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Cloning and identification of a differentially expressed RING E3 ubiquitin-protein ligase gene responding to Jasmonic acid signaling pathway in rubber tree (*Hevea brasiliensis*)

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Abstract

In order to understand the key metabolic pathways and key genes involved in the latex biosynthesis process in rubber tree, we studied the differential gene expression between latex and leaves of rubber tree by cDNA microarray. The significant analysis of microarray (SAM) method was used, and genes that were differentially expressed in the latex and leaves of rubber tree were selected and subjected to various analyses of KEGG metabolic pathway, clustering, and gene ontology based biological pathways. The results indicated that many differentially expression genes mainly distribute in transcription factor activity, zinc ion binding, transferase activity, ubiquitin-protein ligase activity. One of the zinc finger protein encoded genes, named *HbRBX1*, which involves in transcription factor activity, zinc ion binding, ubiquitin-protein ligase activity, was cloned and further confirmed the expression patterns in rubber tree latex and rubber tree leaves by Semi-quantitative RT PCR. In order to understand whether the *HbRBX1* involve in latex biosynthesis or not, the *HbRBX1* expression patterns were also analyzed in response to exogenous jasmonic acid and ethylene, the two types of phytohormone have been proved to involve in latex biosynthesis in rubber tree, the results showed that *HbRBX1* can respond to exogenous jasmonic acid while cannot respond to exogenous ethylene, suggested that *HbRBX1* maybe participate in the latex biosynthesis through the jasmonic acid pathway.

Keywords: rubber tree, RING E3 ubiquitin-protein ligase, jasmonic acid pathway, latex biosynthesis.
Abbreviations: KEGG –Kyoto Encyclopedia of Genes and Genomes, SAM –The significant analysis of microarray, GO –Gene Ontology, NR –Natural rubber, JA –Jasmonic acid, ET –Ethylene, GA –Gibberellin, REF –Rubber elongation factor, SRPP –Small Rubber Particle Protein.

Introduction

The ubiquitination proteins include three enzymes: an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2), and an ubiquitin-protein ligase (E3) (Stone et al., 2005). The three enzymes followed their functions in order, E1 activated the ubiquitin, then ubiquitin is transferred to an E2 enzyme, E3 can bind the E2 and the target protein, and facilitate the transfer of the ubiquitin molecule to the target (Gray et al., 2002), ubiquitination cycle continues until the level of ubiquitin reached to a certain extent, and the target protein is to be transported to the 26S proteasome for degradation (Gray et al., 2002; Stone et al., 2005). The E3 protein plays core role on the target protein ubiquitination. E3 ubiquitin-protein ligases are diverse. Two main types are divided according to the mechanism of the E3 ubiquitin-proteins, one is RING-finger family (Liu et al., 2008; Barlow et al., 1994; Borden and Freemont 1996; Borden 2000), and another is HECT (homologous to E6APC terminus) family (Liu et al., 2008). With more and more new E3 ubiquitin-protein ligases found, the research on E3 ubiquitin-protein ligases becomes a hotspot on understanding their functions of structural basis, substrate proteins and action mechanisms. In *Arabidopsis* genome, there is predicted to encode 475 RING-type E3 ligases (Mach, 2008). However, only few of them were analyzed their functions and identified their target proteins. For example, SINAT5 (Xie et al., 2002), COP1 (Hardtke et al., 2000; Osterlund et al., 2000), KEG (Stone et al., 2006), AIP2 (Zhang et al., 2005), HOS1 (Dong et al., 2006), DRIP1, and DRIP2 (Qin et al., 2008), RHF1a, and RHF1b (Liu et al., 2008), RBX1 (Gray et al., 2002). RBX1 is a RING E3 ubiquitin-protein ligase that interacts with Cullins proteins, and RBX is an evolutionarily conserved protein that contains a RING-H2 finger like motif (Kamura et al., 1999). In *Arabidopsis*, there are two *RBX1* like genes, and protein RBX1a is a subunit of SCF complexes, which function is to bind the ubiquitin-conjugating enzyme E2 and bring it into close proximity with the E3 substrate, the mutation in the *AtRBX1a* gene will results in *Arabidopsis* insensitive to JA suggested that *AtRBX1a* is a component of JA pathway (Gray et al., 2002). Moreover, the levels of *RBX1a* expressions will change plant morphology and defects in Auxin response (Gray et al., 2002). In additional, protein RBX1, which can interact with Cullin3 Proteins and BTB Proteins to form multi-subunit E3 ubiquitin ligase, also can affect plant embryo development (Figueroa et al., 2005). And also, RBX1 can interact with CULLIN4 proteins and the CDD proteins to form an E3 Ubiquitin Ligase that mediated light control of development (Chen et al., 2006). Jasmonic acid (JA) and Ethylene (ET), both are very important phytohormone that widely used in the field of rubber tree to enhance the yield of the latex (Hao et al., 2004; Duan et al., 2010). However, the mechanisms maybe different, JA maybe participate in the process of latex biosynthesis through promotion the laticifer differentiation, and ET maybe have the role on prolong the time of latex produce

Table 1. Some key genes with high expression level in latex compare to leaves in the results of cDNA microarray.

