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A banana fruit transcriptional repressor MaERF10 interacts with MaJAZ3 to strengthen the repression of JA biosynthetic genes involved in MeJA-mediated cold tolerance

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ABSTRACT

Bananas are easily subject to chilling injury (CI) when stored at temperatures below 13 °C and methl jasmonate (MeJA) application is known to alleviate CI symptoms of the fruit. However, the underlying regulatory mechanisms of these processes remain largely unknown. In this study, we identified a transcriptional regulator, *MaERF10*, which was repressed by MeJA treatment in banana fruit under low temperature storage. Electrophoretic mobility shift assays (EMSA) and transient expression analyses indicated that MaERF10 was able to bind to and suppress the promoters of several JA biosynthetic genes, such as *MaLOX7/8*, *MaAOC3*, and *MaOPR4*, whose transcripts were MeJA-inducible. More importantly, yeast two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) assays confirmed that MaERF10 physically interacted with MaJAZ3, a repressor of JA signaling, and the interaction led to deeper repression of JA biosynthetic genes by MaERF10. Taken together, these findings suggest that MaERF10 may act as a transcriptional repressor to modulate MeJA-induced cold tolerance of bananas possibly through recruiting MaJAZ3 to strengthen the repression of JA biosynthetic genes, which provides new insights into a transcriptional regulatory network of MeJA-induced cold tolerance of banana fruit.

1. Introduction

Bananas, like many other tropical and subtropical fruits, are highly sensitive to chilling injury (CI) when exposed to temperatures below 13 °C. This sensitivity greatly limits the application of low temperature storage in bananas, which is employed most widely for marketing horticultural products. CI symptoms in banana include pitting and discoloration of the peel, abnormal ripening of the pulp and increased susceptibility to fungal decay, resulting in severe postharvest losses (Chen et al., 2008). The appearance of such symptoms relies on many factors, such as cultivar, degree of maturity, time and temperature of exposure, and environmental conditions during and after cold storage (Wang, 1990). Some strategies that are effective to alleviate CI of bananas are modified atmosphere (Nguyen et al., 2004), heat treatment (Chen et al., 2008), UV-C illumination (Pongprasert et al., 2011), and application of nitric oxide (NO) (Wu et al., 2014), ethylene (Shan et al., 2014), or methl jasmonate (MeJA) (Peng et al., 2013; Zhao et al., 2013).

It is widely recognized that plant hormones and growth regulators are involved in the development of CI. Several naturally occurring phytohormones, such as gibberellins (GAs), salicylic acid (SA), ethylene, brassinosteroids (BR), and MeJA, have been associated with the response to cold stress in fruits (Ding et al., 2015). Among these phytohormones, jasmonic acid (JA) has been investigated for several decades, and is probably the best-characterized (Wasternack, 2015). The oxylipin JA and its derivatives such as MeJA, collectively referred to as jasmonates, are important plant signaling molecules that play an important role in biotic and abiotic stress responses as well as aspects of growth and development (Wasternack, 2015).

In higher plants, JA is synthesized in the chloroplast *via* the octadecanoid pathway starting from α-linolenic acid (18:3) (Vick and Zimmerman, 1983). The α-linolenic acid is converted to 13-hydroperoxylinoleic acid (13-HPOT) by 13-lipoxygenase (13-LOX), and then allene oxide synthase (AOS) produces 12,13-epoxyoctadecatrienoic acid, which is acted on by allene oxide cyclase (AOC). The AOC product *cis*(+)-12-oxophytodienoic acid (OPDA) is catalyzed by a peroxisomal OPDA reductase (OPR) to

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form 3-oxo-2(2′[Z]-pentenyl)cyclopentane-1-octanoic acid (OPC-8:0), which is then converted to JA by three cycles of β-oxidation (Browse, 2009). The regulation of JA biosynthesis is determined by substrate availability and expression of genes encoding critical steps of the pathway including *LOX*, *AOC*, *AOS* and *OPR*. In banana, MeJA treatment alleviated CI partially due to enhanced endogenous JA content and elevated levels of *MaLOX1/2*, *MaAOS1/2* and *MaAOC1* expression (Zhao et al., 2013).

After biosynthesis, JA is perceived by a set of genes, including the SCF-type E3 ubiquitin ligase SCF^{COI1}, jasmonate ZIM-domain (JAZ) repressor proteins that are targeted by SCF^{COI1} for degradation by the ubiquitin/26S proteasome pathway, and transcription factors (TFs) that positively regulate the expression of JA-responsive genes (Katsir et al., 2008). JAZs belong to a large family of TIFY proteins that is defined by the highly conserved signature sequence TIF[F/Y]XG located in the so-called ZIM domain (Chini et al., 2007). Accumulating evidence indicated that JAZ proteins functioned as repressors and bound to TFs, including MYC2, MYC3 and MYC4, which were involved in JA responses in vegetative tissue, presumably repressing their transcriptional activity (Boter et al., 2004; Chini et al., 2007). Therefore, discovery of the interaction partners of JAZ repressors may help understand the JA signaling pathway from hormone perception to transcriptional reprogramming. Besides MYCs, however, other potential interacting proteins of JAZs need to be further explored.

