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ORIGINAL ARTICLE

Genome-wide identification, characterization, and expression analysis of *SnRK2* family in *Hevea brasiliensis*

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Abstract The sucrose non-fermenting 1-related protein kinase 2 (SnRK2) gene family belongs to a group of plant-specific serine/threonine kinase family involved in abscisic acid (ABA) signaling and biotic and abiotic stress response. Although genome-wide analyses of the *SnRK2* gene family have been conducted in some species, little is known about the *SnRK2* gene family in rubber tree (*Hevea brasiliensis*). In this study, we identified 10 *SnRK2s* designated as *HbSnRK2.1* to *HbSnRK2.10* in the rubber tree genome. The subsequently constructed phylogenetic tree demonstrated that HbSnRK2s have three subfamilies that correlate well with those of *Arabidopsis* sp. and rice subfamilies. All *SnRK2* genes contained nine exons and eight introns. Although the C-terminus was divergent, eight conserved motifs were found. Motifs 1–6 were common to all HbSnRK2s. Expression analysis results showed that 7 of the 10 *HbSnRK2s* were highly expressed in latex. *HbSnRK2.7* was predominantly expressed and simultaneously regulated by abscisic acid, jasmonic acid, and ethylene treatment in laticifers. *HbSnRK* identification and characterization provided further understanding on the role of ABA signal in the rubber tree.

Keywords *Hevea brasiliensis* · *SnRK2* family · Abscisic acid

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Introduction

Abscisic acid (ABA) is a classical plant hormone involved in abiotic stress response. ABA is important to plant growth and development, particularly to cell division and elongation, embryo maturation, seed dormancy, germination, root growth, floral induction, and biotic and abiotic stress response to osmotic stress, chilling, high salinity, drought, pathogen attack, and UV radiation (Finkelstein 2013; Miyakawa et al. 2013; Sah et al. 2016; Finkelstein et al. 2002, 2008; Yoshida et al. 2014; Lopez-Molina et al. 2001). As a stress hormone, ABA acts through regulatory pathways that control stomatal closure and gene expression (Zhu 2002; Wasilewska et al. 2008; Lee and Luan 2012). The ABA signaling pathway consists of three major protein classes, namely, ABA receptors (PYR/PYL/RCARs), type 2C protein phosphatases (PP2Cs), and sucrose non-fermenting 1-related protein kinase 2 (SnRK2; Wasilewska et al. 2008; Zhang et al. 2015; Cutler et al. 2010). Signaling starts with molecular recognition of ABA by the ABA receptor protein family. ABA binds to a PYR/PYL/RCAR protein, thereby inhibiting the phosphatase activity of PP2Cs and relieving inhibition of SnRKs required to activate downstream gene expression that convert ABA signals into appropriate cellular responses (Vilela et al. 2015; Park et al. 2009; Raghavendra et al. 2010; Ma et al. 2009).

SnRK2s belong to a group of plant-specific serine/threonine kinase family. As major contributors in ABA signaling and osmotic stress response, SnRK2s are well studied

(Coello et al. 2011; Fujii and Zhu 2009; Kobayashi et al. 2004; Nakashima et al. 2009; Shao et al. 2014; Wang et al. 2015). SnRK2 families had been identified in some plants, such as *Arabidopsis* (Hrabak et al. 2003; Saha et al. 2014), rice (Kobayashi et al. 2004), maize (Huai et al. 2008), *Malus prunifolia* (Shao et al. 2014), *Brachypodium distachyon* (Wang et al. 2015), and *Brassica rapa* (Huang et al. 2015). All SnRK2 members have a conserved N-terminal catalytic domain that is similar to SNF1/AMP kinases and a short C-terminal regulatory domain that is not highly conserved. These families are classified into three subgroups according to amino acid sequences. The C-terminal domain contains stretches of acidic amino acids, either glutamic acid (group I) or aspartic acid (groups II and III; Kulik et al. 2011). The C-terminal consists of two subdomains. Domain I is present in all SnRK2 family members and is located 20 amino acids away from the catalytic domain. Domain II is essential for ABA response and specific to group III (Kobayashi et al. 2004; Belin et al. 2006; Yoshida et al. 2006). In general, ABA strongly activates SnRK2 group III members, weakly induces group II members, and moderately triggers group I (Kobayashi et al. 2004, 2005; Huai et al. 2008; Boudsocq et al. 2004; Fujita et al. 2009).

The rubber tree (*Hevea brasiliensis* Muell. Arg) is a tropical tree that belongs to the Euphorbiaceae family and is wildly cultivated to produce natural rubber (cis 1,4-polyisoprene) from the tree latex. Latex is a cytoplasm of highly specialized cells, known as laticifers, in rubber tree (Hao and Wu 2000). Laticifers in rubber tree barks are vital to rubber biosynthesis and defense against pathogen attacks (Chow et al. 2007). Furthermore, plant hormones are crucial to natural rubber biosynthesis. Laticifers are responsive to jasmonic acid (JA) because exogenous jasmonate specifically induces their differentiation (Hao and Wu 2000). JA signaling may also regulate natural rubber biosynthesis in laticifers (Peng et al. 2009; Tian et al. 2010; Pirrello et al. 2014). Ethylene (ET) is widely used for the stimulation of latex production (Zhu and Zhang 2009; Tungngoen et al. 2009). ABA-treated rubber trees exhibit early significant response, leading to significant increases in latex yield (Tungngoen et al. 2011) and suggesting that ABA signaling may also regulate latex production. In addition, ABA controls *H. brasiliensis* small rubber particle protein (HbSRPP; Guo et al. 2014), and *Taraxacum brevicorniculatum* small rubber particle protein (TbSRPP) gene (Fricke et al. 2013), indicating the possible regulation of natural rubber biosynthesis by ABA. HbSRPP is a major component of *H. brasiliensis* latex and apparently participates in natural rubber biosynthesis (Chow et al. 2007; Oh et al. 1999). However, knowledge on ABA signaling pathway in rubber trees is currently limited.