Gene description	Gene name	Highest similar sequence from NCBI with A*rabidopsis*	Average Ratio of expression value of latex/leaves
Heavy-metal-associated domain-containing protein	HIPP21	At5g17450	59.3479
Homeobox protein knotted-1 like 1	KNAT1	At4g08150	43.5574
Glycosyl transferase family 2 protein	β-glucosyltransferase	At2g39630	20.6513
Trehalose-6-phosphate phosphatase	TPPA	At5g51460	18.6221
Glycosyl transferase family 8 protein	GAUT2	At2g46480	14.8656
Zinc finger (C3HC4-type RING finger) family protein	LOG2	At3g09770	14.4757
zinc finger (C3HC4-type RING finger) family protein	LUL4	At3g06140	12.3748
Thioredoxin h-type 1	TRX-H-1	At3g51030	11.3756
Sterol 24-c-methyltransferase	SMT1	At5g13710	11.1237
Trehalose-6-phosphate phosphatase	TPPG	At4g22590	7.5119
Protein transport protein sec61 gamma subunit	secE/sec61-gamma protein	At4g24920	7.1513
Superoxide dismutase	CSD3	At5g18100	6.8938
Dolichol phosphate-mannose biosynthesis regulatory protein	DPMS2	At1g74340	5.6487
Hypoxia-responsive family protein / zinc finger (C3HC4-type RING finger) family protein	C3HC4-type RING finger	At3g48030	5.4395
Elongation factor Tu	EF1A	At1g35550	4.0568
Mitochondrial import inner membrane translocase (family zinc finger)	TIM8	At5g50810	3.0574

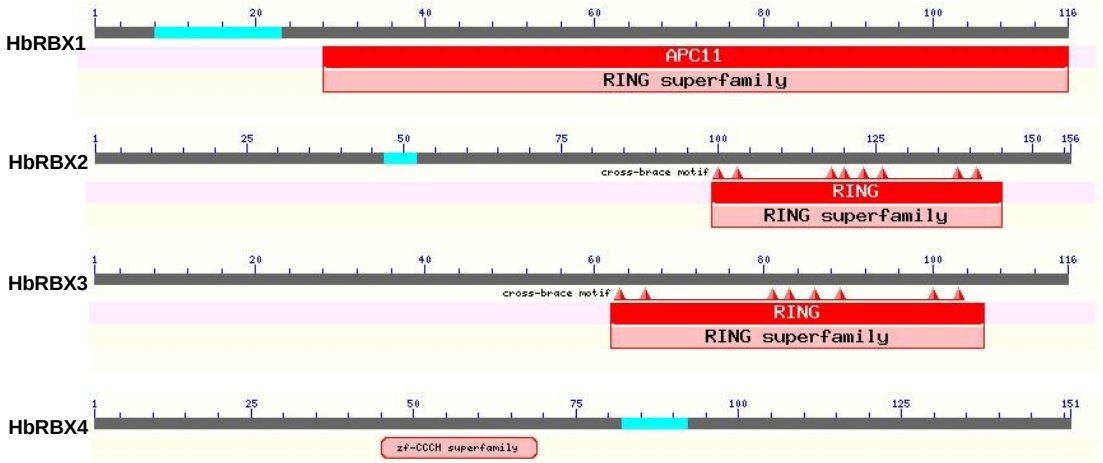


Fig 1. The conserved domain analysis of the four zinc finger proteins in rubber tree
Analysis of putative zinc finger proteins sequence using the Conserved Domain Database (CDD) shows that HbRBX1 contains a Component of SCF ubiquitin ligase and anaphase-promoting complex domain comprising amino acid residus 28–116, HbRBX2 contains a RING-finger (Really Interesting New Gene) domain comprising amino acid residus 99–145, HbRBX3 contains a RING-finger (Really Interesting New Gene) domain comprising amino acid residus 62–106, HbRBX4 contains a zf-CCCH super family domain comprising amino acid residus 45–69.

