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cDNA Cloning and Expression Analysis of Three Components of SCF^{COI1} Complex in Hevea brasiliensis

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To gain a deeper understanding of jasmonate signaling in specific tissues, three of full-length cDNAs, referred to as HbCUL1, HbSKP1 *and* HbRBX1 *were isolated from laticifers of rubber tree by the rapid amplification of cDNA ends (RACE) method and analysed by sequence analysis and real-time RT-PCR. The putative proteins,* HbCUL1*,* HbSKP1 *and* HbRBX1*, had specific domains of CULLIN superfamily and Cullin Nedd8, Skp1-POZ and Skp1, and RING superfamily, respectively.* HbCUL1 *and* HbSKP1 *were most related to AtCUL1 and AtASK1 among the members of Cullins and ASKs from* Arabidopsis*, respectively. Methyl jasmonate was more effective than ethylene on up-regulating the expression of* HbCUL1*,* HbSKP1 *and* HbRBX1. *These data suggest that* HbCUL1, HbSKP1 *and* HbRBX1 *may be the invariant components of SCF^{COI1} complex of jasmonate signaling in laticifers of rubber tree.*

Keywords: *Hevea brasiliensis* Muell. Arg.; jasmonate signaling; laticifer; SCF^{COI1} complex

Abbreviations: CTAB, cetyl trimethyl ammonium bromide; Cul1, Cullin1; DTT, dithiothreitol; EDTA, ethylene diaminetetra-acetic acid; PVP, polyvinyl-polypyrolidone; RACE, rapid amplification of cDNA ends; RBX1, Ring box1; SCF, Skp1-Cdc53-F-box protein; SKP1, S-phase kinase-associated protein 1.

The SKP1-Cullin/Cdc53-F-box protein ubiquitin ligases (SCF) target many important regulatory proteins for degradation by the 26 S proteasome and play vital roles in diverse cellular processes[1–3]. The SCF complex consists of three invariant components, SKP1 (or ASK), CDC53 (or Cullin), the RING finger protein RBX1 (Ring-Box 1, also known as Roc1 or hrt1) and an interchangeable subunit, an F-box protein[4,5]. Mutation of any one of the components causes malfunction in embryogenesis and other development progress[2,6,7]. In SCF complex, the C-terminal region of scaffold-like Cullin binds to RBX1 and its N-terminal region binds to ASK[8]. ASK protein binds a diverse array of substrate-

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specific factors called F-box proteins which involve hormone signaling, leaf senescence and flower development[3,9]. The function of SCF complex is also regulated by COP9 signalosome (CSN) which mediates the deneddylation of CUL1 at the site of histidine[10,11]. Another regulator, TIP120A functions as a negative regulator of the assembly of SCF complex by binding to the central region of nonneddylated CUL1 in nucleus[12,13]. In *Arabidopsis* genome, there are 11 Cullins[7], 2 RBX1, 21 ASKs[14] and more than 700 F-box proteins[15]. Among these only AtCUL1, AtCUL2, AtASK1, AtASK2, AtRBX1 and several F-box proteins have been shown to participate in SCF complex mediating hormone signaling in *Arabidopsis*, such as SCF^{TIR1} in auxin signaling pathway[16,17], $SCF^{EBF1/2}$ in ethylene signal pathway responses[18] and SCF^{SLY} in gibberellin signaling pathway[19]. SCF^{COI1} and the JASMONATE ZIM-DOMAIN (JAZ) proteins act as central components in the perception of and transcriptional response to JA-Ile in jasmonate signal pathway[20–23]. JAZ proteins bind transcription factors such as MYC2 before JA-Ile perception. The formation of COI1-JAZ complex leads to ubiquitination and subsequent degradation of JAZ proteins and permits the expression of a number of jasmonate regulated genes[23].

