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Genome-wide identification and characterization of *WRKY* gene family in *Hevea brasiliensis*

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ABSTRACT

WRKY proteins constitute a large family of transcription factors. In this study, we identified 81 WRKY genes (named *HbWRKY1* to *HbWRKY81*) in the latest rubber tree genome. Tissue-specific expression profiles showed that 74 *HbWRKYs* were expressed in at least one of the tissues and the other 7 genes showed very low expression in all tissues tested, which suggested that *HbWRKYs* took part in many cellular processes. The responses of 20 selected *HbWRKYs* to jasmonic acid (JA) and ethylene (ET) were analyzed in the latex. 17 *HbWRKYs* responded to at least one treatment, which included 15 *HbWRKYs* responding to JA treatment, 15 *HbWRKYs* to ET, which suggested that these *HbWRKYs* were regulated by JA and ET. We also observed that HbWRKY3, 14, and 55 bind *HbSRPP* promoter and activate the transcription in yeast. This study suggests that HbWRKY proteins maybe involved in the transcriptional regulation of nature rubber biosynthesis.

1. Introduction

WRKY proteins constitute a large family of transcription factors in plants [1–4]. WRKY proteins are so named because of the highly conserved WRKY domain, which contains the almost invariant WRKYGQK sequence at the N-terminus followed by a CX4-5CX22-23HX1H or CX7CX23HXC zinc-finger motif [1]. The WRKY domain consists of a four-stranded β-sheet, with the zinc coordinating Cys/His residues forming a zinc-binding pocket [4]. Most functional studies on WRKY proteins indicate a binding to the conserved nucleotide consensus sequence TGAC(C/T) of the W-box. Based on the number of WRKY domains present and on the type of zinc-finger WRKY proteins can be classified into three groups (1, 2 and 3). Group 1 proteins typically contain two WRKY domains including a C2H2 motif. Group 2 proteins have a single WRKY domain and a C2H2 zinc-finger motif and can be further divided into five subgroups (2a–2e). Group 3 proteins also have a single WRKY domain, but their zinc-finger-like motif is C2-H-C [4,5]. A growing body of evidence suggests that WRKY transcription factors are involved in many significant physiological and biochemical processes and have important roles in diverse biological processes in plants [1–5], such as the regulation of plant responses to abiotic stresses, plant hormone signaling [6–12], and secondary metabolism [13–17]. In addition, several WRKY proteins are involved in the regulation of plant growth and developmental processes including trichome development [18], seed development and germination [19], embryogenesis [20–22] and leaf senescence [23].

Extensive studies of the WRKY gene family in various plant species have provided a better understanding of this gene family. In the *Arabidopsis* genome, 72 WRKY genes have been predicted and they can be divided into three groups on the basis of the WRKY domain sequence [1]. There are 109 WRKY genes in rice (*Oryza sativa*) [24], 57 in cucumber (*Cucumis sativus*) [25], 47 in castor bean (*Ricinus communis*) [26], 105 in poplar (*Populus trichocarpa*) [27] and 58 in physic nut (*Jatropha curcas* L.) [28]. However, little is known about this gene family in rubber tree (*Hevea brasiliensis*) [17].

Rubber tree is an important perennial wood crop. Natural rubber, a *cis* 1,4-polyisoprene, is obtained commercially from the latex of rubber tree [29]. Laticifers in rubber tree are the sole site for natural rubber biosynthesis and storage. Latex is the cytoplasmic content of laticifers [30]. Rubber biosynthesis occurs on the surface of a special type of organelle (rubber particle) in the cytoplasm (latex) of the rubber-producing laticifers or latex vessels. The economic importance of natural rubber has prompted investigation into the biochemical, cell biological, and molecular aspects of latex biogenesis [31]. In *H. brasiliensis*, the general metabolic pathway leading to rubber biosynthesis is now clear, but the molecular regulation of natural rubber in *H. brasiliensis* is not well known [32,33]. Therefore, the identification and functional study of regulation of natural rubber biosynthesis-related gene may elucidate

Abbreviations: ET, ethylene; JA, jasmonic acid; RT-qPCR, real-time quantitative PCR; SRPP, small rubber particle protein.

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the molecular mechanisms of natural rubber biosynthesis in rubber tree.

A draft of the *H. brasiliensis* genome sequence was reported recently [34]. The genome-wide of WRKY genes can now be identified and described. In the present study, genome sequence was searched so that HbWRKY genes could be identified in order to predict protein domain architectures and to assess the extent of conservation and divergence in the rubber tree WRKY family. Tissue-specific analysis was performed and jasmonic acid (JA) and ethylene (ET) response expression profiles were generated so that genes, which could potentially participate in the JA, ET signal transduction pathway in rubber tree, could be identified. This extended analysis is the first comprehensive study of the WRKY gene family in rubber tree and provides valuable information for further exploration into the functions of this significant gene family in rubber tree.

2. Results

2.1. Identification and sequence conservation of rubber tree WRKY genes

Seventy-two *Arabidopsis* WRKY proteins and the consensus protein sequences of the WRKY domain, Hidden Markov Model (HMM) profile (PF03106.7), were employed as a query to search against the rubber tree genome database (DDBJ/EMBL/GenBank under the accession: GenBank: AJJZ01000000) using the BlastP program. A total of 88 genes in the rubber tree genome were identified as possible members of the HbWRKY family. To confirm putative WRKY genes in the rubber tree genome, the amino acid sequences of all 88 proteins were searched for the presence of the WRKY domain by Pfam and SMART. Following an extensive search for WRKY genes, 81 typical WRKY genes (named *HbWRKY1* to *HbWRKY81*) were confirmed from the original data. These 81 *HbWRKYs* were subjected to further analyses (Supplementary Table S1).