Despite the elucidation of several SnRK2 gene functions in *Arabidopsis* and other model species, less information on this gene family in rubber tree is available. Sequencing the rubber tree genome (Rahman et al. 2013; Tang et al. 2016) allows the identification and description of ABA signaling pathway genes. In this study, a total of 10 SnRK2 genes (designated as *HbSnRK2.1–10*) were identified in the genome of rubber tree. We also analyzed their phylogenetic relationships, gene structures, protein motifs, and expression patterns in five different tissues. Our results indicated that *HbsnRK2s* are highly expressed in latex, and ABA, JA, or ET regulated these genes. Our study provided a basis for further investigation of various *HbSnRK2* gene functions in rubber trees.

Materials and methods

Plant materials and treatments

Rubber trees (*H. brasiliensis* cultivar RY 7-33-97) were planted in the experimental farm of the Chinese Academy of Tropical Agricultural Sciences, Hainan, China. Rubber tree shoots were treated with 0.5 *w*/*v*% ethrel, 0.1 *v*/*v*% methyl jasmonate, or 100 μM abscisic acid, according to a previous method (Hao and Wu 2000). Latex samples were collected at 1, 3, 6, 9, 12, 24, and 48 h after treatments from 12 shoots for each interval, and stored at −80 °C for RNA extraction. For latex RNA extraction, the latex was dropped directly into liquid nitrogen. Rubber tree roots, flowers, leaves, and barks were washed with double-distilled H_2O and immediately frozen in liquid nitrogen.

Genome-wide identification of SnRK2 gene family in rubber tree

Multiple database searches were conducted to identify the SnRK2s in the rubber trees. Annotated (predicted) genes and proteins of the rubber trees were obtained from the tree genome data (DDBJ/EMBL/GenBank under the accession nos. AJJZ01000000 and LVXX01000000; Rahman et al. 2013; Tang et al. 2016). The *SnRK2* family genes of *Arabidopsis* and rice were acquired from Phytozome v10.1 (https://phytozome.jgi.doe.gov/pz/portal.html#). *SnRK2* complementary DNA (cDNA) sequences from *Arabidopsis* and rice served as queries for the search against rubber tree genome databases. Default Blast settings were used, but the low complexity filter and redundant sequences were removed manually. All search hits of the candidate sequences were analyzed using the NCBI Conserved Domain Search database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) to confirm that each gene belonged to the *SnRK2* family. The specific primers (Table 1) were designed according to the annotated (predicted) HbSnRK2 genes, and the cDNA sequences of the *HbSnRK2* genes were amplified and sequenced. Basing on the BlastP and BlastN search results from

Table 1 Primers used for cloning of *HbSnRK2s*

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
HbSnRK2.1	AGTTTGCAGACACACAGAGG	GCGGCGAAACAAGGATTACA
HbSnRK2.2	TGCTACTTGCTTCTTGCAACT	AAAGGGATTGATACGCCACG
HbSnRK2.3	ACAGCAGCTAGCCAATAGCAA	ACAAGGCTTTCATGGCTAAGGA
HbSnRK2.4	TTTAAGCCCAGGAAGAAGCGG	AGTTCTGTGAGATTCAGACTGCT
HbSnRK2.5	AGCGGGTTTAATTGGAGCTT	CACTGGTATCTGAGAAGGAGCA
HbSnRK2.6	TTGTGGTTAGCTGTGGGAAA	GCAGGTATCCGATAAAGGGCA
HbSnRK2.7	GAGGGCCAAATACGAAGCGT	ACATGCCTGAAGAACCACCC
HbSnRK2.8	GTAGAGGGGCAAATCCGTGG	TGTGAGCATGACAGACCCAA
HbSnRK2.9	TGACGATAATGTGAGCGATTGTC	GCCAAAGAAATGGTGCAGGTT
HbSnRK2.10	CGATCTCTCTCGATCAGGGC	TTTGGCAGCAACTGAGCAGC

the rubber tree genome database, we obtained information on cDNA and genomic sequences. The molecular weight (MW) and isoelectric point (pI) of each protein sequence were calculated using ExPASy (http://web.expasy.org/compute_pi/).

Multiple sequence alignment and phylogenetic analysis

The multiple sequence alignments for the HbSnRK2 proteins were performed using ClustalW (Larkin et al. 2007). Further processing of the alignment data was carried out using ESPript 3.0 (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi; Robert and Gouet 2014) with default parameter settings. An unrooted phylogenetic tree of SnRK2 protein sequences was constructed using MEGA 6.0 (Tamura et al. 2013) through the neighbor joining (NJ) method, and bootstrap analysis was conducted using 1000 replicates.

Gene structure analysis and motif detection of HbSnRK2 family genes

The exon-intron structures of the HbSnRK2 family genes were determined on the basis of the alignments of their coding sequences and corresponding genomic sequences, and a diagram was obtained with Gene Structure Display Server (GSDS 2.0; http://gsds.cbi.pku.edu.cn/) (Hu et al. 2015). Motif detection was performed with the MEME program (version 4.11.2, http://meme-suite.org/tools/meme; Bailey et al. 2015). The parameters were as follows: number of