(Hao et al., 2000; Hao et al., 2004). Here, we reported the differential gene expression between leaf and latex in rubber tree by cDNA microarray, the differential expression genes mainly participated in transcription factor activity, zinc ion binding, transferase activity, ubiquitin-protein ligase activity, cysteine-type endopeptidase activity, elongation factor activity, and protein transporter activity. ARING finger E3 ubiquitin-protein ligase gene, we named *HbRBX1*, has higher expression levels in latex while compared to rubber tree leaves, and this gene maybe involve in transcription factor activity, zinc ion binding, ubiquitin-protein ligase activity indicted by Gene Ontology based biological pathways analysis. The expression patterns analysis of *HbRBX1* under the phytohormone jasmonic acid (JA) and ethylene (ET) treatment indicted that the gene can respond to the JA, but a little role on responding to the ET, indicting this gene maybe participating in the pathway of JA. This study maybe contributes towards an understanding of the relation of RING finger E3 ubiquitin-protein ligase gene and JA pathway.

Results

Microarray for transcriptome analyses of latex and leaves of rubber tree

In order to understand the molecular events happened in rubber tree latex, we studied the differential gene expression between latex and leaves of rubber tree using the cDNA microarray. The

Table 2. The differential gene expression between rubber tree latex and leaves participating in GO-Biological Process analysis

GO-Biological Process	Total	P value	Q value
GO:0004197, cysteine-type endopeptidase activity	10	5.9E-5	1.17E-4
GO:0003746 translation elongation factor activity	9	0.001958	0.002697
GO:0004842 ubiquitin-protein ligase activity	30	0.007768	0.009362
GO:0008270 zinc ion binding	56	0.055195	0.048553
GO:0008565 protein transporter activity	6	0.07322	0.062223
GO:0016758 transferase activity	33	0.282078	0.194751
GO:0003700 transcription factor activity	156	0.341621	0.225463

Note: Total means the differential expression gene number in the pathway, P-value - the significance p-value of the gene enrichment of the considered GO category or annotation cluster, calculated with a unilateral Fisher exact test, Q-value - the estimated value of the false discovery rate (FDR) by using the Benjamini & Hochberg (2001) method.

