Molecular cloning and characterization of *S*-adenosylmethionine decarboxylase gene in rubber tree (*Hevea brasiliensis*)

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Abstract *S*-Adenosylmethionine decarboxylase (SAMDC) is a key rate-limiting enzyme involved in polyamines biosynthesis, and it plays important roles in plant growth, development and stresses response. However, no *SAMDC* gene was reported in rubber tree. Here we report characteristics of an *SAMDC* gene (*HbSAMDC1*) in rubber tree. *HbSAMDC1* contains a 1080 bp open reading frame (ORF) encoding 359 amino acids. Quantitative real-time PCR analyses revealed that *HbSAMDC1* exhibited distinct expression patterns in different tissues and was regulated by various stresses, including drought, cold, salt, wounding, and H₂O₂ treatments. *HbSAMDC1* 5' untranslated region (UTR) contains a highly conserved overlapping tiny and small upstream ORFs (uORFs), encoding 2 and 52 amino acid residues, respectively. No introns were located in the main ORF of *HbSAMDC1*, whereas two introns were found in the 5' UTR. In transgenic tobaccos, the highly conserved small uORF of *HbSAMDC1* is found to be responsible for translational repression of downstream β -glucuronidase reporter. To our knowledge, this is the first report on molecular cloning, expression profiles, and 5' UTR characteristics of *HbSAMDC1*. These results lay solid

foundation for further elucidating *HbSAMDC1* function in rubber tree.

Keywords Rubber tree · Polyamines · *S*-Adenosylmethionine decarboxylase · Translational regulation · Genomic structure

Introduction

As small molecules, positive charged at physiological conditions, polyamines (PAs) are of great importance for most prokaryotes, eukaryotes, and viruses (Fuell et al. 2010; Takahashi and Kakehi 2010). Spermine, spermidine, and putrescine are the major PAs involved in fundamental cellular processes and environmental stress response in plants (Kusano et al. 2007; Wimalasekera et al. 2011; Tiburcio et al. 2014; Liu et al. 2015). *S*-Adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50), which catalyzes the conversion of *S*-adenosyl methionine to *S*-adenosylmethioninamine, is the key enzyme for the biosynthesis of PAs. SAMDC expression is tightly controlled at both the transcriptional and post-transcriptional levels (Hu et al. 2005).

SAMDC genes have been isolated and characterized in different plant species, such as potato (Mas Arif et al. 1994; Kumar et al. 1996), cotton (Mo et al. 2016), pea (Marco and Carrasco 2002), apple (Hao et al. 2005), *Arabidopsis* (Ge et al. 2006; Marco et al. 2014; Wi et al. 2014), tobacco (Mellidou et al. 2016), tomato (Sinha and Rajam 2013), rice (Roy and Wu 2002; Basu et al. 2014), etc. The results from the aforementioned studies suggested that *SAMDC* genes played critical roles in plant growth, development, and response to environmental stress. It is well known that high-level PAs are harmful to plant cells, so PA

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concentrations need to be strictly controlled in plant. Plant *SAMDC* genes usually contain a long 5' untranslated region (UTR), in which tiny and small upstream open reading frames (uORFs) are present. Tiny uORFs usually are 3–4 codons in size, whereas small uORFs encode 50–54 amino acids (Lee et al. 1997; Franceschetti et al. 2001; Hanfrey et al. 2002). Interestingly, the tiny and small uORFs are overlapped by one nucleotide, thus the last nucleotide of the tiny uORF stop codon is the first nucleotide of the small uORF start codon (Franceschetti et al. 2001; Hanfrey et al. 2002). This one base overlapping arrangement is highly conserved from moss to higher plants, suggesting the important roles of uORFs in transcriptional regulation of *SAMDC* expression. The peptides encoded by small uORFs are highly conserved among different plant species (Franceschetti et al. 2001; Hanfrey et al. 2002). Further analyses showed that the highly conserved small uORF was required for the translational repression. Eliminating small uORF from *SAMDC* genes resulted in an increase in translational efficiency of the *SAMDC* proenzyme in transgenic plants (Hanfrey et al. 2002, 2005; Hu et al. 2005).