Table 2 Primers for qRT-PCR

Gene	Sense primer	T_m	Anti-sense primer	T_m	Product length	Primer efficiency	Ct (root)
HbSnRK2.1	AGTGGAGAATTCGTGTGCCC	60.32 °C	TGCGGCGAAACAAGGATTAC	59.20 °C	135	1.917	24.05
HbSnRK2.2	GTAGCGATTTCGTTTGCCCT	58.92 °C	ACGCCACGTGTCTATACAAAG	58.40 °C	148	1.984	22.95
HbSnRK2.3	GGTCCCAAAATGGGTGGTCT	59.89 °C	CCCTGTACATGACAAGAATGGC	59.32 °C	160	1.975	23.93
HbSnRK2.4	GTCCAGGCAAGTGGAGAGTA	58.73 °C	ACAAAGTGCAGTTCTGTGAGAT	58.19 °C	102	2.003	26.19
HbSnRK2.5	GTTAAGGAGGCGCAAGCAAG	59.83 °C	CACTGGTATCTGAGAAGGAGCA	59.24 °C	74	1.938	23.18
HbSnRK2.6	AGAGTTAAGGAGGCACAAGCA	59.30 °C	GCAGGTATCCGATAAAGGGC	58.19 °C	75	2.001	22.37
HbSnRK2.7	TGGATAGCAGTGGGGAGATAGT	59.82 °C	ATGCATTCTTTTGGCCAGATTT	58.44 °C	85	1.978	22.70
HbSnRK2.8	GGATAGTAGCGGGGAGATAGTTTAT	59.11 °C	TTTCTATGCTAACCTACCACATTCT	57.80 °C	137	1.905	26.14
HbSnRK2.9	CCGGTGCCCGTAGTCTCAA	60.64 °C	GCCAAAGAAATGGTGCAGGTTC	60.87 °C	160	1.990	22.49
HbSnRK2.10	TGCCCGTGGTCTCAATCAAT	59.67 °C	TTTTTGGCAGCAACTGAGCA	59.18 °C	174	1.987	22.94
Hb actin	CACCACCAGAGAGAAAGTACAG	58.08 °C	GATGGACCAGACTCATCGTATTC	58.45 °C	112	1.986	18.54

Table 3 Basic information of *HbSnRK2* family and their putative proteins

Gene	Accession no.	Gene length (bp)	ORF length (bp)	Exon	Predicted protein		
					Size (aa)	MW (kDa)	pI
HbSnRK2.1	KY211982	2106	1017	9	338	38.56	5.67
HbSnRK2.2	KY211983	2218	1017	9	338	38.18	5.97
HbSnRK2.3	KY211984	2424	1011	9	336	37.96	6.19
HbSnRK2.4	KY211985	3171	1068	9	355	41.03	5.79
HbSnRK2.5	KY211986	3690	1065	9	354	40.52	6.00
HbSnRK2.6	KY211987	4312	1065	9	354	40.71	5.83
HbSnRK2.7	KY211988	5179	1095	9	364	41.38	4.47
HbSnRK2.8	KY211989	5543	1089	9	362	41.12	4.54
HbSnRK2.9	KY211990	4922	1092	9	363	41.25	4.56
HbSnRK2.10	KY211991	4752	1092	9	363	41.20	4.60

repetitions, any; maximum number of motifs, 20; and optimum motif widths, between 9 and 30 residues. Other options used default values.

RNA extraction and gene expression assay by qRT-PCR

Total RNA was extracted according to a previous method by Tang (Tang et al. 2007). Table S1 lists the quality assessment of RNAs preferably RIN values. cDNA strand was synthesized using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Lithuania). RT-PCR was conducted with primers (Table 2) and rubber tree actin gene (GenBank HQ260674), which served as an internal control. RT-PCR was performed using the fluorescent dye SYBR-Green (TaKaRa, China), and the melt curve analysis of amplification products was conducted in Stratagene Mx3005P Real Time Thermal Cycler (Agilent, USA). The RT-PCR conditions were as follows: 5 min at 95 °C for denaturation, 45 cycles for 8 s at 95 °C, 30 s at 58 °C, and 20 s at 72 °C for amplification. Three biological replicates were used per treatment or control.

Statistical analysis

Relative RNA expression levels were determined through the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Values are expressed as means ± SE of three different experiments with three replicate measurements. ANOVA was used to compare the statistical difference based on Fisher LSD test, at a significance level of $P < 0.05$ and $P < 0.01$.

Results

Identification of *SnRK2* gene in rubber tree

To extensively identify rubber tree *SnRK2* genes, we used BLAST and hidden Markov model to search the rubber tree

Table 4 The percentage of HbSnRK2 amino acid identity

	HbSnRK2.2	HbSnRK2.3	HbSnRK2.4	HbSnRK2.5	HbSnRK2.6	HbSnRK2.7	HbSnRK2.8	HbSnRK2.9	HbSnRK2.10
HbSnRK2.1	89.94	82.63	67.06	69.64	68.45	69.82	69.73	71.89	72.19
HbSnRK2.2		81.74	66.17	67.86	67.26	68.64	68.84	69.53	69.82
HbSnRK2.3			68.36	69.46	67.37	69.05	69.85	71.13	72.32
HbSnRK2.4				82.77	80.51	65.89	65.79	67.35	67.64
HbSnRK2.5					95.48	68.42	68.04	69.59	70.76
HbSnRK2.6						66.37	65.98	68.71	69.88
HbSnRK2.7							96.41	85.67	86.78
HbSnRK2.8								83.98	84.81
HbSnRK2.9									95.32

genome database with *SnRK2* sequences from *Arabidopsis* and rice as queries. After removing redundant sequences, we identified 10 *SnRK2* genes that have complete serine/threonine protein kinase catalytic domains (Table 3) and designated them as *HbSnRK2.1–10*. The identified *HbSnRK2* genes in rubber tree encode proteins with amino acid residues ranging from 338 (HbSnRK2.1, HbSnRK2.2, and HbSnRK2.3) to 364 (HbSnRK2.7). Table 1 lists the other characteristics of the *HbSnRK2* genes, including gene and open reading frame length, pI, MW, and exons. The gene length ranged from 2096 bp (*HbSnRK2.1*) to 5193 bp (*HbSnRK2.8*). The MWs were from 38.18 (HbSnRK2.2) to 41.38 kDa (HbSnRK2.7), and all proteins have low pI values (pI <7.0) which ranged from 4.47 (HbSnRK2.7) to 6.19 (HbSnRK2.3).

The conserved residues of *HbSnRK2s*

To confirm the identity of putative full-length coding sequences, we performed RT-PCR amplifications on cDNAs from rubber tree latex. The 10 complete sequences were obtained and validated through sequencing. Amino acid sequence alignment indicated that 10 HbSnRK2s had at least 65.79% amino acid identity, and the maximum percentage of amino acid sequence identities was between HbSnRK2.7 and HbSnRK2.8 at 96.41% (Table 4).