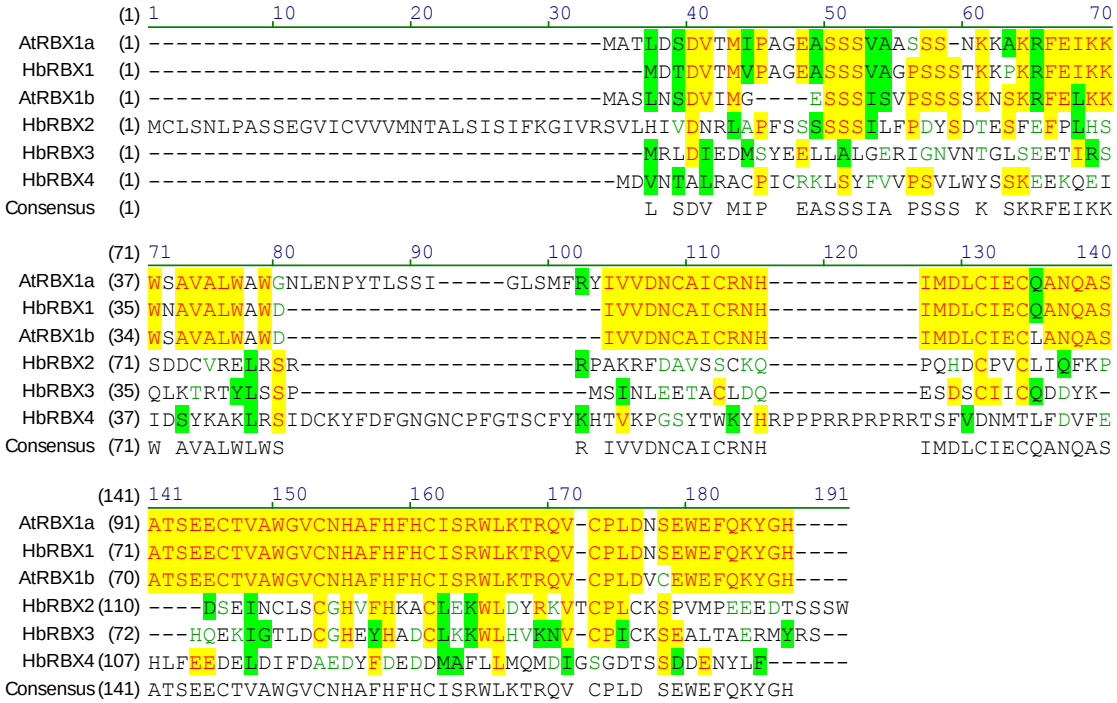


Fig 2a. RBX proteins in rubber tree and *Arabidopsis*. Alignment of RBX proteins in rubber tree (Hb) with *Arabidopsis* (At) orthologs, *Arabidopsis* RBX1a is encoded by At5g20570 and RBX1b is encoded by At3g42830. rubber tree RBX1, RBX2, RBX3, RBX4 are encoded respectively by EC606590, AY221983, EC607538, EC605705. Identical residues in all six proteins are indicated with red font.

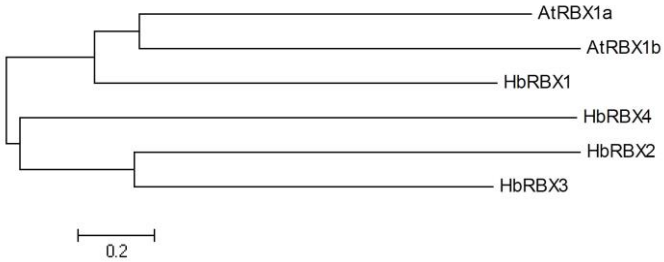


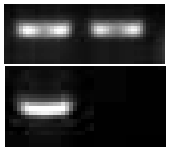
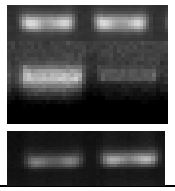
Fig 2b. The Phylogenetic analysis of RBX protein in rubber tree and *Arabidopsis.*Neighbor-Joining (NJ) method was used to analyze phylogenetic tree. HbRBX1 is more similar with the proteins AtRBX1a and AtRBX1b.

significant analysis of microarray (SAM) method was used, and genes that were differentially expressed in the latex and leaves of rubber tree were selected and subjected to various analyses of KEGG metabolic pathway, clustering, and Gene Ontology based biological pathways.

Metabolic pathway analyses showed differential gene expression between latex and leaves of rubber tree

The results indicated that there are 305 genes significant up regulation in the rubber tree latex, and 44 genes have very significant up regulation in the rubber tree latex（P≤0.01）, such as elongation factor gene, thioredoxin H-type 1 gene, four zinc finger family protein genes and so on (Table 1). We analyzed these differentially expression genes by the gene ontology analysis, the results indicted that these differentially expression genes mainly participated in transcription factor activity (156 genes), zinc ion binding (56 genes), transferase activity (33 genes), ubiquitin-protein ligase activity (30 genes), cysteine-type endopeptidase activity (10 genes), translation

Table 3. Confirmation the results of cDNA microarray by SQ-PCR

Gene name	Gene description	Ratio of the cDNA Microarray results	SQ-PCR Confirmation latex Leaves	Target gene
HbRBX1	Zinc finger (C3HC4-type RING finger) family protein, RBX1-like protein	14.4757		*HbRBX1* *HbACT*
HbRBX2	Zinc finger (C3HC4-type) family protein, RING zinc finger protein	12.3748		*HbRBX2* *HbACT*
HbRBX3	RING/U-box domain containing protein,	5.6487		*HbRBX3* *HbACT*
HbRBX4	Zinc finger (C3HC4-type RING finger) family protein, E3 ubiquitin-protein ligase makorin	3.0574		*HbRBX4* *HbACT*

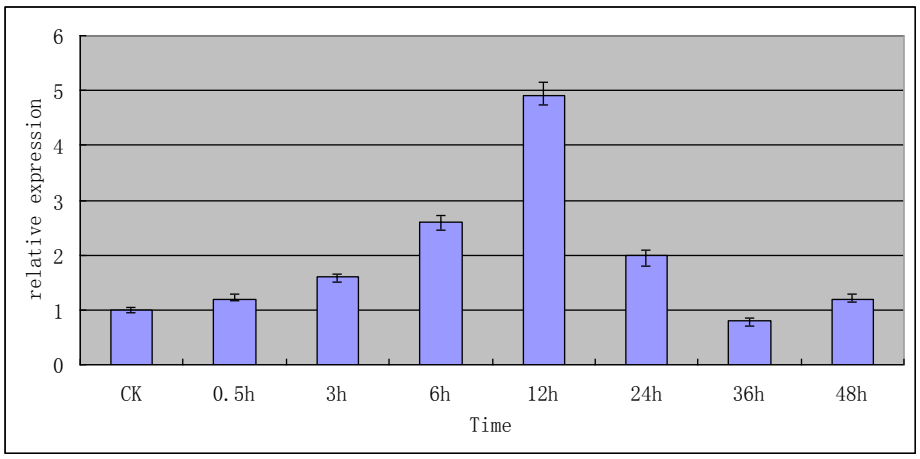


Fig 3. The expression pattern of *HbRBXI* under the Jasmonic acid treatment.