Rubber tree originating from the Amazon region is a perennial tropical tree, and it is the only one species cultivated commercially for harvesting latex. China belongs to non-traditional rubber growing regions, in which rubber trees are often subjected to various abiotic stresses, such as low temperature, seasonal drought, typhoon, etc. In view of functional conservation of plant *SAMDC*s, we speculate that *SAMDC* genes might be related to stress response in rubber tree. In the present study, we cloned a new *SAMDC* gene from *Hevea brasiliensis*, named as *HbSAMDC1*. The expression patterns of *HbSAMDC1* in different tissues, developmental stages of leaves, and response to various stresses were further analyzed. Additionally, gene structure and 5' UTR characteristics of *HbSAMDC1* were analyzed in details. Our studies not only contribute to understanding the characterization and expression profiles of *HbSAMDC1*, but also lay solid foundation for studying *HbSAMDC1* function in rubber tree.

Materials and methods

Plant materials and treatments

Rubber tree clone, Reyan 7-33-97, was cultivated at the experimental farm of Chinese Academy of Tropical Agricultural Sciences. The samples including stem apexes, leaves, latex, barks, male and female flowers were harvested from 17-year-old Reyan 7-33-97. In addition, the leaves from different developmental stages were sampled from 6-year-old trees (Reyan 7-33-97). The virgin trees (7-

year-old) were selected for H₂O₂ and wounding treatments, both of which are important factors involved in laticifer differentiation and tapping panel dryness in rubber tree (Hao and Wu 2000; Chen et al. 2003; Putranto et al. 2015; Tian et al. 2015). The H₂O₂ treatment (2%) was performed according to the method from Hao and Wu (2000). The wounding treatment was carried out as described by Tang et al. (2010). The latex was directly collected into liquid nitrogen for total RNA extraction after the first few drops of latex containing the debris were removed. For abiotic stress treatments, 1-year-old seedlings of Reyan 7-33-97 were treated with 16% PEG (drought), 8 °C (cold), and 1 M NaCl (salt), respectively, as described elsewhere (Liu et al. 2016). The leaves were harvested at indicated time points after treatments.

RNA extraction

According to Xu's method (Xu et al. 2010), we extracted total RNA from all samples and treated them with RNase-free RQ1 DNase (Promega, USA). The concentration and quality of the extracted RNA were analyzed by the spectrophotometer (ThermoScientific NanoDrop 2000, USA) and gel electrophoresis.

Molecular cloning of *HbSAMDC1* full-length cDNA

The first strand cDNA was synthesized with total RNA from the latex as template using SuperScript II reverse transcriptase (Invitrogen, USA). The 3' and 5' rapid amplification of cDNA ends (RACE) of *HbSAMDC1* was performed with the SMART RACE cDNA amplification kit (Clontech, USA) according to the manufacturer's instruction. The 5'- and 3'-RACE-1 and -2 specific primers are listed in Table 1. The PCR products were cloned in pGEM-T easy vector and sequenced. After aligning and assembling the sequences of internal EST sequence, 3'-RACE and 5'-RACE products, we obtained the full-length cDNA of *HbSAMDC1* and testified it by sequencing its PCR products. The PCR cycling conditions for amplifying *HbSAMDC1* full-length cDNA were as follows: 94 °C for 4 min, then 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 2 min, followed by 72 °C for 10 min.

qRT-PCR

In each quantitative real-time PCR (qRT-PCR) reaction, the gene-specific primers were used, and the primer sequences of internal control and *HbSAMDC1* were given in Table 1. qRT-PCR was carried out using the LightCycler 2.0 system (Roche Diag-nostics, Germany) and SYBR-Green fluorescent dye (TaKaRa, China) according to the manufacturers' instructions. The qRT-PCR cycling

Table 1 Primer sequences used in this study

Primer name	Primer sequences (5'-3')
5' or 3' RACE	
<i>HbSAMDC1</i> 3'-RACE-1	CTGATATCCTCAACTAGGCTG
<i>HbSAMDC1</i> 3'-RACE-2	CTGCTTCTGCAACCGATCTC
<i>HbSAMDC1</i> 5'-RACE-1	GAGATCGGTTGCAGAAGCAG
<i>HbSAMDC1</i> 5'-RACE-2	CAGCCTAGTTGAGGATATCAG
Validating for full-length cDNA	
<i>HbSAMDC1</i> FLF	CTCTCAAGAGCAATTTTCGTA
<i>HbSAMDC1</i> FLR	GAAAAACCAAAAACACATATTTAG
qRT-PCR analyses	
<i>18S rRNA</i> RTF	GCTCGAAGACGATCAGATACC
<i>18S rRNA</i> RTR	TTCAGCCTTGCGACCATAC
<i>HbSAMDC1</i> RTF	CAACTCTGAAATGCTGCTGG
<i>HbSAMDC1</i> RTR	GAGAGCCACATCTGGTAATG