Ethylene response factors (ERFs), which belong to the family of APETALA2/ERF, constitute one of the largest plant-specific TFs (Dey and Vlot, 2015). The typical feature of AP2/ERF family is characterized by possessing one or two copies of the highly conserved DNA-binding domain, AP2/ERF, which specifically binds to GCC-box (AGCCGCC) (Hao et al., 1998). ERFs can activate or repress gene expression involved in developmental processes or in response to environmental stimuli, depending on the sequences outside the AP2/ERF domain (Mizoi et al., 2012; Licausi et al., 2013). Compared to the ERF activators, the ERF repressors in general contain an ERF-associated amphiphilic repression (EAR) motif at their C-terminal regions (Ohta et al., 2001). Previous findings revealed that ERF repressors are induced by pathogen infection, mechanical wounding or different phytohormones such as JA, ethylene and abscisic acid (ABA), and generally act as negative regulators of plant response to stresses (Fujimoto et al., 2000; McGrath et al., 2005; Song et al., 2005; Yang et al., 2005). For example, the EAR-repressor AtERF4 binds to the GCC-box of *PDF1.2*, a gene encoding an antimicrobial peptide, and represses its jasmonate-ethylene-dependent expression. Overexpression of *AtERF4* in *Arabidopsis* renders the plants more susceptible to the wilt pathogen *Fusarium oxysporum* (McGrath et al., 2005). Nevertheless, little is known about the possible roles of EAR-motif containing ERFs in cold stress response of plant and economical fruits.

In this study, we characterized one repressor type *ERF* gene from banana fruit, *MaERF10*, which was suppressed by MeJA, and elucidated its possible involvement in MeJA-induced cold tolerance of banana fruit. MaERF10 can bind *in vitro* to the GCC-box motifs in promoters of JA biosynthetic genes, *MaLOX7/8*, *MaAOC3*, and *MaOPR4*, which were induced by cold and MeJA treatment. More interestingly, MaERF10 physically interacted with MaJAZ3, and the interaction enhanced the MaERF10-mediated transcriptional repression of JA biosynthetic genes. Our results advance our understanding of transcriptional regulatory network underlying MeJA-induced cold tolerance of banana fruit.

2. Materials and methods

2.1. Material and treatments

Pre-climacteric banana (*Musa acuminata* AAA Group, cv. Cavendish) fruit at the 75–80% plump stage were obtained from a local commercial plantation near Guangzhou, south-eastern China. Hands were separated into individual fingers, and fruit of uniform weight, shape, and maturity as well as free of visual defects, were selected. These fruit were first surface sterilized by dipping in a 1% hypochloride solution for 1 min and then immersed in 0.05% Sporgon (with 46% Prochloraz–Mn; Aventis, Valencia, Spain) for 3 min to prevent fungal disease. They were then allowed to air-dry at 25 °C for 2 h and treated as follows.

The selected banana fruit were randomly divided into two groups of 150 fingers each for the following treatments: 30 min in 10 L of distilled water containing 0 (control) or 0.1 mM MeJA, under a reduced pressure of about 0.1 MPa as described by Chen et al. (2008) and Zhao et al. (2013). Both control and MeJA-treated fruit were subsequently placed into ten individual unsealed polyethylene plastic bags (0.01 mm thickness) and stored at 7 °C (chilling temperature) and 90% relative humidity (RH) for 5 days. Sampled were taken at 0, 2, 6, 12 h and 1, 3, 5, 7 d, and banana peel were collected.

All of the samples were frozen in liquid nitrogen immediately after sampling, and stored at −80 °C for further use. All assessments were conducted in three biological replicates.

2.2. Quantitative analysis of gene expression by real-time PCR (qPCR)

Synthesis of first strand cDNA and all RT-qPCR analysis were performed as described previously (Chen et al., 2011). The sequences of all primers used for RT-qPCR analysis are listed in Supplementary Table 1. *MaRPS4* (*ribosomal protein 4*) was selected as the reference gene according to our previous study on the selection of reliable reference genes under different experimental conditions (Chen et al., 2011). All RT-qPCR reactions were normalized using Ct value corresponding to the reference gene. The relative expression levels of target gene were calculated with the formula $2^{-\Delta\Delta CT}$. Three independent biological replicates were used in the analysis.

2.3. Electrophoretic mobility shift assays (EMSA)

Purified GST-MaERF10-N recombinant protein was used for EMSA, which was generated by introducing the N-terminus (1-102 aa) of MaERF10 consisting of the DNA-binding domain into the pGEX-4T-1 (Amersham Biosciences) vector. GST-MaERF10-N was expressed in *Escherichia coli* BL21 and purified using the GST-Tagged Protein Purification Kit (clontech) following the instructions. EMSA was performed with the LightShift Chemiluminescent EMSA Kit (Thermo Scientific). The 60-bp promoter probes of *MaLOX7/8*, *MaAOC3*, and *MaOPR4* each containing a GCC-box motif were synthesized and labeled with biotin at the 3′end (Thermo Scientific). Cold competitor probes were generated from dimerized oligos of each promoter region containing either the wild-type GCC-box (GCCGCC) or mutated G-box (AAAAAA) motifs, respectively (Supplementary Table 1). The binding reaction was carried out in a total volume of 20 μL according to manufacturer's recommendation. Biotin-labeled DNA was detected by the chemiluminescence method according to the manufacture's protocol on Chemiluminescent Nucleic Acid Detection Module Kit (Thermo Scientific).