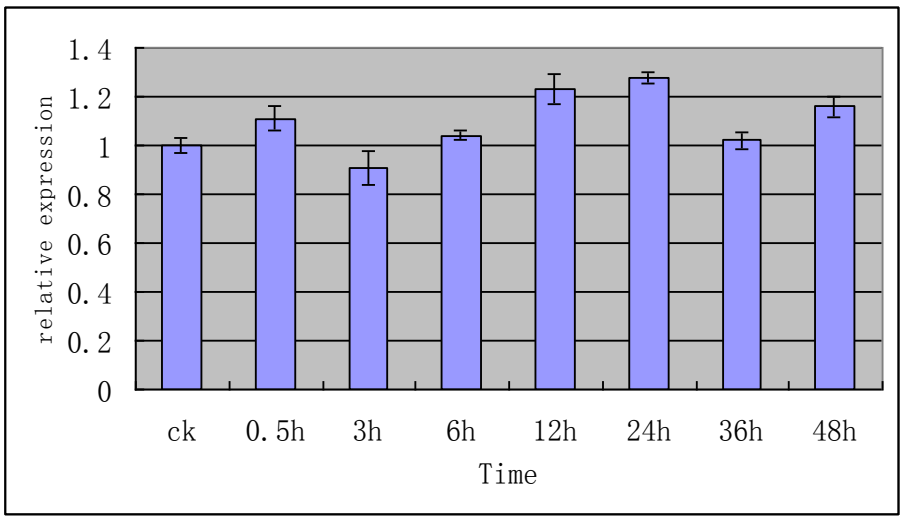


Fig 4. The expression pattern of *HbRBXI* under the ethylene treatment.

elongation factor activity (9 genes), protein transporter activity (6 genes) (Table 2). S pecially, many differentially expression genes mainly distribute in transcription factor activity, zinc ion binding, transferase activity, ubiquitin-protein ligase activity.

Zinc finger proteins have higher expression level in rubber tree latex, compared to rubber tree leaves

We are very interested in the zinc finger protein expressed in the rubber tree latex, because many zinc finger proteins have the activity of zinc ion binding, ubiquitin-protein ligase activity and transcription factor activity. There are four zinc finger proteins highly expressed in latex according to the results of cDNA microarray, we named as *HbRBX1*, *HbRBX2*, *HbRBX3* and *HbRBX4*, the gene expression ratios in latex while compared to rubber tree leaves of the four zinc finger proteins respectively are 14.4757, 12.3748, 5.4395 and 3.0574. And we used the semi-quantitative RT PCR to confirm the four zinc finger proteins expression patterns of the cDNA microarray, the results of the semi-quantitative RT PCR confirmed the correct expression patterns of the cDNA microarray (Table 3). And we also cloned the full-length of the four zinc finger genes. The conserved domain analysis indicts that the four zinc finger proteins all belong to the RING finger family proteins (Fig. 1). *HbRBX1* has the highest expressed ratio according to the results of cDNA microarray and semi-quantitative RT PCR, we cloned the fragment and discovered that the fragment has high sequence similarity to RING finger E3 ligase *RBXIa* in *Arabidopsis*, indicted that *HbRBX1* is a putative orthologue of *AtRBXIa*. From the results of the sequence analysis, the sequence of *HbRBX1* has 93.3% nucleotide sequence identity to *AtRBXIa*, and has 82.8% nucleotide sequence identity to *AtRBXIb* (Fig. 2).

