Postharvest Biology and Technology 116 (2016) 8–15

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Two banana fruit ripening-related C2H2 zinc finger proteins are transcriptional repressors of ethylene biosynthetic genes

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ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 10 November 2015
Accepted 31 December 2015
Available online 8 January 2016

Keywords:
C2H2 zinc finger protein
Banana
Fruit ripening
Transcriptional repressor
Ethylene biosynthesis

ABSTRACT

C2H2 zinc finger proteins (C2H2-ZFPs), as one of the largest TF families in eukaryotes, play an essential role in plant development and stress responses, but their involvement in the ripening of economically important fruits, as well as their transcriptional regulatory mechanisms, remain largely unclear. In this study, two C2H2-ZFPs, named as *MaC2H2-1/2*, were identified and characterized from banana fruit. MaC2H2-1/2 contain a typical ERF-associated amphiphilic repression (EAR) motif at their C-terminus. Subcellular localization demonstrated that MaC2H2-1/2 proteins were both localized to the nucleus. Dual-luciferase reporter assay (DLR) showed that MaC2H2-1/2 were transcriptional repressors *in vivo*. Gene expression analysis revealed a significant induction of *MaC2H2-1/2* transcripts during the ripening of banana fruit with three different ripening characteristics caused by natural, ethylene-induced, and 1-methylcyclopropene (1-MCP)-delayed treatments, which correlated well with ethylene production. More importantly, electrophoretic mobility shift assay (EMSA) and transient expression showed that MaC2H2-1/2 bound to the promoters of the key ethylene biosynthetic genes *MaACS1* and *MaACO1*, and repressed their activities. Collectively, these results suggest that MaC2H2-1/2 are transcriptional repressors and may mediate a finely tuned regulation of ethylene production during banana fruit ripening, possibly via transcriptional repression of ethylene biosynthetic genes, expanding the knowledgement of the involvement of C2H2 zinc finger proteins in fruit ripening.

1. Introduction

Transcription factors (TFs) play an important role in the regulation of plant development, differentiation, cell growth, and response to environmental signals through recognizing specific *cis*-regulatory DNA sequences at the promoters of their target genes (Meshi and Iwabuchi, 1995). Many TF families have been identified and studied in plants, such as MYCs, NACs, WRKYs, MADS-boxes, ERFs and zinc finger proteins (ZFPs), which accounts for 6%–10% in the whole genome depending on the databases (Franco-Zorrilla et al., 2014). ZFPs are characterized based on the number and the order of the Cys (cysteine) and His (histidine) residues that bind the zinc ion. Among these different ZFPs types, C2H2-ZFPs (also called the TFIIIA-type ZFPs), which are often described as $CX_{2-4}CX_{12}HX_{2-8}H$, constitute one of the largest TFs with finger domains in eukaryotes, and display a wide range of functions, from DNA or RNA binding to the involvement in protein–protein interactions (Takatsuji, 1999; Englbrecht et al., 2004; Agarwal et al., 2007; Gourcilleau et al., 2011). In particular, most plant C2H2-ZFPs contain a QALGGH motif inside the zinc finger domain which are thought to be important for C2H2-ZFPs binding to DNA targets (Kubo et al., 1998; Takatsuji, 1999; Ciftci-Yilmaz and Mittler, 2008), whereas yeast and animals do not.

Accumulating studies have shown that C2H2-ZFPs function in plant development regulation and are involved in stress responses. For example, SUPERMAN (one zinc finger of C2H2 type) is involved in regulation of flower development in *Arabidopsis* (Bowman et al., 1992; Sakai et al., 1995), while ZFP15, which is the fiFP1 characterized C2H2-type ZFP in rice, might play a regulatory role in spike development (Huang et al., 2005). Recently, Sendon et al. (2014) found that *Atdwa1* mutant which displayed severe dwarfism, loss of apical dominance and other pleiotropic defects, was caused by synergistic manifestation of concomitant activation of At5g54340–At5g54360 which encoded proteins related to C2H2-type ZFPs, suggesting that ZFPs might play a role in regulation of plant growth and development in *Arabidopsis*. In

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http://dx.doi.org/10.1016/j.postharvbio.2015.12.029
0925-5214/

addition, some C2H2-ZFPs are implicated to mediate stress responses. SCOF-1 (for soybean cold-inducible factor-1), functions as a positive regulator to enhance cold tolerance of plants (Kim et al., 2001). *Arabidopsis* AZF2 and STZ which belong to ZPT2-related proteins containing two canonical Cys-2/His-2-type zinc-finger motifs, are two transcriptional repressors and increase stress tolerance following growth retardation (Sakamoto et al., 2004). Potato StZFP1 might be involved in salt and dehydration stresses via an ABA-dependent pathway (Tian et al., 2010). Similarly, rice *ZFP182* is required for ABA-induced antioxidant defense and its expression is regulated by MAPKs in ABA signaling pathway (Zhang et al., 2012). Interestingly, C2H2-ZFPs showed altered expression after the breaker stage and were identified as one of the “late response” genes during tomato fruit ripening (Rohrmann et al., 2011), indicating the possible involvement of C2H2-ZFPs in fruit ripening, however, their transcriptional regulation mechanisms is poorly understood, especially in other economically important fruit.