Figure 1 shows the multiple sequence alignment of the HbSnRK2 proteins. As stated previously, the HbSnRK2s have highly conserved N-terminal kinase domains and divergent C-terminal domains that contain acidic amino acid-rich regions

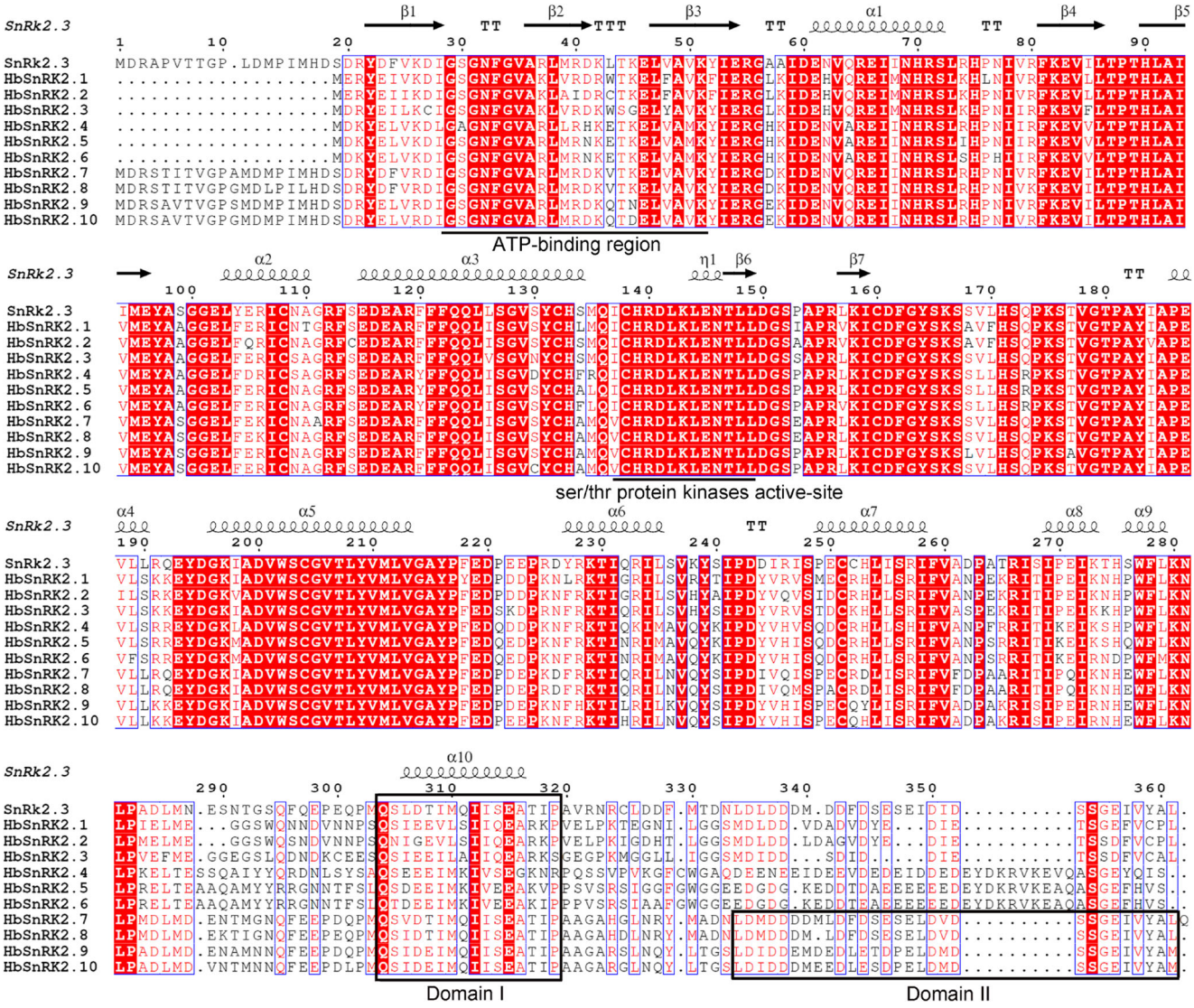

Fig. 1 Sequence and secondary structure alignment of HbSnRK2 proteins. The predicted secondary structure of the HbSnRK2 proteins was indicated, and the crystallographic structure of SnRK2.3 was used as a model (Protein Data Bank Code 3UC3). Espript interface (http://espript.ibcp.fr/) was used. *Underlined* stretches represent a conserved ATP-binding region and Ser/Thr protein kinase active site. *Black boxes* indicate the domain I and domain II at C-terminus