Real-time PCR results suggested HbRBX1 can respond to exogenous JA

In order to understand the roles on the *HbRBX1* in rubber tress latex biosynthesis, we examined the *HbRBX1* gene expression patterns under the different phytohormone treatments. Two types of phytohormone, ET and JA, are chosen for *HbRBX1* gene expression patterns analysis. Total RNA samples isolated from different time after different types of phytohormone treatments were used for analysis, eight samples were selected for analyzing *HbRBX1* gene expression pattern in latex biosynthesis process, including 0h, 3h, 6h, 12h, 24h, 30h, 36h, 48h latex sample after phytohormone treatment. The results of quantitive PCR showed that the *HbRBX1* gene expressed in all selected latex samples (Fig. 3, 4). In JA treatment, the higher levels of *HbRBXI* mRNA were detected in 6h and 12h latex sample after JA treatment, and the highest levels of *HbRBXI* mRNA were detected in 12h latex sample (Fig. 3). This result is consistent to the 24h tapping latex system after the phytohormone treatment in rubber tree, the results suggested important roles on *HbRBXI* in latex biosynthesis. In ethylene treatment, each sample has almost the same expression level, the relative expression value is between 0.91 to 1.28, the higher levels of *HbRBXI* mRNA were detected in 12h and 24h latex sample, the highest levels of *HbRBXI* mRNA were detected in 24h latex sample, the relative expression is 1.28 fold compared with the control, which indicted that ethylene has a little regulation role on *HbRBXI* (Fig. 4).

Discussion

Zinc finger protein gene maybe participate in rubber tree latex biosynthesis

The results of the cDNA microarray indicted that the genes up-regulated in latex including rubber elongation factor, zinc finger protein gene, and the two types of gene expression patterns in our results are consistent with the previous research of Oh et al. (Oh et al., 1999; Ko et al., 2003). And according to the results of the Ko et al., the zinc finger protein genes related EST have high abundance expressions in the latex expression library that they had constructed (Ko et al., 2003). In rubber tree, the production of rubber latex maybe the result of responding to stress, and there is a high abundance of zinc finger protein expressed in latex that maybe relate to the processes of the latex producing, discharge latex, and lengthen the time of the latex production. In our research, we discovered that four zinc finger protein gene have higher expression levels in latex compared to leaves. In addition, there are many genes are up-regulated in latex, such as sterol 24-C-methyltransferase，thioredoxin H-type 1，glycosyl transferase family 8 protein，glycosyl transferase family 2 protein and so on (Table 1). All these results have important role on revealing the mechanism of the rubber tree latex biosynthesis.

RING E3 ubiquitin-protein ligase maybe participating in rubber tree latex biosynthesis process through phytohormone signaling pathway

RING E3 ubiquitin-protein ligase complex mediated protein degradation pathway has been proved to participate in phytohormone transduction pathway, for example, JA, auxin, GA, and ET signaling pathways, and the common core components of those phytohormone signaling transduction pathway are RING E3 ubiquitin-protein ligase complexs (Chini et al., 2009)，which indicted the important role of RING E3 ubiquitin-protein ligase on phytohormone signaling transduction pathway. In *Arabidopsis*, the mutation of RING E3 ubiquitin-protein ligase complexs component *AtRBX1* resulted in the plants insensitive to exogenous JA treatment (Xu et al., 2002; Chini et al., 2009)，indicting that *RBX1* has important regulation on JA signaling transduction pathway. JA and ET, both are very important phytohormone that widely used in the field of rubber tree to enhance the yield of the latex. However, the mechanisms maybe different, JA maybe participate in the process of latex biosynthesis, and ET maybe have the role on prolong the time of latex produce (Hao et al., 2004). As our results indicted that *HbRBXI* can respond to JA while has a little role on responding to ET, we can give a hypothesis on that *HbRBXI* maybe participate in the process of latex biosynthesis through the pathway of JA. In addition, Ko et al. (Ko et al., 2003) analyzed 20,000 transcription fragment based on cDNA-AFLP, the results indicted that rubber tree REF, SRPP, and zinc finger protein have higher expression levels in the cDNA library of latex. As we all know, REF (Priya et al., 2007) and SRPP (Oh et al., 1999) gene have been proved to have very important function on latex biosynthesis, however, the function of zinc finger gene on latex biosynthesis has to be determined. In *Arabidopsis*, many zinc finger proteins function as E3 ligase, and in *Arabidopsis* genome, there are about 475 zinc finger protein gene function as E3 ligase.

So many zinc finger protein genes that have high expression levels in latex cDNA library maybe mean zinc finger protein gene is the key factor that participated in the latex biosynthesis in rubber tree.