conditions were as follows: 94 °C for 30 s, followed by 45 cycles of 94 °C for 5 s, 60 °C for 20 s, and 72 °C for 20 s. The primer specificity was verified by determining the melting curves. The relative expression level was calculated according to LightCycler Relative Quantification Software 4.05 instructions. In this study, all qRT-PCR experiments were repeated at least three times with independent cDNA preparations, and the relative expression levels were indicated as mean $\pm$ SD.

Multiple alignments and bioinformatic analyses

The SAMDCs were obtained from NCBI database, and sequence alignment was carried out with ClustalX1.8b software. The phylogenetic tree was constructed using MEGA 4.1 program by the neighbor-joining method with 1000 bootstrap replicates (Kumar et al. 2008).

Site-directed mutagenesis and plasmid construction

The site-directed mutants within *HbSAMDC1* 5' UTR were constructed according to the method of Gao et al. (2006). The wild type of *HbSAMDC1* 5' UTR (SAM) was amplified with the primer pairs (F: 5'-CTCCCATGGCGACCTCATCATTTATCGATTG-3' and R: 5'-GAAGATCTACCATCAGAAATTATTACAGTGAAGAGTG-3'). The PCR reactions were carried out as following: 94 °C for 3 min, then 25 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 3.5 min, followed by 72 °C for 6 min. The PCR products were linked with the pMD18-T vector and sequenced. The correct plasmids were digested with *Dpn* I.

With the corrected SAM as templates, the site-directed mutant (MUT) was amplified with two primer pairs (F: 5'-GGATGATCTTTTGGAGTCAAAAGGTG-3' and R: 5'-CACCTTTTGACTCCAAAAGATCATCC-3' as well as F: 5'-CCTCAACTACGCTGCCTGCCACCAC-3' and R: 5'-GTGGTGGCAGGCAGCGTAGTTGAGG-3'). The other site-directed mutant (TAG) was amplified with primer pairs (F: 5'-CCTTGGTTAGAGCATTGAAGACATTC-3' and R: 5'-GAATGTCTTCAATGCTCTAACCAAGG-3'). The corrected fragments of SAM, MUT, and TAG were digested with *Nco* I and *Bgl* II and linked with the *Nco* I and *Bgl* II digested pCAMBIA1301 vector to construct pCAMBIA1301-SAM, pCAMBIA1301-MUT, and pCAMBIA1301-TAG.

Plant transformation and GUS enzyme assay

pCAMBIA1301-SAM, pCAMBIA1301-MUT, and pCAMBIA1301-TAG introduced into *Agrobacterium tumefaciens* strain LBA4404 separately were used to transform tobacco with the leaf disc method described previously (Burtin and Michael 1997). Once transgenic plantlets rooted, they were transferred to soil and grown in a greenhouse at 25 °C under a 16 h photoperiod. The transgenic plants were detected by PCR method and analyzed the β -glucuronidase (GUS) expression with qRT-PCR method. The GUS activities of the transgenic and wild-type plants were assayed with the GUS-Light assay system as described by Hanfrey et al. (2002). Total protein contents of the extracts were determined by the Bradford method (Bradford 1976), and GUS activity was expressed as relative light units per μ g of protein.