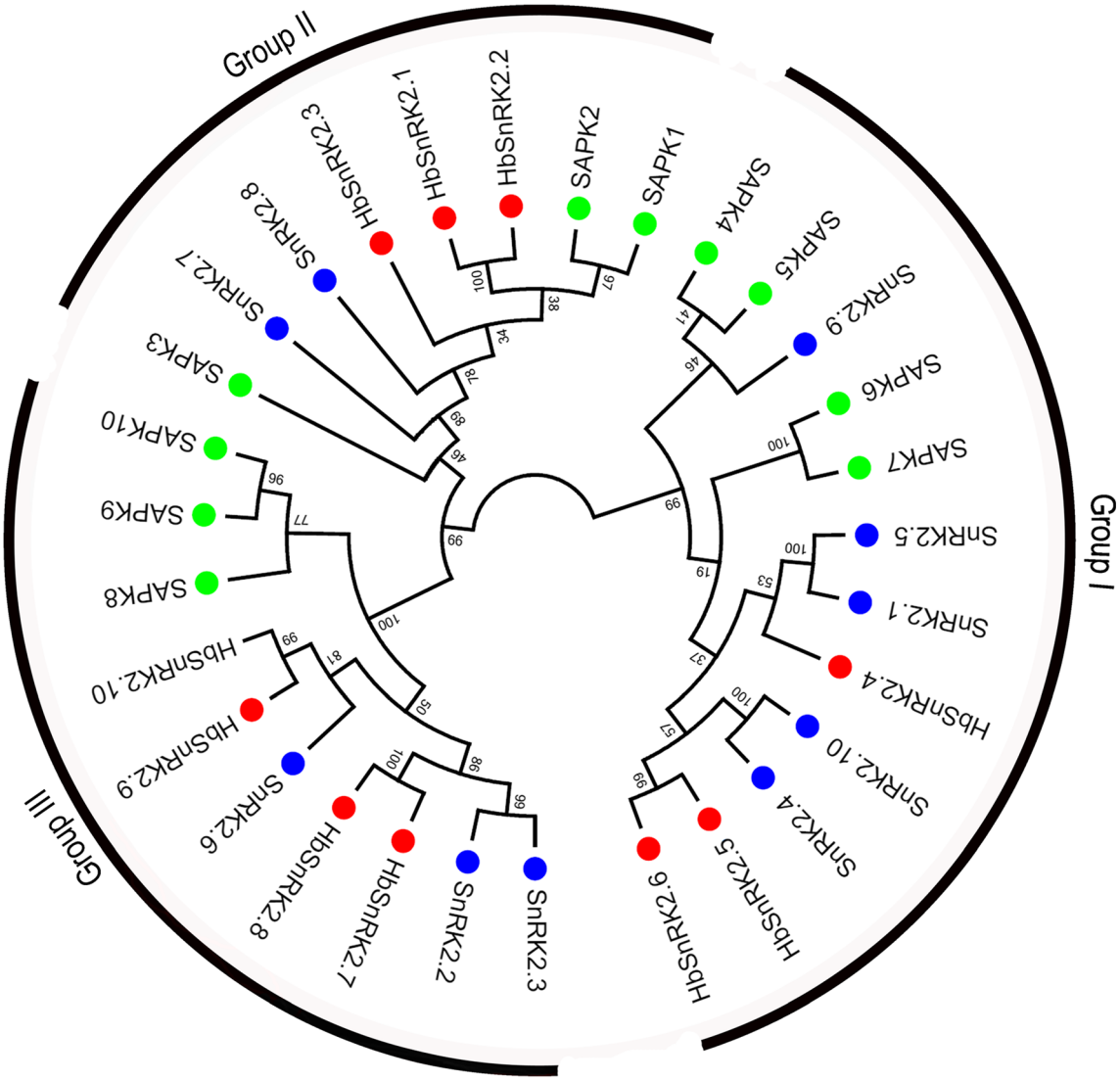

Fig. 2 Phylogenetic analysis of SnRK2 proteins from rubber tree, *Arabidopsis*, and rice. A total of 30 SnRK2s from rubber tree, *Arabidopsis*, and rice were used to create the NJ tree with 1000 bootstrap. The SnRK2 proteins are classified into three groups. GenBank accession numbers of selected SnRK2 proteins from Arabidopsis (*blue*) and rice (*green*) used for drawing phylogenetic tree: SnRK2.1–2.10 NP_196476, NP_190619, NP_001318893, NP_001031021, NP_201170, NP_567945, NP_195711, NP_974170, NP_179885, and NP_176290 and SAPK1–10 BAD17997, BAD17998, BAD17999, BAD18000, BAD18001, BAD18002, BAD18003, BAD18004, BAD18005, and BAD18006

(Halford and Hardie 1998). All the members of the HbSnRK2 family have two conserved signatures in the kinase domains of their N-terminal regions. The first conserved signature is an ATP-binding region signature with a lysine residue as ATP-binding site, and the second is a serine/threonine protein kinase active-site signature with an aspartic acid residue as active site. These two signatures possibly belong to the protein kinase domain (Fig. 1). The C-terminal also contained two distinct domains. Domain I, which is necessary for activation by osmotic stress, was conserved within all SnRK2s, whereas domain II was only present in strongly ABA-responsive kinases. The domain II in the HbSnRK2 family was observed in HbSnRK2.7, HbSnRK2.8, HbSnRK2.9, and HbSnRK2.10 (Fig. 1).

Phylogenetic analysis

To characterize phylogenetic relationships between HbSnRK2s and SnRK2 family members from *Arabidopsis* and rice, phylogenetic analysis was conducted using MEGA version 6 by comparing the full-length protein sequences of 10 HbSnRK2s with SnRK2s from *Arabidopsis* and rice. All SnRK2 proteins were clustered into three groups, denoted as groups I, II, and III, which contain HbSnRK2.4 to HbSnRK2.6, HbSnRK2.1 to HbSnRK2.3, and HbSnRK2.7 to HbSnRK2.10, respectively (Fig. 2). Four HbSnRK2s (HbSnRK2.7 through HbSnRK2.10) that contain the domain II of the C-terminal were present in group III, suggesting that they are necessary for ABA response (Kulik et al. 2011).

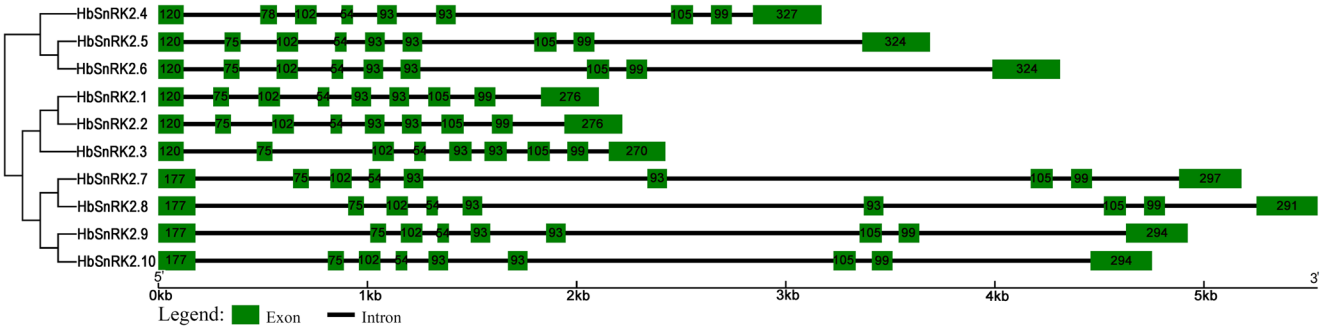

Fig. 3 Exon-intron structure of *HbSnRK2s* based on evolutionary relationship. The NJ evolutionary tree was created with 1000 bootstraps based on the full-length sequences of HbSnRK2s. Exon-intron analyses of *HbSnRK2* genes were carried out with GSDS. *Black lines* and *green boxes* represent the introns and exons, respectively. The length of each exon is indicated