Banana is one of the most popular fruits grown in tropical and subtropical regions with high nutritional and economical values. As a typical climacteric fruit, the ripening of banana fruit is characterized by an autocatalytic increase in ethylene biosynthesis just prior to the initiation of ripening (Liu et al., 1999). Once the banana fruit is ripened it has a very short shelf life due to the soft texture and susceptibility to diseases, leading to severe postharvest loss. Therefore, understanding the ripening mechanism of banana is imperative for developing effective practices to prolong the shelf life and maintain the quality of the fruit. Since ethylene plays an important role in banana fruit ripening, much attention has been paid on the involvement of ethylene biosynthesis and signaling pathway in fruit ripening (Liu et al., 1999; Inaba et al., 2007; Mbéguié-A-Mbéguié et al., 2008; Yan et al., 2011; Hu et al.,

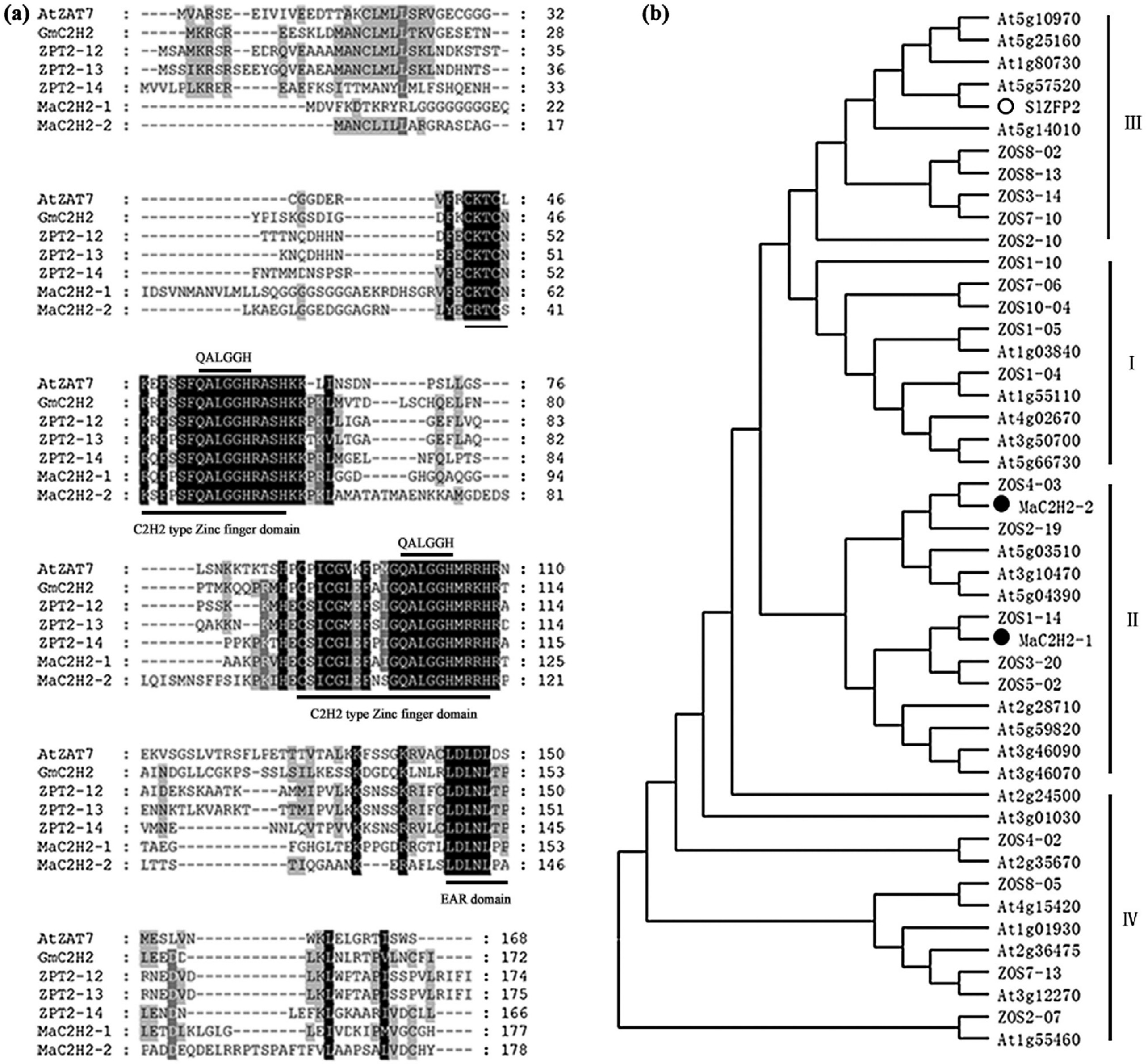

Fig. 1. Sequence alignment (a) and phylogenetic tree (b) of MaC2H2-1/2. C2H2 type Zinc finger domain and EAR domain were underlined, while QALGGH motif was overlined. Multiple alignments were carried out using CLUSTALW and the phylogenetic tree was constructed with MEGA5.0 using a bootstrap test of phylogeny with Neighbor–Joining test and default parameters. In phylogenetic tree (b), MaC2H2-1/2 are indicated by solid black circle, and the ripening-associated SlZFP2 (Weng et al., 2015) is marked with empty black circle. Sequences used for the phylogenetic analysis are listed in Supplementary Text 1.