The most productive source of natural rubber, a *cis* 1,4-polyisoprene, is exploited from rubber tree (*Hevea brasiliensis* Muell. Arg.) through tapping of trunk bark and ethrel (an ethylene releaser) stimulation[24]. Considering that laticifer differentiation is specifically induced by exogenous jasmonic acid and its precursor linolenic acid[25], natural rubber biosynthesis in the differentiated laticifers may be also regulated by JA signaling[26]. Characterisation of the jasmonate signaling in specialised tissues is essential for understanding how the conserved SCF^{COI1}-JAZ-MYC2 module regulates diverse responses of plants to biotic and abiotic stresses as well as different stages of plant growth and development. We have characterised *HbCOI1* and *HbJAZ1* from laticifers of rubber tree, which are related to the *COI1* and *AtJAZ1/AtJAZ2* from *Arabidopsis*[26,27]. The present study focuses on the characterisation of cDNAs encoding the three invariant components of SCF^{COI1} complex so as to gain a deeper understanding of the jasmonate signaling in laticifiers, a tissue specific for natural rubber biosynthesis.

MATERIALS AND METHODS

Planting Material and Treatments

Plants of rubber tree clone CATAS7-33-97 budded on rootstocks were grown in the experimental farm of Chinese Academy of Tropical Agricultural Sciences (CATAS) in Hainan Island. They were planted in the nursery to multiply the clonal scions for budding. The plants were pruned each year and epicormic shoots grew from the latent buds on the pruned trunks. Stem of the epicormic shoots were respectively treated with 0.5% ethrel and 0.1% methyl jasmonate (Me-JA). Latex samples were collected at 2 h, 6 h, 1 d, 3 d, and 5 d after treatments from ten shoots for each interval. Because the amount of latex collected from each shoot was too small, total RNA were extracted from the mixed latex samples from ten shoots.

Isolation of Total RNA

Total RNA was extracted from latex according to the method of Tang *et al*.[28] The quality and concentration of the extracted RNA were checked by agarose gel electrophoresis and measured by a spectrophotometer (GE Ultrospec™ 2100 pro UV/Visible Spectrophotometer, USA).

Screening of Internal Conserved Fragments of *HbCUL1*, *HbRBX1*, and *HbSKP1*

An EST library of latex from virgin tree of rubber tree clone CATAS7-33-97 was constructed by our laboratory. The conserved fragments of *HbCUL1, HbRBX1*, and *HbSKP1* were found by screening the EST library with the Blast Search Tool online (http://www.ncbi.nlm.nih.gov), among which the full length cDNA of *HbRBX1*, and an EST for *HbSKP1* containing a start codon but truncated at the 3' of the clone were found.

3'-RACE (Rapid Amplification of cDNA ends) of *HbCUL1*, and *HbSKP1*

The 3'-ready cDNA was synthesised by reversely transcribing 1 μg of total RNA with oligo dT-3 site adaptor primer (5'-CCA GTG AGC AGA GTG ACG AGG ACT CGA GCT CAA GCT TTT TTT TTT TT-3') supplied by 3'-RACE kit (Takara, Dalian, China). For cloning of HbSKP1 and HbCUL1, the specific primers (*Table 1*) for 3'-RACE were designed as primer 3HbSKP1, 3HbCUL1 and the nested primer 3HbSKP2, 3HbCUL2 based on the cloned internal conserved fragments, respectively. The first round of 3'-RACE was performed using the universal primer (5'-CCA GTG AGC AGA GTG ACG-3') and the specific primer 3HbSKP1 or 3HbCUL1 in a total volume of 50 μL under the conditions of 94ºC for 5 min, 55ºC for 5 min, 72ºC for 40 min followed by 31 cycles of amplification (94ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min). The PCR product was diluted ten-fold as a template for the second round of 3'-RACE using the universal primer (5'-GAG GAC TCG AGC TCA AGC-3') and the specific primer 3HbSKP2 or 3HbCUL2 under the same conditions as the first round. The products were purified and cloned into pGEM-T easy vector, followed by sequencing.

5'-RACE (Rapid Amplification of cDNA ends) of *HbCUL1*

An aliquot of 1 μg of total RNA were reversely transcribed with PCR reaction. The 5'-ready cDNA was obtained using 5'-CDS primer A and SMART II A oligonucleotide provided in the SMARTIM RACE cDNA