To gain insight into the rubber tree WRKY domains and zinc-finger motif, sequence logos were produced to examine how well conserved the WRKY domain and zinc-finger motif were in the WRKY proteins within each residue position. As shown in Fig. 1, conserved amino acid residues were identical among the members detected in the WRKY regions and zinc-finger motif, respectively.

2.2. Phylogenetic analysis of the rubber tree WRKY family

The phylogenetic relationship between the HbWRKYs was examined by multiple sequence alignment of their WRKY domain with bootstrap analysis. The 81 members of the HbWRKY family were subdivided into 3 subgroups, designated group 1 to group 3 (Fig. 2). 16 HbWRKY proteins were classed in the group I WRKY subfamily, members of which contain two WRKY domains, an N-terminal WD (NTWD) and a C-terminal WD (CTWD) [1]. 51 HbWRKY proteins were classed in the group I WRKY subfamily, which can be subdivided into additional seven subgroups (IIa–e). 14 HbWRKY proteins were classed into group III. In comparison with rice and *Arabidopsis*, rubber tree has more WRKY proteins in group IIe and less WRKY proteins in group IIIb (Table 1). To compare the two phylogenetic trees on the basis of HbWRKY domains and complete protein sequences, respectively, similar subgroups were analyzed, though the classifications of only a few members varied (Fig. 3). This indicated that the conserved WRKY domain was an important unit in HbWRKY protein. We subsequently performed an exon–intron structure analysis to support the phylogeny reconstruction. The *HbWRKYs* within the same groups of the phylogenetic tree all showed similar exon–intron structures, intron position, and consensus domain (Fig. 3). The schematic structures revealed that each coding sequence of *HbWRKYs* is disrupted by two to eight. 77 out of 81 *HbWRKYs* had more than two exons.

2.3. Expression profiles for HbWRKYs in different tissues

Real-time quantitative RT-PCR was used to detect the expression patterns for all *HbWRKYs* in the roots, barks, leaves, flowers, and the latex. Tissue-specific expression profiles showed that 74 rubber tree WRKY genes were expressed in at least one of the tissues and the other 7 genes showed very low expression in all tissues tested (Fig. 4). Fifty-six genes (*HbWRKY 1–8*, *10–13*, *16–18*, *20–23*, *31*, *32*, *35–43*, *46*, *48*, *49*, *52*, *54–57*, *59–65*, *67–71*, *73*, *74*, *76*, *77*, *80*, *81*) were expressed

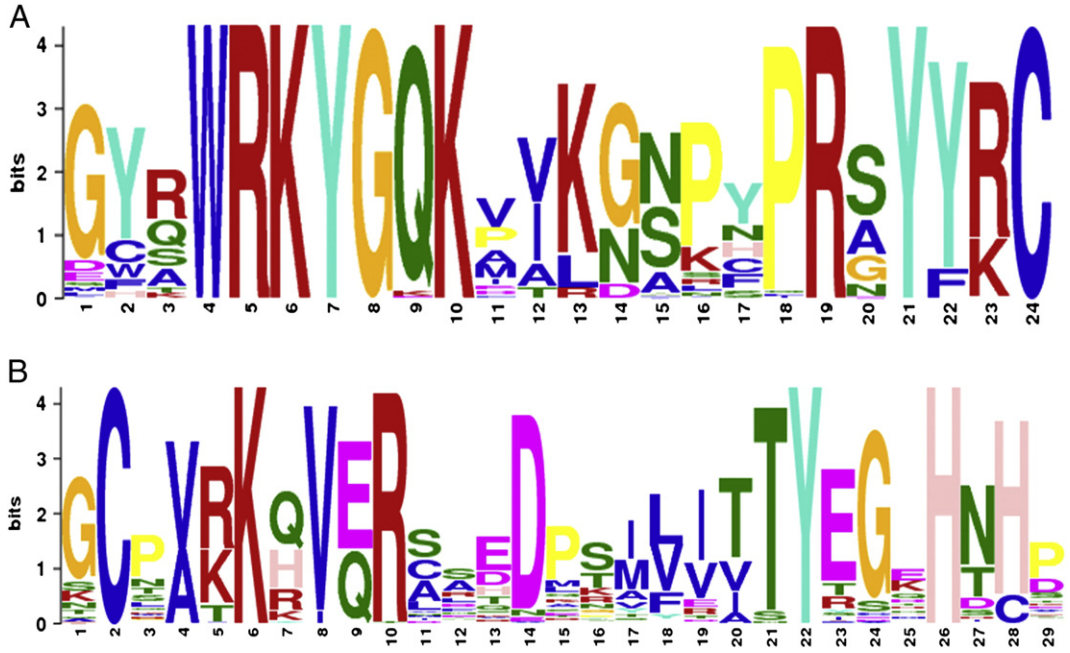

Fig. 1. Predicted domains in the HbWRKY protein. The conserved domains were carried out by MEME using the protein sequences of HbWRKYs. This online software was used to create the logo representations of the WRKY domain (A) and the zinc finger motif (B). On the Y axis (measured in bits) depicts the overall height of the stack indicating the sequence conservation at that position, while the height of symbols within the stack indicates the relative frequency of each amino or nucleic acid at that position.