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Research article

Genome-wide identification and expression analysis of the metacaspase gene family in *Hevea brasiliensis*

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ABSTRACT

Metacaspases, a family of cysteine proteases, have been suggested to play important roles in programmed cell death (PCD) during plant development and stress responses. To date, no systematic characterization of this gene family has been reported in rubber tree (*Hevea brasiliensis*). In the present study, nine metacaspase genes, designated as *HbMC1* to *HbMC9*, were identified from whole-genome sequence of rubber tree. Multiple sequence alignment and phylogenetic analyses suggested that these genes were divided into two types: type I (*HbMC1*–*HBMC7*) and type II (*HbMC8* and *HbMC9*). Gene structure analysis demonstrated that type I and type II *HbMCs* separately contained four and two introns, indicating the conserved exon–intron organization of *HbMCs*. Quantitative real-time PCR analysis revealed that *HbMCs* showed distinct expression patterns in different tissues, suggesting the functional diversity of *HbMCs* in various tissues during development. Most of the *HbMCs* were regulated by drought, cold, and salt stress, implying their possible functions in regulating abiotic stress-induced cell death. Of the nine *HbMCs*, *HbMC1*, *HbMC2*, *HbMC5*, and *HbMC8* displayed a significantly higher relative transcript accumulation in barks of tapping panel dryness (TPD) trees compared with healthy trees. In addition, the four genes were up-regulated by ethephon (ET) and methyl jasmonate (MeJA), indicating their potential involvement in TPD resulting from ET- or JA-induced PCD. In summary, this work provides valuable information for further functional characterization of *HbMC* genes in rubber tree.

1. Introduction

Programmed cell death (PCD) is a conserved and genetically controlled cell death process. In plants, PCD includes two broad categories, developmentally regulated PCD and environmentally induced PCD (Gunawardena, 2008). Developmentally regulated PCD covers a wild range of tissues and organs, such as leaf, xylem, embryo, etc. (Bollhoner et al., 2013; Huang et al., 2014; Wertman et al., 2012), and it is initiated by the internal factors and occurs at a predictable time and location (Gunawardena, 2008). In contrast, environmentally induced PCD is triggered by external biotic or abiotic signals, such as pathogen, heat shock, and water stress (Duan et al., 2010; Kim et al., 2013; Li et al., 2012; Olvera-Carrillo et al., 2015).

PCD is essential for plant development and survival against pathogen invasion and environmental stresses. Despite the importance of PCD in plants, the molecular mechanisms involved in this process are largely unclear. However, in animals, the molecular mechanisms of PCD have been well elucidated by studying the model system *Caenorhabditis elegans* (Lord and Gunawardena, 2012). In animal cells, caspases (cysteine aspartic-specific proteases) play central role in signaling and executing PCD (Grutter, 2000). However, no orthologous caspases have been identified in plants. The only plant gene family closely resembling caspases is the metacaspase family (Uren et al., 2000). Although metacaspases have similar morphology and secondary structure as caspases, they

Abbreviations: EST, expressed sequence tag; ET, ethephon; LSD, lesion-simulating disease; MC, metacaspase; MeJA, methyl jasmonate; ORF, open reading frame; PCD, programmed cell death; qRT-PCR, quantitative real-time PCR; TPD, tapping panel dryness; TSA, transcriptome shotgun assembly.

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cannot be defined as caspases, since metacaspases do not have aspartate-specific proteolytic activity (Tsiatsiani et al., 2011). Plant metacaspases were divided into type I and type II based on their sequence and structure similarity (Tsiatsiani et al., 2011; Fagundes et al., 2015). Both type I and II metacaspases have a putative conserved caspase-like catalytic domain composed of p10 and p20 subunits, which contain conserved catalytic histidine/cysteine (His/Cys) dyad (Fagundes et al., 2015; Vercammen et al., 2007). The catalytic histidine lies in the (H/Y)(Y/F)SGHG sequence and the catalytic cysteine in the active-site, pentapeptide D(A/S)C(H/Y)S sequence (Fagundes et al., 2015; Zhang et al., 2013). Besides, type I metacaspases could present or not present a prodomain rich in proline, include a zinc finger motif in the N-terminus region. Whereas type II metacaspases lack the prodomain and the zinc finger motif, but harbor a longer linker region than that found in type I metacaspases, which connects the p10 and p20 subunits (Fagundes et al., 2015).

Metacaspases play important roles in plant PCD (Fagundes et al., 2015; Lam and Zhang, 2012). Several metacaspase genes have been demonstrated to be essential for different types of PCD in plants. In *Arabidopsis*, there are three type I (*AtMC1*–*AtMC3*, also known as *AtMCP1a*-*AtMCP1c*) and six type II (*AtMC4*–*AtMC9*, also known as *AtMCP2a*–*AtMCP2f*) metacaspase genes (Tsiatsiani et al., 2011). Among the type I metacaspases, *AtMC1* and *AtMC2* antagonistically control hypersensitive response-associated cell death in *Arabidopsis*. *AtMC1* is a positive regulator of cell death, whereas *AtMC2* negatively regulates cell death (Coll et al., 2010). Among the type II metacaspases, *AtMC4* plays a positive regulatory role in biotic and abiotic stress-induced PCD (Watanabe and Lam, 2011), and *AtMC8* is required for cell death triggered by UVC and H_2O_2 (He et al., 2008). Additionally, *AtMC9* is essential for efficient progression of autolysis during vessel cell death (Bollhoner et al., 2013; Tsiatsiani et al., 2013). In wheat, the metacaspase gene *TaMCA4* functions in PCD induced by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (Wang et al., 2012). Moreover, the pepper metacaspase gene *Camc9* plays a role as a positive regulator of pathogen-induced cell death via the regulation of reactive oxygen species production and defence-related gene expression in plants (Kim et al., 2013). In Norway spruce (*Picea abies*), the metacaspase gene *mcII-Pa* is required for both progression of vacuolar cell death and suppression of necrosis (Minina et al., 2013). These results indicate that metacaspases are essential for cell death regulation in plants.