TABLE 1. SEQUENCES OF THE OLIGO NUCLEOTIDES USED FOR FULL LENGTH cDNA CLONING AND REAL-TIME PCR

Primer Name	Sequence (5' to 3')	Primer Name	Sequence (5' to 3')
3HbCUL1	AGGCTTCTGCACTGTTGCTGTTCAACT	CUL1	ATGACAATGAATGAGCGGAAGAC
3HbCUL2	ATGACTCAACTGAACTTGACCGATGATG	CUL2	CAAGGCCACATTACGCCAAGTAT
3HbSKP1	ATGTCGTCCACCTCCGGCGACCACG	SKP1	ATGTCGTCCACCTCCGGCGAC
3HbSKP2	TCACTCTCAAGACTGCAGACGCCAACT	SKP2	GTCTTCATCAACACCCTCAAAAG
5HbCUL1	CGTTACCTTTCTGACCATCCCTTCCATC	RBX1	ATGGACACCGACGTGACCATG
5HbCUL2	TCCTCAATGGCTTCATCACTCAGCTTCT	RBX2	GTGGCCATACTTTTGGAATTCC
YRBXNF	TGCTTTTCACTTCCACTGCATC	YCULNF	AGCCCGACTTCAAGGCAATC
YRBXNR	TCAGGTGATGATTCACACAACTTC	YCULNR	TGGATAATCAACAAGGCCACAT
YSKPNF	CGGATCAAGAACAAGAGCGTTG	Y18Sf	GCTCGAAGACGATCAGATACC
YSKPNR	GGAAGAAGAAGAGGAAACTGATTCAG	Y18Sr	TTCAGCCTTGCGACCATAC

Amplification Kit (Clontech, Palo Alto, CA). The specific 5'-RACE primer 5HbCUL1 and nested primer 5HbCUL2 were designed based on the cloned internal conserved fragment (*Table 1*). Primary 5'-RACE PCR was performed with 5HbCUL1 and UPM primer (provided in the kit) in a total volume of 25 µL and was denatured at 94ºC for 3 min, followed by 31 cycles of amplification (5 s denaturation at 94ºC, 1 min annealing at 55ºC, and 1 min extension at 72ºC). The product of the primary PCR was diluted 50-fold, and was used as a template for nested PCR performed with primer 5HbCUL2 and NUP (provided in the kit) under the same conditions of the primary 5'-RACE PCR. The products were purified and cloned into pGEM-T easy vector, followed by sequencing.

Cloning of the Full-length cDNA

After alignment and assembly of the sequences of the internal conserved sequence, the 3'-RACE and 5'-RACE products, the full-length cDNA sequences were deduced and subsequently obtained by PCR. The open reading frames (ORFs) were amplified by RT-PCR using primers CUL1, CUL2, SKP1, SKP2, RBX1, and RBX2 with the PCR kit (Takara, Dalian, China). The products were purified and cloned into pGEM-T easy vector, and sequenced.

Multiple Alignment and Bioinformatic Analyses

The full-length cDNA sequence was compared to the non-redundant peptide database using DNAMAN version 6.0 and Basic Local Alignment Search Tool (BLAST) at the National Centre for Biotechnology Information (NCBI) (http://www.ncbi. nlm. nih.gov).

Real-time RT-PCR

Real time RT-PCR was referred to Zhao *et al*.[29]. Prior to cDNA synthesis, contaminating DNA was removed from RNA samples using the kit of Ambion DNA-free DNase treatment and removal reagents. First strand cDNA was synthesised from 2 µg of RNA with M-MuLV reverse transcriptase and random hexamer primers (Takara, Dalian, China) according to the manufacturer's instructions. The cDNA was diluted 1:20 with nuclease-free water. Aliquots of the same cDNA sample were used for Real-time RT-PCR with primers designed for HbCUL1 (YCULNF/YCULNR), HbSKP1 (YSKPNF/YSKPNR), HbRBX1 (YRBXNF/YRBXNR), and 18S (Y18Sf/Y18Sr) which was used as a housekeeping gene (*Table 1*). Reactions were performed in a 25 µL volume containing 500 nM of each primer and 1×SYBER Green PCR master mix (TIANGEN, Beijing, China). 18S rRNA primer was used at the concentration of 50 nM. Real-time RT-PCR was performed on the Roche lightcycler 2.0 (Applied Biosystem, Switzerland) using parameters recommended by the manufacturer : (1) pre-denaturation at 95ºC for 30 s with 1 cycle; (2) denaturalisation of 45 cycles at 95ºC for 5 s, annealing at 58ºC for 20 s, extension at 72ºC for 20s, 45 Cycles; (3) 1 cycle of dissociation at 95ºC for 15 s, 60ºC for 30 s, 95ºC, 15 s, 1 Cycle. Each PCR reaction was done in triplicate and no-template controls were included. Accumulation of PCR products was detected in real time and the results were analysed with Exor3 System Software (Applied Biosystem, Switzerland) and presented as E^{dCt}, where *d*Ct was the value of the Ct values of *HbSKP1, HbCUL1* and *HbRBX1* minus that of the housekeeping gene. The statistical significance of the values was determined by the *t*-test. The P values<0.05 were considered to be significant in all cases.