Materials and methods

Plant materials

Hevea brasiliensis 7-33-97 trees were planted at the Experimental Farm of the Chinise Academy of Tropical Agriculture, five-year-old virgin trees were selected to collect samples. For genomic DNA and RNA isolation, latex and leaves samples were collected and immediately frozen in liquid nitrogen, and the frozen leaf powder was stored at -70°C or was used immediately. For rubber tree latex RNA extraction, rubber trees were tapped using a taping knife, the first a few drops of latex, which contained mostly debris form the plant, were discarded, the latex was allowed to drop directly into liquid nitrogen in an ice kettle, the frozen latex powder was stored at -70°C or was used immediately.

cDNA microarray

The *Arabidopsis* Genome Oligo Set Version 3.0 (Operon) array comprising 29110 of 70mer Oligo DNA probes were used (Żmieńko et al., 2011). Each chip represents 26173 protein encoding genes and 28964 transcripts. Details about the oligo library can be found in: http://www.operon.com/products/ microarrays/oparrays_down load .aspx.

Labeling, hybridization and scanning of the microarray slides

Total RNA (5μg) was converted into cDNA by reverse transcription using T7-Oligo (dT) primer. cRNA was prepared by in vivo transcription from double stranded cDNA using the T7 RiboMAX Express Large Scale RNA Production System (Promega). Labeled cDNA molecules were generated by reverse transcription of cRNA (2ug) by CbcScriptⅡenzyme with random primer and by subsequent Klenow Fragment Polymerase labeling. The transgenic plant samples were labeled with Cy5, and the wild type with Cy3. The labeled cDNA was dissolved in 35 uL hybridization buffer consisting of 3 × SSC, 0.12% SDS, 5×Denhart and 25% Formamide. Microarray hybridization was conducted overnight at 42°C. Upon completion of hybridization, the slides were washed at 42°C in a buffer of 0.12% SDS, 2 × SSC solutions for 5 min, followed by another 5 min wash in 0.12×SSC at room temperature. The slides were then dried by spinning in a centrifuge before immediately proceeding to scanning.

Rubber tree genomic DNA and RNA isolation

Genomic DNA was isolated from rubber tree leaves by the method described by Dellaporta et al. Total RNA was extracted according to the Tang method (Tang et al., 2007). The quality and concentration of the extracted DNA and RNA were checked by agarose gel electrophoresis and measured with a spectrophotometer (DU-70, Beckman, Fullerton, CA).

Cloning zinc finger protein Ortholog from Rubber tree

Four rubber tree nucleotides (GenBank accession number EC606590, AY221983, EC607538, EC605705) with high sequence homology to GenBank accession number NM_122064.2, AF339689.1, NM_104198.4, NM_202526.1 of Arabidopsis thaliana were identified in the NCBI GenBank database with the NCBI BLAST program. Gene-specific primers (AY221983 forward: 5'- TCTTCCCTTCTACTTCC-ACA-3', AY221983 reverse: 5'- CCAAGAGCTAGATGTA-TCCTCT-3', EC606590 forward: 5'- CTGTCTCTGTGACCT-TACCG-3', EC606590 reverse: 5'- TAGGGTCGGAAGA-CTGCTG-3', EC607538 forward: 5'- AACTGATGGGGTAG-CTGTACT -3', EC607538 reverse: 5'- GCAATGCACCATT-TAACC -3', EC605705 forward: 5'- GAGGCATTAAAA-CGTAGTCAAG-3', EC605705reverse: 5'- CGCCTGATCCA-ATATCCA-3') were synthesized according to the four nucleotide sequence. Four nucleotides were amplified from rubber tree latex cDNA library with PCR. The amplified fragments were sequenced. The putative Four nucleotides coding sequence were also amplified and cloned from rubber tree genomic DNA and completely sequenced.

Semi-Quantitative RT-PCR

For Semi-quantitative RT-PCR analysis, total RNA was extracted from 5 years old rubber tree leaves and latex with Tang method. Total RNAs were treated with RNase-free DNase I (TaKaRa Biotechnology Co., Ltd., Dalian, CHN) to remove residual DNA. The first strand cDNAs for each sample were generated using cDNA Synthesis Kit (TaKaRa Biotechnology Co., Ltd., Dalian, CHN). The RT product was used in semi-quantitative PCR reactions with primers for the gene-specific primers (AY221983 forward: 5'-TCTTCCCTTCTACTTCCACA-3', AY221983 reverse: 5'-CCAAGAGCTAGATGTATCCTCT-3', EC606590 forward: 5'- CTGTCTCTGTGACCTTACCG-3', EC606590 reverse: 5'-TAGGGTCGGAAGACTGCTG-3', EC607538 forward: 5'-AACTGATGGGGTAGCTGTACT -3', EC607538 reverse: 5'-GCAATGCACCATTTAACC -3' , EC605705 forward: 5'-GAGGCATTAAAACGTAGTCAAG-3', EC605705reverse: 5'- CGCCTGATCCAATATCCA-3') were synthesized according to the four nucleotide sequence. The semi-quantitative PCR conditions were 10 min pre-denaturation at 95°C, 23 cycles of 95°C (10 sec), 52°C (15 sec) and 72°C (30 sec). A rubber tree 18S rRNA gene was used as a control in the semi-quantitative PCR with specific primers (5-AAGACGAACAACTGCGAAAGC -3 and 5- ATGTTGCTT-TTAGGACTCCGC -3).

