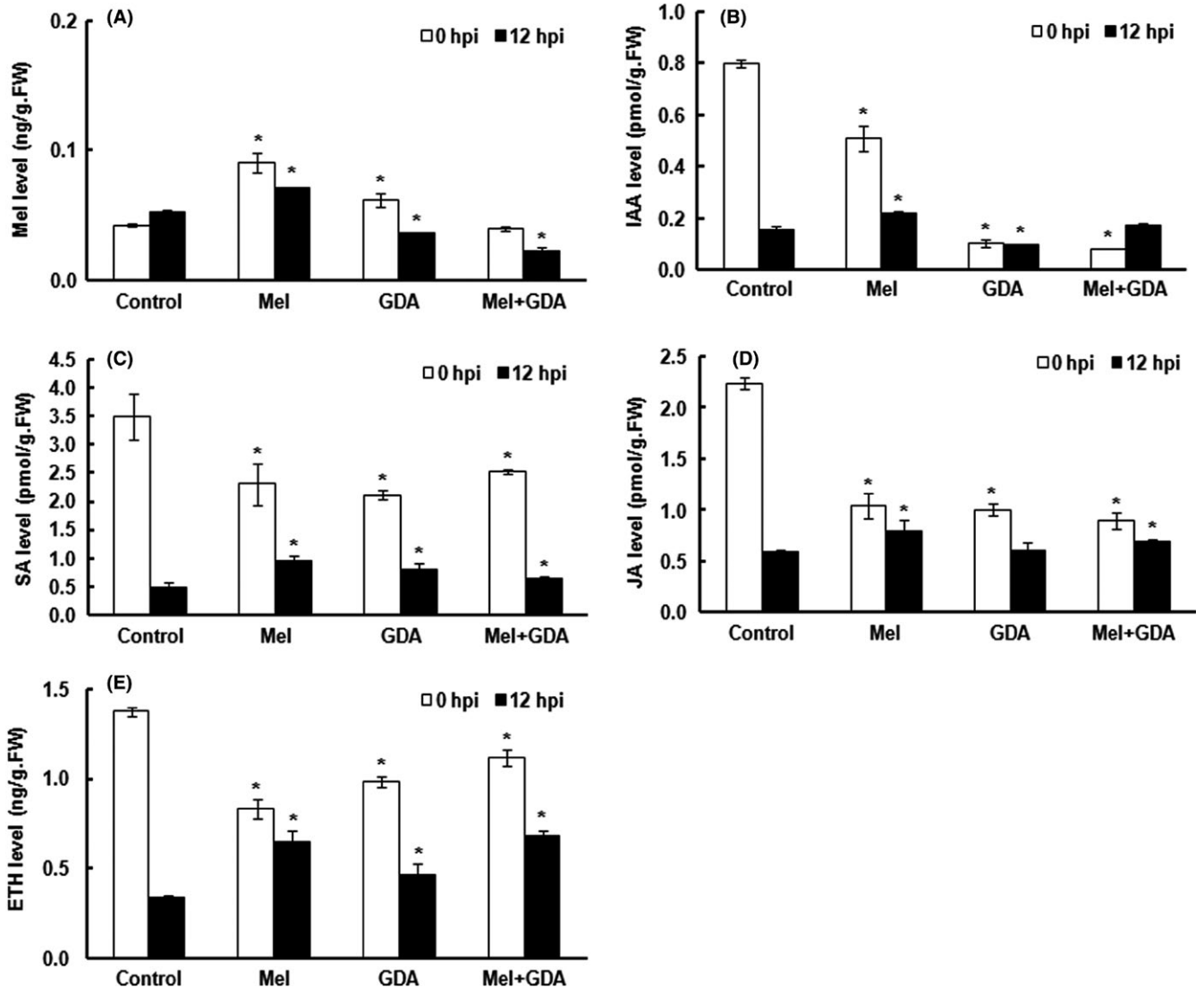


FIGURE 9 The effects of melatonin and HSP90 inhibitor (GDA) on endogenous levels of melatonin and plant hormones in banana. Quantification of melatonin (A), IAA (B), SA (C), JA (D), and ETH (E) in banana roots after different treatments. For the assay, five-leaf stage banana roots were pretreated by control, 100 μmol L^{-1} melatonin, 10 μmol L^{-1} GDA, 100 μmol L^{-1} melatonin plus 10 μmol L^{-1} GDA for 2 d, thereafter were inoculated by *Foc4* for 0 and 12 h. Asterisk symbol (*) indicates a significant difference in comparison with control treatment