RESULTS

Isolation and Characterisation of *HbCUL1*, *HbRBX1*, and *HbSKP1*

A 729-bp fragment was found in the EST library which showed high similarity with the CUL genes from *Arabidopsis* as revealed by a BlastX search. 3'-RACE and 5'-RACE generated an 809-bp fragment and a 1375-bp fragment, respectively. By alignment and assembly of these three sequences, the full-length cDNA sequence was deduced, and its open reading frame (ORF) was amplified by PCR and confirmed by sequencing. The cDNA, referred to as *HbCUL1* (GenBank accession No.: GQ369509), was 2762-bp in length and contained a 2235-bp ORF encoding a putative protein of 744 amino acids, flanked by a 153-bp 5'-UTR (untranslated region) upstream of the start codon and a 374-bp 3'-UTR including a poly (A) tail of 18 bp downstream of the stop codon. The predicted molecular mass of the protein is 87 kDa, with a pI of 6.56. By the SignalP 3.0 Server (http://genome.cbs.dtu.dk/services/SignalP/), there is great probability for the existence of signal peptide in the deduced protein and the cleavage site is between pos. 24 and 25 amino acid residues. Blast analysis showed that the putative *HbCUL1* protein was homologous with the CUL members (*Figure 1A*) and shared 81.99%, 82.26%, 87.77%, 95.97%, 97.31% and 97.45% with CUL homologs from *Nicotiana tabacum, Arabidopsis, Picea sitchensiss, Vitis vinifera, Populus trichocarpa* and *Ricinus communis*, respectively (*Figure 2A*).

A 638-bp fragment was found in the EST library which showed high similarity with the RBX genes from *Arabidopsis* as revealed by a BlastX search. The ORF was identified in this fragment and confirmed by sequencing. The cDNA, referred to as *HbRBX1* (GenBank accession No.: HM640271), was 638-bp in length and contained a 315-bp ORF encoding a putative protein of 104 amino acids, flanked by a 32-bp 5'-UTR upstream of the start codon and a 255-bp 3'-UTR. The predicted molecular mass of the protein is 13 kDa, with a pI of 6.2. By the SignalP 3.0 Server, there is great probability for the existence of signal peptide in the deduced protein and the cleavage site was between pos. 21 and 22 amino acid residues. Blast analysis showed that the putative *HbRBX1* protein was homologous with the other RBX members (*Figure 1B*). It also shared 80.17%, 88.24%, 89.08%, 89.92%, 90.08%, 91.60%, and 92.44% identity with RBX homologs from *Drosophila melanogaster, Populus trichocarpa, Picea sitchensis, Arabidopsis, Vitis vinifera, Arachis hypogaea*, and *Glycine max*, respectively (*Figure 2B*).

A 486-bp fragment was found from the EST library which showed relatively high similarity with the SKP genes from *Arabidopsis* as revealed by a BlastX search. 47bp 5' UTR and 441 bp ORF were found in this fragment. 3'-RACE generated a 658-bp fragment. By alignment and assembly of these two sequences, the full-length cDNA sequence was deduced, and its open reading frame (ORF) was amplified by PCR and confirmed by sequencing. The cDNA, referred to as *HbSKP1* (GenBank accession No.: HM640272), was 778-bp in length and contained a 543-bp ORF encoding a putative protein of 180 amino acids, flanked by a 47-bp 5'-UTR upstream of the start codon and a 188-bp 3'-UTR. The predicted molecular mass of the protein is 20.3 kDa, with a pI of 4.38. By the SignalP 3.0 Server, there is great probability for the existence of signal peptide in the deduced protein and the cleavage site is between pos. 62 and 63 amino acid residues. Blast analysis showed that the putative *HbSKP1* protein had