Results

Isolation of *HbSAMDC1* full-length cDNA

Four Transcriptome Shotgun Assembly (TSA) sequences (JR348215, JR347648, JR362696, and JR349936) with significant homology to plant SAMDCs were obtained by analyzing rubber tree de novo transcriptome sequencing data (Li et al. 2012). The four TSA sequences were further assembled into one gene with a complete ORF. In the present study, this gene was designated as *HbSAMDC1*. Based on the assembled *HbSAMDC1* sequence, the 3' and 5' UTRs of *HbSAMDC1* were amplified and sequenced. After aligning and assembling 3' and 5' UTRs and the assembled TSA sequences, *HbSAMDC1* full-length cDNA sequence was obtained and further confirmed by sequencing its PCR products. The full-length cDNA of *HbSAMDC1* is 1888 bp with an ORF of 1080 bp. The 5' and 3' UTRs are 547 and 261 bp with 27 bp poly(A) tail,

b

A circular phylogenetic tree showing the relationships between SAMDC proteins from various species. The tree is rooted at the top and branches out to include the following species: *O. sativa* (OsaSAMDC1, OsaSAMDC2), *T. maritima* (TmsSAMDC), *Z. mays* (ZmsSAMDC1, ZmsSAMDC2), *A. thaliana* (AtsSAMDC1, AtsSAMDC2), *S. cerevisiae* (SisSAMDC1, SisSAMDC2, SisSAMDC3), *D. rerio* (DrsSAMDC), *P. falciparum* (PfsSAMDC), *H. sapiens* (HhsSAMDC), *R. solanaceus* (RssSAMDC), *V. vinifera* (VvsSAMDC), and *G. max* (GmsSAMDC). A scale bar of 0.05 is provided for reference.

Fig. 3 *HbSAMDC1* expression patterns in different tissues and developmental stages of leaves. **a**, **b** *HbSAMDC1* expression profiles in different tissues and developmental stages of leaves, respectively. BA, LA, LE, FF, MF, and SA represent barks, latex, leaves, female flowers, male flowers, and stem apexes, respectively. RL, PL, LL, ML and SL separately are red leaves, pale-green young, light young, mature and senescence leaves. The standard bars were obtained from at least three independent replications and the results were given as mean $\pm$ SD

contrast, *HbSAMDC1* did not show expression change in the corresponding control materials (Fig. 4). The results mentioned above suggested that *HbSAMDC1* was involved in stress responses in rubber tree.

Conservation of tiny and small uORFs

Like other plant *SAMDC* genes usually containing a long 5' leader sequence, the 5' UTR of *HbSAMDC1* is 547 bp in size and possesses the tiny and small uORFs (Fig. 1). We further analyzed the small uORFs in 24 plant *SAMDC*s including 23 angiosperms and 1 gymnosperm (Fig. 5a). The results indicate that the amino acids encoded by the small uORFs are highly conserved. Moreover, an upstream tiny uORF usually overlaps with the small uORF in the plant *SAMDC* 5' leader sequences (Fig. 5). In all the plant *SAMDC* genes analyzed in Fig. 5b, the last nucleotide of the tiny uORF stop codon is the first nucleotide of initiation codon of the small uORF. Interestingly, the +4 position from the initiation codon (ATG) of the tiny uORFs is never optimal G, whereas the corresponding position of the small uORFs is always G. In contrast, relative

Fig. 4 *HbSAMDC1* expression patterns under different stress treatments. **a**, **b** qRT-PCR analyses of *HbSAMDC1* expression profiles under low temperature, drought and salt as well as hydrogen peroxide and wounding, respectively. The standard bars were obtained from at least three independent replications and the results were given as mean $\pm$ SD. Compared with the control, one asterisk shows significant difference with a $P < 0.05$, and two asterisks show very significant difference with a $P < 0.01$

little sequence conservation is found outside the overlapping uORFs between *SAMDC* family members.

Genomic structure of *HbSAMDC1*

According to *HbSAMDC1* full-length cDNA sequence, we designed primer pair to amplify the genomic sequence of *HbSAMDC1*, and obtained genomic sequences by sequencing its corresponding PCR products. Comparing the full-length cDNA sequence with the corresponding genomic DNA sequence, three exons and two introns were identified. As shown in Fig. 6, none introns exist in 3' UTR and main ORF of *HbSAMDC1*, but a short intron is found within the small uORF. In addition, a longer intron is located just upstream of *HbSAMDC1* tiny uORF.