Gene structure and conserved motifs in the C-terminus

Intron/exon structures were detected according to their evolutionary relationships to understand the structural features of the *HbSnRK2* genes. Each gene structure was obtained by comparing its coding sequences to its genomic sequences. As shown in Fig. 3, all *HbSnRk2* genes have eight introns and nine exons, and the exons have strictly conserved exon lengths. The lengths of the second until the eighth exons (except the second exon of *HbSnRK2.4*) were 75, 102, 54, 93, 93, 105, and 99 bp, respectively. In addition, the first and ninth exons of the *HbSnRK2* genes in each subfamily have the same length. This finding supports the close evolutionary relationships among these genes and the classification of subfamilies.

Although the C-terminal regions of *SnRK2s* were divergent in comparison with the highly conserved N-terminus, 10 conserved motifs were identified (Fig. 4). Motifs 1, 2, and 3 were present in all *HbSnRK2* members. Motifs 5, 6, and 4 were unique to groups I, II, and III *HbSnRK2s*, respectively. Motifs 7 and 8 existed in group I *TaSnRK2s* except *HbSnRK2.4*, and motifs 9 and 10 were unique to *HbSnRK2.4*.

Expression profiles of *HbSnRK2s* in different tissues

The qRT-PCR of *HbSnRK2s* were performed in the roots, barks, leaves, flowers, and latex to investigate the expression patterns of *HbSnRK2s* in rubber tree. Figure 5 reveals that tissue expression profiles have differential patterns. The 10 *HbSnRK2s* were expressed in at least one of the five tissues, but their expression levels were relatively weak in the roots. The expression levels of four genes (*HbSnRK2.2*, *7*, *8*, and *9*) were significantly higher in latex than in other tissues, but the content of the three genes (*HbSnRK2.1*, *3*, and *4*) were either extremely low or not expressed in the latex. The amounts of *HbSnRK2.1* and *HbSnRK2.3* were significantly higher in flowers than in other tissues. *HbSnRK2.5*, *6*, and *10* showed high expression levels in leafs, flowers, and latex. With the exception of latex, *HbSnRK2.7* had the highest expression levels in barks among other tissues.

Expression patterns of *HbSnRK2s* in the latex respond to JA, ET, and ABA treatment

Regular application of ET on the rubber tree trunks stimulates latex yield. JA is also a key factor related to the production of rubber trees. ABA is an ubiquitous hormone that regulates plant growth, development, and environmental stress responses. Given that *SnRK2s* are important in abiotic stress responses, we examined the expression levels of *HbSnRK2s* in latex by treating the rubber tree shoots with JA, ET, or ABA. In this study, 7 of 10 *HbSnRK2s* (except for *HbSnRK2.1*, *3*, and *4*) had relative high levels of transcript abundance in latex under JA, ET, and ABA

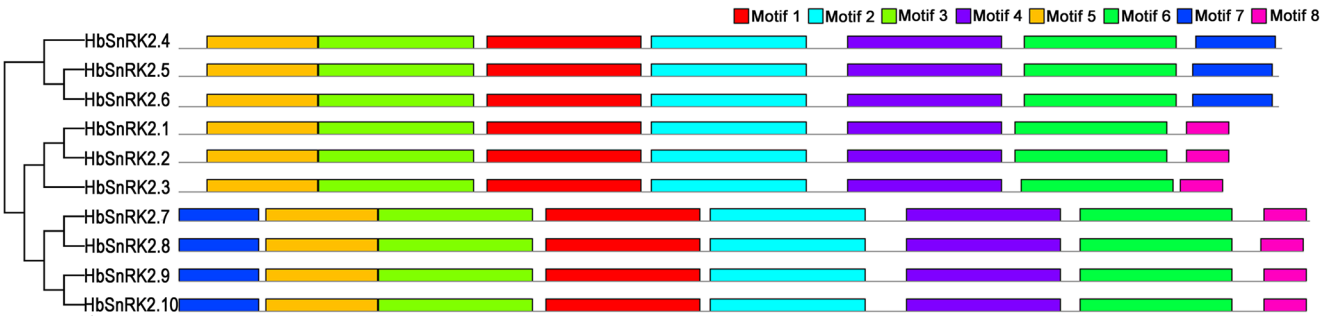

Fig. 4 Distribution of the conserved motifs in the C-terminus of SnRK2s according to evolutionary relationship. Conserved motifs in the HbSnRK2 proteins were identified by MEME software. *Gray lines* represent the non-conserved sequences, and *different colored boxes* represent each motif