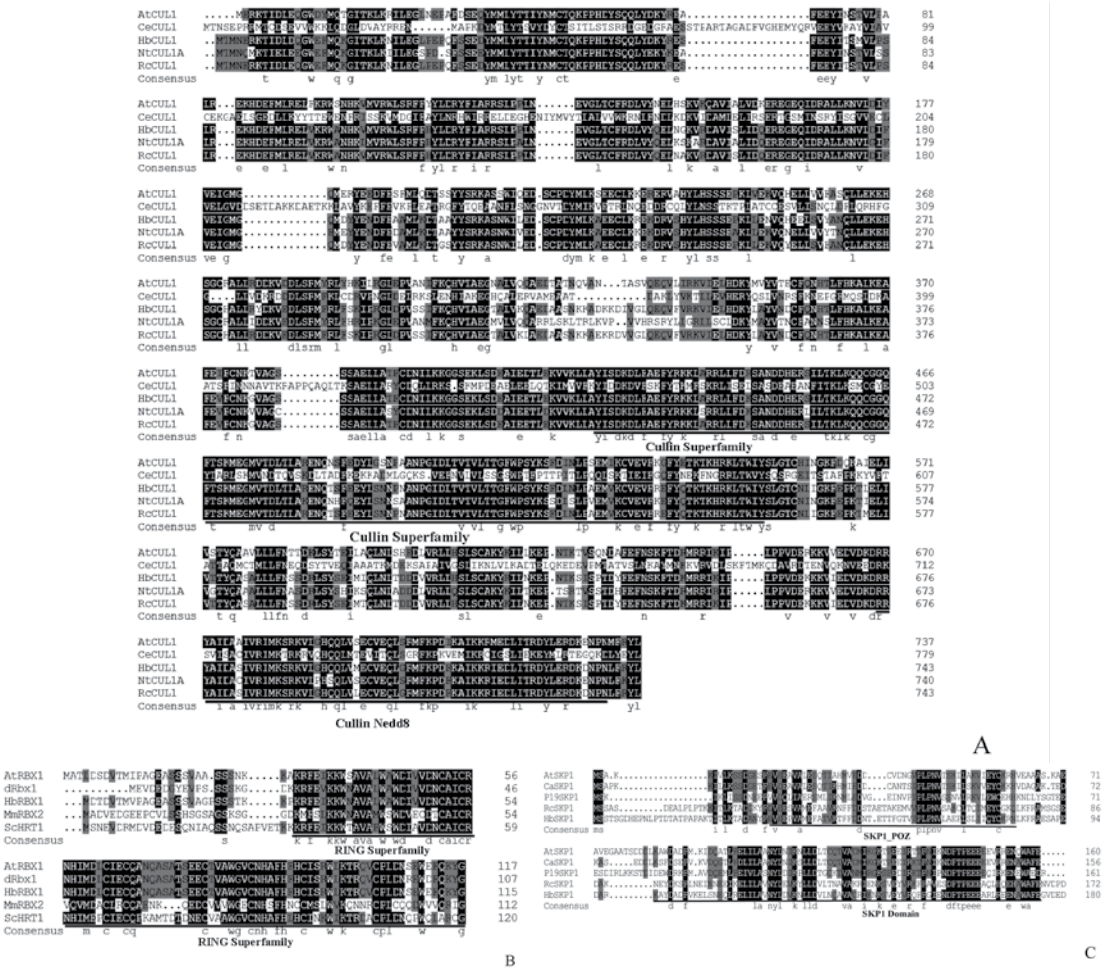
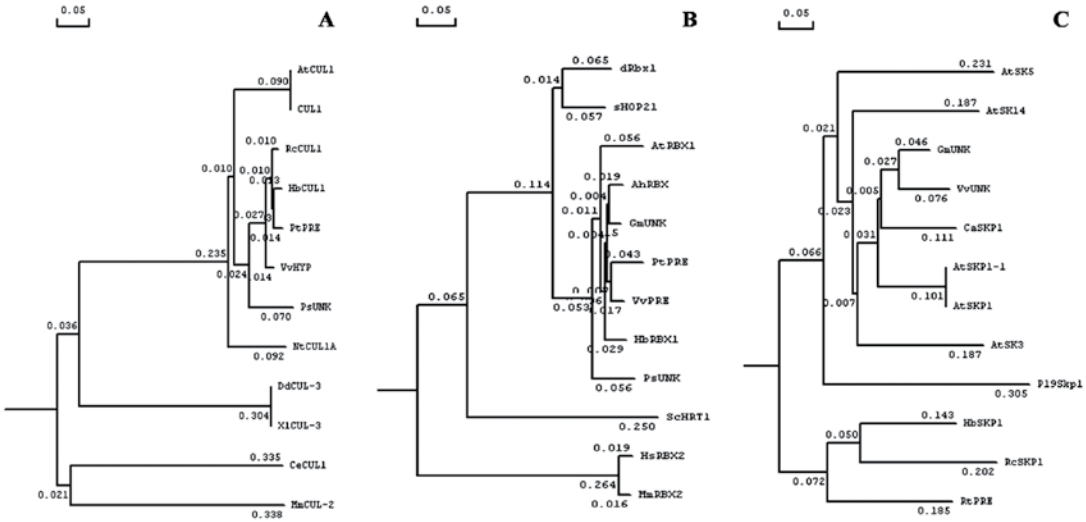