Small uORF of *HbSAMDC1* mediates translational repression of downstream GUS in transgenic tobaccos

The small uORF plays a pivotal role in translational regulation of the downstream cistron (Hanfrey et al. 2002; Hu

Fig. 5 Alignment of deduced amino acids of small uORF in plant *SAMDC* mRNA 5' leader sequences. **a** Alignment of small uORF amino acids deduced from mRNA and ESTs (accession numbers in parentheses): HbsuORF, *Hevea brasiliensis* SAMDC1 (KT454961); RcsuORF, *Ricinus communis* SAMDC (XM_002514488); NtsuORF, *Nicotiana tabacum* SAMDC (AF033100); GmsuORF, *Glycine max* SAMDC (EST-AI442381); PtsuORF, *Pinus taeda* SAMDC (EST-AI725223); VvsuORF, *Vitis vinifera* SAMDC (AJ567368); DcsuORF, *Dianthus caryophyllus* SAMDC (U38527); Zm1suORF, *Zea mays* SAMDC1 (NM_001155794); Zm2suORF, *Zea mays* SAMDC2 (NM_001112243); TmsuORF, *Triticum monococcum*

SAMDC (DQ862828); At1suORF, *Arabidopsis thaliana* SMADC1 (U63633); At2suORF, *Arabidopsis thaliana* SMADC2 (AJ251899); Os1suORF, *Oryza sativa* SMADC1 (Y07766); Os2suORF, *Oryza sativa* SMADC2 (AJ251899). **b** cDNA sequences of the junction between tiny and small uORFs in plant *SAMDC* mRNA 5' leaders. The sequence of the tiny uORF is indicated in lowercase letters with frames and that of the beginning of the small uORF is given in capital letters. The termination codon of the tiny uORF is underlined. For an explanation of the genes and accession numbers see legend to Fig. 5a. The junction sequences of two *Arabidopsis* SAMDCs are identical

Fig. 6 Schematic representation of the genomic structure of *HbSAMDC1*. The relative positions of the introns, overlapping tiny and small uORFs and main ORF are depicted. I1, I2, E1, E2, and E3 separately represent intron 1, intron 2, exon 1, exon 2, and exon 3. The bar represents 200 bp

et al. 2005). To characterize the role of *HbSAMDC1* mRNA 5' leader small uORF, chimeric genes consisting of the wild type or site-directed mutants of 5' leader sequence flanked by the 35S promoter and GUS coding sequence were constructed (Fig. 7a). SAM construct contained the wild type *HbSAMDC1* 5' leader sequence. MUT construct had a leader sequence in which the small uORF was eliminated and replaced by the tiny uORF, which was extended downstream to 68 codons. TAG construct possessed a leader sequence with the small uORF C-terminal truncated to 25 codons by introducing a stop codon (Fig. 4a). Furthermore, we generated transgenic tobacco

plants bearing these constructs and compared the GUS translational efficiency in different transgenic plants. As shown in Fig. 7b, abolishing the small uORF (MUT) led to a threefold increase of GUS translational efficiency in transgenic tobacco plants. Interestingly, the GUS translational efficiency of TAG transgenic plants increased more than sixfold compared to SAM transgenic plants. These results suggest that the small uORF of *HbSAMDC1* is responsible for translational repression of the downstream GUS cistron, and its C-terminal sequence plays a critical role in this regulation.

Discussion

The typical characteristic of plant *SAMDC* genes is the presence of overlapping tiny and small uORFs in 5' UTR. The tiny uORF contains 3 or 4 codons, and the small uORF has 51–55 codons (Franceschetti et al. 2001; Hanfrey et al. 2002). Consistent with the aforementioned results, *HbSAMDC1* also possess the typical characteristic of plant *SAMDC* genes. The tiny and small uORFs of *HbSAMDC1*

Fig. 7 The structure of site-directed mutants and downstream GUS translational efficiency in transgenic tobaccos. **a** White arrow represents the CaMV 35S promoter, hatched block represents downstream ORF, black box represents tiny uORF and derivatives, and white box represents small uORF and derivatives. **b** GUS translational efficiency in leaves of transgenic tobaccos. GUS translational efficiency was calculated as GUS activity divided by GUS mRNA level for each transformant. GUS mRNA levels were normalized to *ubiquitin* mRNA level in each sample. Mean value of the plants containing the SAM construct was set at 1.0 (n refers to the number of transformants for each construct). 35Sp, CaMV 35S promoter

consists of 3 and 53 codons, respectively (Fig. 1). The amino acid sequences encoded by the small uORFs are highly conserved between monocot and dicot plants. In mammalian, the peptide MAGDIS encoded by the small uORF is essential for translational regulation in response to polyamine levels (Ruan et al. 1996). Further study demonstrated that it is the small uORF amino acids rather than DNA sequence that are responsible for translational inhibition of mammalian SAMDC activity (Mize et al. 1998).