Four zinc finger proteins expression pattern analysis in Latex at different type phytohormone treatment detected by Real-time Quantitative PCR

For detecting the expression levels of four zinc finger proteins expression pattern analysis in Latex at different type phytohormone treatment, an rubber tree18S rRNA was selected as an endogenous control. The standard curves were constructed using the purified PCR products of the five genes generated for each specific primer pairs. Each PCR reaction also included a reverse transcription negative control (without reverse transcriptase) to confirm the absence of genomic DNA, a non template negative control to check for primer-dimer and a Rubber tree genomic DNA control to verify no specific amplification with the primers. QPCR was performed with a Stratagene (La Jolla, CA) Brilliant SYBR Green QRT-PCR master mix kit on a Stratagene Mx3000P. Real-time RT-PCR was performed in a 25 μL reaction mixture containing 12.5 μL FastStart Universal Probe Master (ROX) (Roche Ltd.), 1.0 μL forward primer (50 uM), 1.0 μL reverse primer (50 uM),8.0 μL ddH2O, and 2 μL of template cDNA (5 ng μL^{-1}). The PCR conditions were 10 min pre-denaturation at 94°C, 40 cycles of 15 sec at 95°C and 1 min at 60°C. The MxPro^{TM} QPCR Software Version 3.00 (STRATAGENE) was used for data

collection and analysis. Quantification results were expressed in terms of the cycle threshold (CT) value determined according to the manually adjusted baseline. Relative gene expressions in samples were determined using a method previously described (Zhang et al., 2007). Briefly, differences between the CT values of target genes and control genes were calculated as $\Delta CT = CT^{target} - CT^{control}$, and expression levels of target genes relative to control genes were determined as $2^{-\Delta CT}$. PCR was repeated three times, and the average values of $2^{-\Delta CT}$ were used to determine difference in expression between samples and control (Zhang et al., 2007).

Phytohormone treatment

Hevea brasiliensis 7-33-97 trees were planted at the Experimental Farm of the Chinise Academy of Tropical Agriculture, five-year-old virgin trees were selected to collect samples. Phytohormone treatment prior to their first tapping of rubber tree was treated according to the Hao method (Hao et al., 2004). The concentrations of ethephon and JA were 0.2% and 0.1% individually. The control entreated with the water. Latex was collected on 0.5, 3, 6, 12, 24, 36 and 48 h after phytohormone treatment. Three times repeated for each phytohormone treatment were carried out, each time point sample were collected the latex from five different rubber trees.

Statistical analysis

The hybridized microarray chips were scanned on a LuxScan 10K, a double channel scanner (CapitalBio). The SpotData image analysis software (CapitalBio) was used to analyze the images, and convert them into numerical data. Lowess (Workman *et al.* 2002) normalization method was used to normalize the data. Differential gene expression was identified by significant analysis of microarray (SAM) method (Tusher *et al.* 2001). And genes that were differentially expressed in the latex and leaves of rubber tree were selected and subjected to various analyses of KEGG metabolic pathway, clustering, and gene ontology based biological pathways.

Conclusion

This study mainly focuses on the differential gene expression between latex and leaves of rubber tree. Genes that were differentially expressed in latex and leaves of rubber tree were selected and Gene Ontology based biological pathways also analyzed. Many differentially expression genes mainly distribute in transcription factor activity, zinc ion binding, transferase activity, ubiquitin-protein ligase activity. One of the zinc finger protein encoded genes, named *HbRBX1*, which involved in transcription factor activity, zinc ion binding, ubiquitin-protein ligase activity indicted by gene ontology analysis, was cloned and further research for expression patterns in rubber tree latex and rubber tree leaves by Semi-quantitative RT PCR. And Jasmonic acid and ethylene treatments demonstrated that *HbRBX1* can respond to exogenous jasmonic acid while can not respond to exogenous ethylene, suggested that *HbRBX1* maybe participate in the latex biosynthesis through the jasmonic acid pathway.

Acknowledgments

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