Figure 1. Amino acid alignment of HbCUL1, HbRBX1 *and* HbSKP1 *with other Cullin proteins, Ring-box proteins and SKP-like proteins, respectively. The identical amino acids were shaded in dark and the well-conserved residues were shaded in gray. The conserved domain was underlined. A. The aligned Cullin proteins were* HbCUL1 *(GenBank accession No.: GQ369509) from* Hevea brasiliensis, AtCUL1 *(GenBank accession No.: GI: 68052236) from* Arabidopsis thaliana, CeCUL1 *(GenBank accession No.: GI: 2493900) from* Caenorhabditis elegans, NtCUL1A *(GenBank accession No.: GI: 34481799) from* Nicotiana tabacum *and* RcCUL1 *(GenBank accession No.: GI: 255551707) from* Ricinus communis. *B. The aligned Ring-box proteins were* HbRBX1 *(GenBank accession No.: HM640271) from* Hevea brasiliensis, AtRBX1 *(GenBank accession No.: GI: 18420256) from* Arabidopsis thaliana, DmRBX1 *(GenBank accession No.: GI: 37538005) from* Drosophila melanogaster, MmRBX2 *(GenBank accession No.: GI: 37538006) from* Mus musculus, *and* ScHRT1 *(GenBank accession No.: GI: 37537921) from* Saccharomyces cerevisiae. *C. The aligned SKP proteins were* HbSKP1 *from* Hevea brasiliensis *(GenBank accession No.: HM640272),* AtSKP1 *(GenBank accession No.: GI: 18410982) from* Arabidopsis thaliana, CaSKP1 *(GenBank accession No.: GI: 62467589) from* Capsicum annuum, P19SKP1 *(GenBank accession No.: GI: 74626243) from* Schizosaccharomyces pombe, *and* RcSKP1 *(GenBank accession No.: GI: 255544596) from* Ricinus communis.

conserved domains of Skp1-POZ and Skp1 (*Figure 1C*) and shared 40.96%, 42.08%, 42.31%, 43.96%, 51.11% and 64% identity with SKP homologs from *Arabidopsis, Capsicum annuum, Vitis vinifera, Glycine max, Populus trichocarpa* and *Ricinus communis*, respectively (*Figure 2C*).

Effect of Me-JA and Ethrel on the Expression of *HbCUL1*, *HbSKP1*, and *HbRBX1*

The relative expression of *HbCUL1* was the highest among the three genes after treatment with methyl jasmonate (Me-JA) in the epicormic shoots (*Figure 3A*). The *HbCUL1*



Protein	Accession numbers	Protein Similarity
RcCUL1	GI: 255551707	97.45%
PtPRE	GI: 224123204	97.31%
VvHVP	GI: 225432272	95.97%
PsPRE	GI: 148909489	87.77%
CUL1	GI:68052236	82.26%
AtCUL1	GI: 18411983	82.26%
NtCUL1A	GI: 34481799	81.99%
DdCUL-3	GI:74854062	33.33%
XlCUL-3	GI:74854062	33.33%
CeCUL-1	GI:2493900	27.40%
MmCUL-2	GI:38257755	26.84%