with different effects on IAA level especially after fungal pathogen infection, and with the same effects on endogenous levels of SA, JA, and ETH. Because melatonin synthesis and IAA synthesis share the same substrate of tryptophan, the cross talk between melatonin and auxin in defense response should be emphasized. In *Arabidopsis*, tryptophan pathway-related genes, especially for auxin biosynthesis genes, show upregulation in *Fusarium oxysporum*-infected leaves and roots, and auxin signaling and transport are involved in the susceptibility to the root-infecting fungal pathogen *Fusarium oxysporum*.[71] In banana, melatonin and GDA had opposite effect on IAA level in response to *Foc4*, while melatonin and GDA cotreated plants had no significant effect, suggesting the involvement of *MaHSP90s* in the cross talk of melatonin and auxin in response to fungal infection. Thus, we highlight IAA as the key hormone in melatonin- and *MaHSP90s*-mediated fungal resistance in banana. Because melatonin and *MaHSP90s* also had complex effects on other hormones (SA, JA, and ETH) in response to fungal infection in banana, the effects and underlying signalings should be further dissected. As SA, JA, and ETH play important roles in plant disease resistance, the effects of melatonin and *MaHSP90s* on the endogenous levels of SA, JA, and ETH may also contribute to the fungal resistance in banana, at least partially.

Based on the above results, the possible molecular mechanism of *MeHSP90s* in melatonin-mediated banana Fusarium wilt was proposed in this study (Figure 10). In response to fungal pathogen *Foc*, melatonin activates the transcripts of *MaHSP90s*, which is essential for banana resistance to Fusarium wilt. Moreover, melatonin and *MaHSP90s* trigger the reprograming of defense-related plant hormones (IAA,

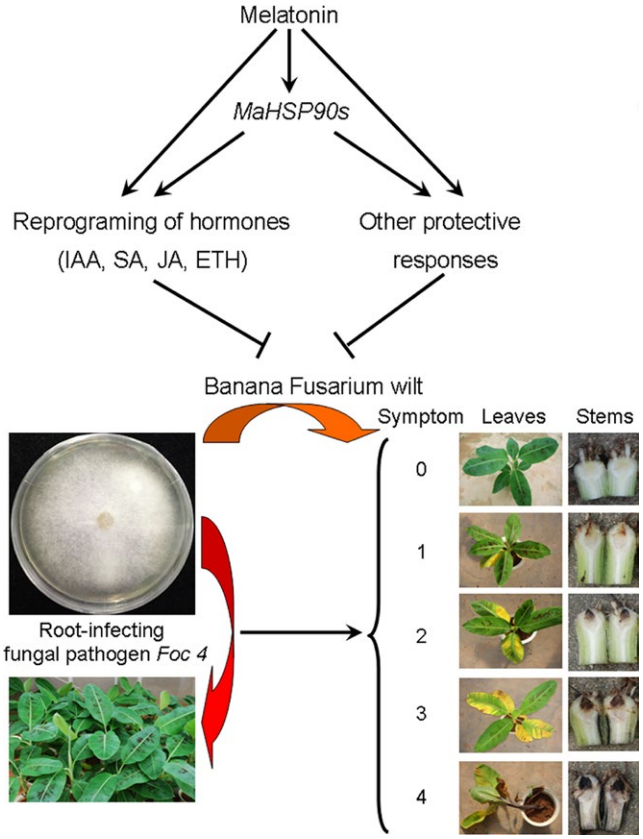


FIGURE 10 The molecular mechanism of *MeHSP90s* in melatonin-mediated banana Fusarium wilt

SA, JA, and ETH) as well as other protective responses. Notably, disease resistance conferred by exogenous melatonin could be reversed by the treatment of HSP90 inhibitor (GDA), indicating that *MaHSP90s* are required for melatonin-induced banana resistance to Fusarium wilt.

5 | CONCLUSION

Taken together, this is the first report about the roles of melatonin and *MaHSP90* gene family in banana resistance to Fusarium wilt. We highlight that *MaHSP90s* are essential for melatonin-conferred disease resistance against banana Fusarium wilt (Figure 10), and both melatonin and *MaHSP90s* may be further used in the biological control of Fusarium wilt in banana.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 31570249), a central financial support to enhance the comprehensive strength of the central and western colleges and universities, the Startup Funding and the Scientific Research Foundation of Hainan University (No. kyqd1531) to Haitao Shi.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Shi H conceived and directed this study, designed and performed the experiments, analyzed the data, wrote and revised the manuscript; Wei Y and Hu W performed the experiments, analyzed the data, and revised the manuscript; Wang Q provided help in the fluorescence intensity analysis; Zeng H, Li X, and Yan Y performed the experiments, Reiter RJ provided suggestions and revised the manuscript, He C designed the experiments, and revised the manuscript. All authors approved the manuscript and the version to be published.

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How to cite this article: Wei Y, Hu W, Wang Q , Zeng H, Li X, Yan Y, Reiter RJ, He C, Shi H. Identification, transcriptional and functional analysis of heat shock protein 90s in banana (*Musa acuminata* L.) highlight their novel role in melatonin-mediated plant response to Fusarium wilt. *J Pineal Res*. 2017;62:e12367. https://doi.org/10.1111/jpi.12367