The metacaspase gene family has been systematically investigated by genome-wide scans in Viridiplantae (Fagundes et al., 2015), *Arabidopsis* (Kwon and Hwang, 2013; Tsiatsiani et al., 2011), grape (Zhang et al., 2013), and rice (Huang et al., 2015; Wang and Zhang, 2014). In addition, several studies on metacaspases have been reported in maize (Ahmad et al., 2012), tomato (Hoeberichts et al., 2003), pepper (Kim et al., 2013), and wheat (Wang et al., 2012). However, to date, no metacaspase gene has been reported in rubber tree (*Hevea brasiliensis*).

Rubber tree is a perennial plant in the *Euphorbiaceae* family and is the sole commercial source of natural rubber because of its high production and rubber quality. Tapping panel dryness (TPD) accounts for 10–40% annual rubber production losses (Gébelin1 et al., 2015), therefore it is one of the most serious threats to natural rubber production. The TPD syndrome is characterized by the partial or complete cessation of latex flow upon tapping (Venkatachalam et al., 2007). Previous studies suggested that PCD in bark cells possibly play a role in TPD occurrence (Chen et al., 2003; Li et al., 2010; Putranto et al., 2015; Venkatachalam et al., 2007). Metacaspases, as important regulators of PCD, may be associated with TPD. The completion of rubber tree genome sequence has made it possible to identify and characterize the metacaspase family genes at a genome-wide level. In the present study, we identified nine rubber tree metacaspase genes (*HbMC1*–*HbMC9*) and analyzed their gene structure, phylogenetic relationship, expression profiles in various tissues, and response to different types of abiotic stress and hormone treatments. Our results lay the foundation for future functional characterization of *HbMC* genes in rubber tree.

2. Materials and methods

2.1. Plant materials and treatments

Rubber tree clone, Reyan 7-33-97, was cultivated under normal field conditions at the experimental farm of Chinese Academy of Tropical Agricultural Sciences in Danzhou, Hainan, China. The fresh tissues or organs including leaf, stem tip, latex, bark, female flower, and male flower were sampled from 20-year-old rubber trees during spring bloom period. Roots were collected from one-year-old tissue culture seedlings of Reyan 7-33-97. Latex and barks were collected from healthy and TPD rubber trees selected according to Li et al. (2010). Three biological replicates were sampled for each tissue, and each replicate was equally harvested from five trees. Samples were immediately frozen in liquid nitrogen and then stored at −80 °C for RNA isolation.

The tissue culture seedlings of Reyan 7-33-97 were used for cold, drought, and salt stress treatments. Cold stress treatment was performed by transferring seedlings to a growth chamber at 8 °C. For drought and salt stress treatments, the seedlings were washed thoroughly with tap water to eliminate substrates, and then transferred into solutions supplemented with 20% (W/V) PEG-6000 (polyethylene glycol 6000) or 1 M NaCl, respectively. Each treatment had three replicates, and each replicate contained three seedlings. Leaf samples were collected at 0, 3, 24, and 48 h after treatments, and then immediately frozen into liquid nitrogen and stored at −80 °C for RNA extraction.

The seven-year-old virgin trees were used for ethephon (ET), methyl jasmonate (MeJA), and wounding treatments. ET and MeJA treatments were carried out according to the methods of Hao and Wu (2000). Latex was harvested at 0, 4, 8, 24, and 48 h after treatments. The wounding treatment was performed as described by Tang et al. (2010). Latex was harvested at 0, 6, 24, and 48 h after treatment. Each treatment had three replicates, and each replicate contained three trees. The first few drops of latex containing the debris were discarded, and then the latex from the treated and control rubber tree was allowed to drop directly into liquid nitrogen in an ice kettle for total RNA extraction.

2.2. RNA isolation and first-strand cDNA synthesis

Total RNA was isolated from the collected samples according to Xu's method (Xu et al., 2010), and then treated with RQ1 RNase-free DNase (Promega, USA) to remove genomic DNA contamination. The quality and quantity of the extracted RNA were checked by agarose gel electrophoresis and measured by a spectrophotometer (Thermo Scientific NanoDrop 2000, USA). First strand cDNA was synthesized with RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's instruction.

2.3. Identification and isolation of metacaspase genes in Hevea brasiliensis

The nine full-length cDNA sequences of *Arabidopsis thaliana* metacaspase genes (*AtMC1*–*AtMC9*) were obtained from TAIR (http://www.arabidopsis.org/) as reported in previous study (Tsiatsiani et al., 2011). The cDNA sequences of these genes were used as queries to search against the Transcriptome Shotgun