Protein	Accession numbers	Protein Similarity
GmUNK	GI: 255631147	92.44%
AhRBX	GI: 83701143	91.60%
VvPRE	GI: 225444714	90.08%
AtRBX1	GI: 18420256	89.92%
PsUNK	GI: 116780716	89.08%
PtPRE	GI: 224068466	88.24%
DmRbx1	GI:37538005	80.17%
sHOP21	GI:37537963	79.31%
ScHRT1	GI:37537921	54.55%
HsRbx2	GI:37538003	43.59%
MmRbx2	GI:37538006	41.88%

Protein	Accession numbers	Protein Similarity
RcSKP1	GI: 255544596	61.54%
PtPRE	GI: 224081401	51.11%
GmUNK	GI: 255641709	43.96%
VvUNK	GI: 225428047	42.31%
CaSKP1	GI: 62467589	42.08%
SKP1-1	GI:71153764	40.96%
AtSKP1	GI: 18410982	40.96%
AtSK14	GI:75318731	37.22%
AtSK3	GI:75206636	35.45%
AtSK5	GI:75335818	30.81%
P19Skp1	GI:74626243	29.79%

Figure 2. Phylogenetic tree analysis of HbCUL1*(A),* HbRBX1*(B), and* HbSKP1*(C) from rubber tree (*Hevea brasiliensis*) and members from different species with indicated GenBank accession numbers and similarities to* HbCUL1, HbRBX1 *and* HbSKP1, *respectively.*

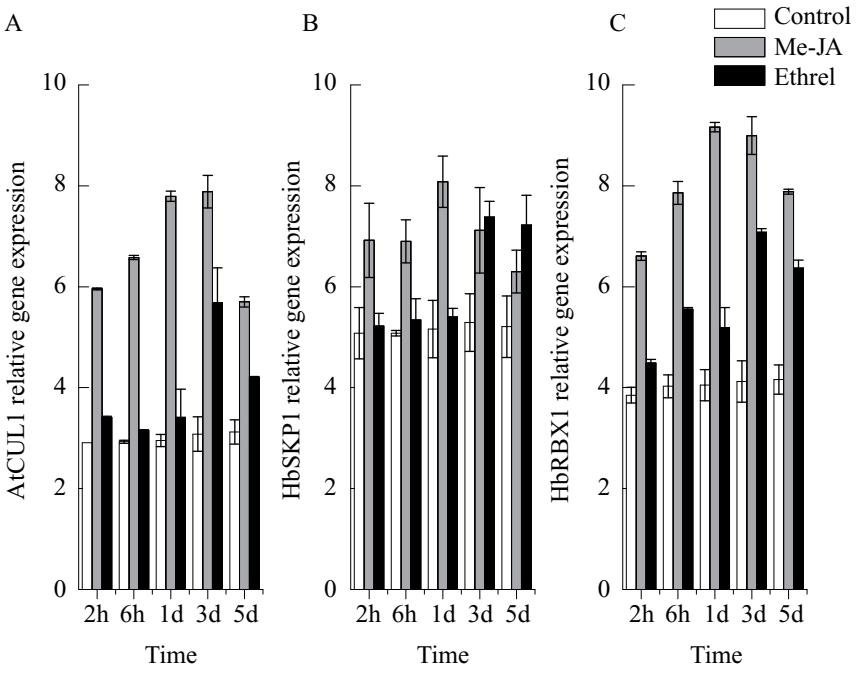


Figure 3. Transcription patterns of HbCUL1 *(A),* HbSKP1 *(B), and* HbRBX1 *(C) in laticifers of epicormic shoots treated with 0.1 % (W/V) Me-JA and 2 % (W/V) ethrel, respectively.*

was remarkably up-regulated at 2 h and a high level of the gene expression was kept within 5 d after Me-JA treatment (*Figure 3A*). The *HbSKP1* was remarkably up-regulated and a high level of the gene expression was kept within 5 d after Me-JA treatment (*Figure 3B*). The level of *HbRBX1* expression was the highest at 1 d after Me-JA treatment in the epicormic shoots and high level of the gene expression was kept within 5 d (*Figure 3C*). The ethrel treatment showed little effect on the expression of *HbCUL1, HbRBX1* and *HbSKP1* within 1 d. The up-regulated expression of *HbCUL1, HbRBX1* and *HbSKP1* caused by ethrel was found at 3 d and 5 d (*Figure 3*). In general, the relative expression level of *HbCUL1, HbRBX1* and *HbSKP1* in response to ethrel was lower than that treated by Me-JA (*Figure 3*).