If the function of plant SAMDC 5' leader sequence is similar to mammalian, plant small uORF should also involved in translational repression in response to internal polyamines changes. Given the sequence differences between plant and mammalian 5' UTR, the mechanisms of plant and mammalian SAMDC genes in response to polyamines and translational repression might be different. Plant SAMDC 5' UTR usually possess the tiny uORF overlapping with small uORF, therefore it is unlikely that the initiation codon of small uORF will be recognized when its upstream tiny uORF is translated. If tiny uORF is translated, its overlapping small ORF cannot be simultaneously translated. The ribosome will probably reinitiate the downstream main SAMDC gene, because it is sufficiently far enough length to permit efficient reinitiation (Kozak 1987). Consistent with the results from Hanfrey

et al. (2002, 2005), we also found that highly conserved small uORF in *HbSAMDC1* mRNA 5' leader was indeed responsible for translational repression of its downstream GUS reporter cistron in transgenic tobaccos.

Comparing with other plant SAMDC genes, we found that the genomic structure of *HbSAMDC1* was very interesting. No introns were identified in downstream main ORF, whereas two introns were located in the 5' UTR of *HbSAMDC1*, around the overlapping tiny and small uORFs. The results are consistent with other plant SAMDCs (Hu et al. 2005). It has been demonstrated that the introns within the in the SAMDC 5' UTR are required for up-regulating SAMDC activity when plant internal spermidine level is low (Hu et al. 2005). In addition, the introns within the 5' UTR of other genes also have been found to play important roles in regulating gene expression (Bouvet and Wolffe 1994). Thus, we speculated that the introns present in the *HbSAMDC* 5' UTR may also involved in regulating SAMDC expression to maintain PA homeostasis.

The natural PAs, namely spermine, spermidine, and putrescine, play essential roles in many different developmental and physiological processes in plants, such as root and shoot development, floral initiation and development, fruit development and ripening, cell division, and stress response (Alcázar et al. 2006; Mattoo and Handa 2008; Tiburcio et al. 2014; Liu et al. 2015). As the key rate-limiting enzyme in spermine biosynthesis, SAMDC genes play vital roles in plant growth and development, and resistance to biotic and abiotic stresses in plants (Marco and Carrasco 2002; Hao et al. 2005; Ge et al. 2006; Sinha and Rajam 2013; Wi et al. 2014; Mellidou et al. 2016). Ge et al. (2006) found that the *Arabidopsis bud2* (*samdc4*) mutant showed a bushy and dwarf phenotype, and the double mutant of *bud2* and *samdc1* was embryo lethal. Several studies have demonstrated that overexpression of SAMDCs improved tolerance to abiotic stresses in different plant species, such as *Arabidopsis*, rice, tomato, and tobacco (Minocha et al. 2014). In the present study, *HbSAMDC1* expression varied in different tissues and development stages of leaves, suggesting that *HbSAMDC1* might be associated with growth and developmental processes in rubber tree. In addition, *HbSAMDC1* expression was highly induced by drought and salt stresses. This is in accord with previous studies on soybean *GmSAMDC1* and rice *SamdC* (Tian et al. 2004; Basu et al. 2014). *GmSAMDC1* and *SamdC* also showed up-regulation under cold stress (Tian et al. 2004; Basu et al. 2014). In apple, *MdSAMDC1* was up-regulated at the early stage of cold stress treatment but was down-regulated at the later stage (Hao et al. 2005). Pillai and Akiyama (2004) showed that the up-regulation of *OsSAMDC* under cold stress could be used as a molecular marker for screening of cold tolerant

genotypes in rice. They found that *OsSAMDC* transcripts displayed a continuous increase in the cold-resistant rice genotype during cold stress, but there was no obvious change in expression of *OsSAMDC* in the susceptible genotype under the same conditions. Rubber tree is a cold-sensitive tropical tree species. Interestingly, *HbSAMDC1* transcripts decreased gradually from 6 to 24 h after cold stress treatment. These results indicate that the increase in *SAMDC* expression may be very important in the acquisition of cold tolerance in plants.

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