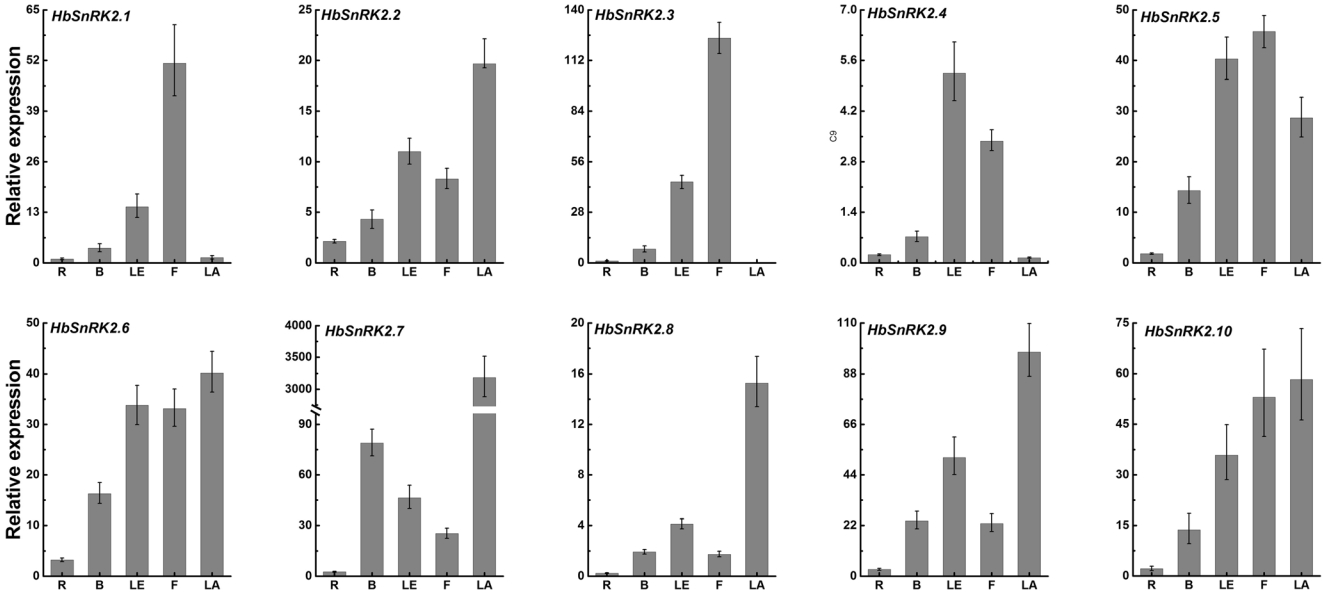

Fig. 5 Expression patterns of *HbSnRK2s* in different tissues. Relative transcript abundances of *HbSnRK2s* were examined by qRT-PCR. The *Y* axis is the scale of the relative transcript abundance level. *X* axis represents the tissues of rubber tree. *R* root, *B* bark, *Le* leaf, *F* flower, *La* latex. Total RNA was isolated from roots, barks, leaves, flowers, and latex. The rubber tree actin gene (GenBank HQ260674.1) served as an internal control

treatment. As shown in Fig. 6, the expression level of eight *HbSnRK2s* was differentially regulated in each of the three different treatments.

Analysis of *HbsnRK2* expression levels in latex treated by ET showed that *HbSnRK2.5*, *7*, *8*, and *10* were upregulated to different degrees upon treatment, whereas *HbSnRK2.6* was downregulated. No obvious change was observed in the expression level of the other two *HbSnRK2s* (*HbSnRK2.2* and *9*) analyzed (Fig. 6a). After the JA treatment, *HbSnRK2.5*, *6–8*, and *10* exhibited elevated expression, whereas *HbSnRK2.2* was downregulated. The expression level of *HbSnRK2.9* had no considerable change (Fig. 6b). For ABA treatment, seven *HbSnRK2s* also showed altered expression levels in latex. In particular, *HbSnRK2.5*, *7–9*, and *10* were markedly upregulated, whereas *HbSnRK2.2* was downregulated. Meanwhile, *HbSnRK2.6* was not responsive to ABA treatment (Fig. 6c).

Discussion

In this study, genome-wide analysis revealed 10 *SnRK2* genes in *H. brasiliensis*. Rubber tree, *Arabidopsis* (Hrabak et al. 2003; Saha et al. 2014), rice (Kobayashi et al. 2004), and *Brassica napus* (Yoo et al. 2016) all have 10 *SnRK2* genes and maize has 11 (Huai et al. 2008). In contrast, the grape genome contains only six *SnRK2s* (Boneh et al. 2012), suggesting the possible expansion of genes in the *SnRK2* family along with whole-genome duplication events after the separation of plant lineages (Yoo et al. 2016).

According to the information obtained from the in silico survey of *H. brasiliensis* genome (Rahman et al. 2013; Tang et al. 2016), we identified the *SnRK2* genes expressed in laticifer cells of rubber trees. Laticifer is a specific tissue essential to natural rubber biosynthesis and storage in rubber trees (Hao and Wu 2000). The transcriptome profiles of laticifers are relatively simple and have high prevalence of rubber biosynthesis-related genes and high level of defense-related genes, which are involved in rubber biosynthesis and defense (Chow et al. 2007). ABA may regulate natural rubber biosynthesis in laticifers (Fricke et al. 2013; Guo et al. 2014). Therefore, ABA signaling can be potential targets for genetic manipulation for the improvement of rubber productivity. In this study, our survey resulted in the identification of 10 *HbSnRK2* genes from *H. brasiliensis*. All *HbSnRK2* genes were mainly expressed in latex except *HbSnRK2.1*, *3*, and *4*, which were barely expressed in laticifers. The expression patterns found in various tissues can provide hints on the functional relevance and significance of *HbSnRK2*. For example, *HbSnRK2.2*, *7*, *8*, and *9* were preferentially expressed in laticifers, and thus, their potential involvement in rubber biosynthesis and defense appears logical. *HbSnRK2.2*, *5*, *6*, and *10* exhibited strong expression in laticifers and had high expression levels in leaf and flower tissues. Thus, we can infer that *HbSnRK2.2*, *5*, *6*, and *10* might be important in rubber biosynthesis and in other leaf and flower functions. *HbSnRK2.1* and *HbSnRK2*.3 expression levels were high in flower, and *HbSnRK2.4* expression was high in leaf, implying their potential functions in flower and leaf development. Nevertheless, further studies are necessary to determine whether *HbSnRKs* are crucial in rubber biosynthesis, defense, and development.