DISCUSSION

Bioinformatic analysis showed that *HbCUL1* protein had conserved Cullin family region (428-569 aa) and Cullin NEDD8 domain (675-738 aa)[30]. The derived amino acid sequence of *HbCUL1* shared high identity with Cullin homologs from other plant species, indicating that *HbCUL1* was a member of Cullin family. Among 11 Cullins in *Arabidopsis*[7], *HbCUL1* shared highest identity with *AtCUL1*, the identified component of SCF complex (*Table 2*). RBX protein family in different species has a conserved RING superfamily domain and contains 108-121 amino acids with molecular weight of 12-16 kDa[31,32]. The *HbRBX1* protein had a RING-finger domain (28-116 aa). It shared high identity with RBX homologs from other plant

TABLE 2. PROTEIN SIMILARITY OF *HbCUL1* AND *HbSKP1* WITH CULLIN AND ASK PROTEINS IN *ARABIDOPSIS*

TAIR number	Other name	Accession numbers	Protein Similarity with *HbCUL1*	TAIR number	Other name	Accession numbers	Protein Similarity With *HbSKP1*
At4g02570	AtCUL1	AAK76704	81.84%	At1g75950	AtASK1	NP_565123	41.49%
At1g02980	AtCUL2	NP_171797	65.11%	At2g03170	AtASK14	O81057	37.22%
At1g43140	F1l21.19	NP_175007	63.77%	At1g20140	AtASK4	NP_564105	37.04%
At1g26830	AtCUL3A	NP_174005	32.20%	At2g03190	AtASK16	NP_565297	36.60%
At1g69670	AtCUL3B	NP_177125	31.94%	At2g25700	AtASK3	AAD31370	35.98%
At5g46210	AtCUL4	AJ318018	28.80%	At1g10230	AtASK18	NP_563864	35.86%
At1g59800	F23H11.12	NP_176189	18.27%	At3g25650	AtASK15	NP_566773	35.18%
At2g04660	APC2	NP_178543	12.33%	At2g20160	AtASK17	AAD24382	32.22%
At4g12100	F16J13.170	NP_192974	8.74%	At3g60020	AtASK5	Q9M1X4	30.81%
At1g59790	F23H11.11	NP_176188	8.42%	At2g03160	AtASK19	NP_565295	29.73%
At3g46910	T6H20.60	NP_190275	5.09%	At5g08590	AtASK2	ABD85157	8.86%

species. SKP1 functions as a bridge linking Cul1/CDC53 to F-box protein. The *HbSKP1* had conserved domains of Skp1-POZ and Skp1. Among 21 *Arabidopsis-SKP1-like* proteins (ASKs)[14], *HbSKP1* shared highest identity with *AtASK1*, the identified component of SCF (*Table 2*). Taken all these data together, *HbCUL1, HbSKP1* and *HbRBX1* should be the invariant components of SCF complex in laticifers of rubber tree.

Exogenous Me-JA is much more effective than ethrel on up-regulating the expression of *HbCUL1, HbSKP1*, and *HbRBX1*, which is similar to the case of *HbCOI1* and *HblMYC1/HblMYC2*. The expression of *HbCOI1* and *HblMYC1/HblMYC2* is remarkably up-regulated by Me-JA, but not by ethrel[27,29]. The components of COI1-JAZ-MYC2 module of jasmonate signaling in *Arabidopsis* have been identified in laticifers of rubber tree[26,27,29]. From the available evidence, it is concluded that *HbCUL1*, *HbSKP1* and *HbRBX1* may be the invariant components of SCF^{COI1} complex and mediate jasmonate signaling in laticifers, a kind of tissues specific for natural rubber biosynthesis in rubber trees.

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