ABA is a major mediator of plant stress responses and many developmental programs. Most SnRK2 genes respond

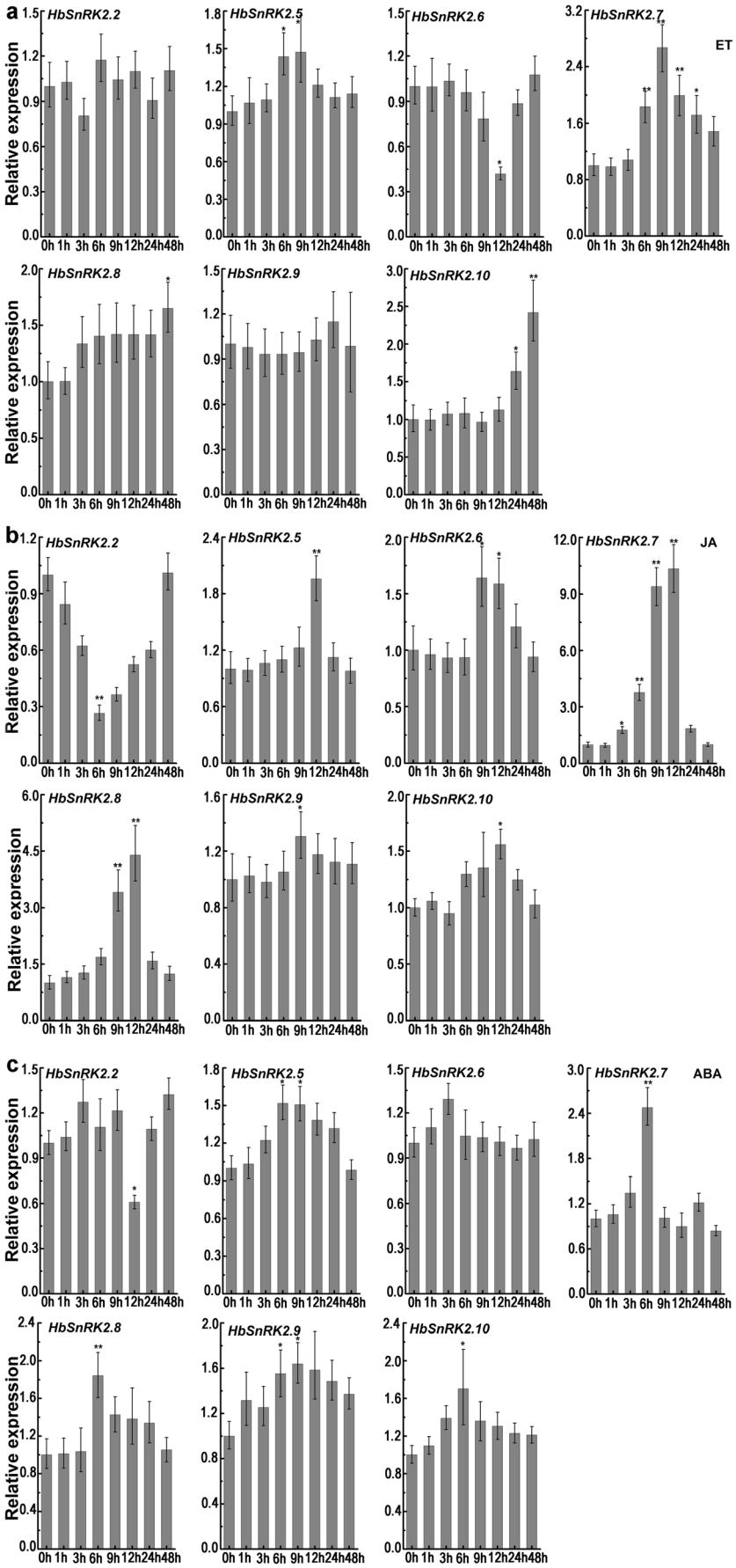

Fig. 6 Expression patterns of the seven *HbSnRK2s* responding to ET, JA, and ABA treatment. Relative transcript abundances of *HbSnRK2s* were examined by qRT-PCR. *Y* axis is the scale of the relative transcript abundance level, and *X* axis is the time course of ET (**a**), JA (**b**), and ABA (**c**) treatment. Rubber tree actin gene (GenBank HQ260674.1) served as internal control. The significant difference was assessed by ANOVA (*single* or *double asterisks* corresponding to $P < 0.05$ and $P < 0.01$)

to various stresses, such as salt, drought, and cold (Yoo et al. 2016). In the present study, we applied ABA, JA, and ET treatment in latex. Among the 10 *HbSnRKs* that have high expression levels in latex, *HbSnRK2.7* was predominant and exhibited strong responses to ABA, JA, and ET. JA and ET contribute to natural rubber biosynthesis and regulation (Hao and Wu 2000; Peng et al. 2009; Pirrello et al. 2014; Zhu and Zhang 2009; Tungngoen et al. 2009), and ABA possibly regulates natural rubber biosynthesis (Fricke et al. 2013; Guo et al. 2014). The interactions between ABA and JA/ET signaling pathways are possibly coordinated and generate combined optimal resistant responses in plants subjected to abiotic and biotic stresses (Lackman et al. 2011; Ahmad et al. 2016; Aleman et al. 2016). ABA, JA, and ET simultaneously regulated the expression of *HbSnRK2.7*, suggesting that *HbSnRK2.7* plays a role in the interactions among ABA, JA, and ET signaling pathways. Thus, elucidating rubber biosynthesis regulated by ABA, JA, and ET as signal molecules may be of great interest in the future.

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Data archiving statement Nucleotide sequences were deposited with the GenBank *HbSnRK2.1* (GenBank accession no. KY211982), *HbSnRK2.2* (GenBank accession no. KY211983), *HbSnRK2.3* (GenBank accession no. KY211984), *HbSnRK2.4* (GenBank accession no. KY211985), *HbSnRK2.5* (GenBank accession no. KY211986), *HbSnRK2.6* (GenBank accession no. KY211987), *HbSnRK2.7* (GenBank accession no. KY211988), *HbSnRK2.8* (GenBank accession no. KY211989), *HbSnRK2.9* (GenBank accession no. KY2119990), and *HbSnRK2.10* (GenBank accession no. KY211991).

Author contributions Shi-Qing Peng and Dong Guo conceived and designed the experiments and drafted the manuscript; Dong Guo, Hui-Liang Li, Jia-Hong, Zhu Ying Wang, An Feng, and Gui-Shui Xie carried out the gene isolation, sequence analysis, and gene expression analysis. All authors read and approved the manuscript.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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