Transcript Profiling Provides Insights into the Molecular Mechanisms of Harvesting-activated Latex Regeneration in Virgin Rubber Trees

Yujie Fan
Hainan University
Xiaohu Xiao
Chinese Academy of Tropical Agricultural Sciences
Jianghua Yang
Chinese Academy of Tropical Agricultural Sciences
Jiyan Qi
Hainan University
Yi Zhang
Hainan University
Shengmin Zhang
Hainan University
Jun Tao
Hainan University
Chaorong Tang (chaorongtang@126.com)
Hainan University

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Abstract

Background: Natural rubber, an important industrial raw material with wide applications, is harvested in the form of latex (cytoplasm of rubber-producing laticifers) from *Hevea brasiliensis* (para rubber tree) by the way of tapping, i.e. removing a slice of trunk bark by a special knife. In regularly tapped rubber trees, latex regeneration consists of one of the main yield-limiting factors for rubber productivity. Conspicuous stimulation on latex production for the first few tappings makes virgin (untapped before) rubber trees an ideal model to investigate the regulatory mechanisms of latex regeneration. To understand the underlying mechanisms, genome-wide transcript profiling was conducted with a silver-staining cDNA-AFLP technology against the latex samples for the first five tappings.

Results: A total of 505 non-redundant differentially expressed (DE) transcript-derived fragments (TDFs) were identified, of which 217 were up-regulated, 180 down-regulated, and 108 bell type-regulated among the five tappings. About 72.5% of these DE-TDFs were functionally annotated, and classified into 11 functional categories, which were discussed with reference to harvesting-stimulated latex regeneration. The importance of sugar metabolism and rubber biosynthesis was highlighted, due to the fact that most of the DE-TDFs annotated in sucrose transport, sugar catabolism, glycolysis, tricarboxylic acid cycle and pentose-phosphate pathway and nine of the ten rubber biosynthesis pathway DE-TDFs were up-regulated by the tapping treatment. More than one tenth of the total DE-TDFs were randomly selected for expression validation by semi-quantitative RT-PCR, and 83.8% showed patterns consistent with their original cDNA-AFLP gel profiles. Moreover, quantitative RT-PCR analysis revealed an 89.7% consistency for the 29 latex-regeneration related DE-TDFs examined.

Conclusions: In brief, our results indicate the tapping treatment incurs extensive physiological and molecular changes in the laticifers of virgin rubber trees. The vast numbers of tapping-responsive DE-TDFs identified here provide a basis for unravelling the gene regulatory network for latex regeneration in regularly harvested rubber trees.

Introduction

Natural rubber (*cis*-1, 4-polyisoprene, NR) is an elastomer with superior properties that cannot be completely replaced by petroleum-derived synthetic rubber, and is used as an important industrial raw material related to national economy and people's livelihood. There are more than 2, 500 kinds of NR-bearing plants. However, due to the advantages of good quality, high yield, and easiness for harvesting, *Hevea brasiliensis* (para rubber tree, *Hevea* thereafter) has become the sole commercial NR source [1]. *Hevea* trees need high temperature and humid climate conditions for normal growth and NR production yet vulnerable to typhoon, thus confining its planting to restricted tropical areas [2].

Many factors are affecting *Hevea* rubber yield. From the physiological perspective, there are three main factors: duration of latex flow after tapping, the capability of latex regeneration between two consecutive tappings, and the ability of laticifer differentiation in bark cambium [3, 4]. The number of laticifer rings in

tapped *Hevea* bark is 1 to 3 times more than un-tapped, and tapping promotes laticifer differentiation [4]. Meanwhile, mechanical injury and jasmonic acid stimulation facilitate laticifer differentiation, latex regeneration and production [6, 7]. Latex is the cytoplasm that flows out of the laticifers after tapping, of which dry weight more than 90% is rubber hydrocarbon, *i.e.* NR [8]. In regularly tapped *Hevea* trees, the expelled latex can be regenerated within 2 to 3 days, latex regeneration thus representing the main metabolic activity occurred in the laticifers of trunk bark where harvesting is conducted.

After tapping, the substance flowing out of the laticifers includes rubber hydrocarbon, organelles, sugars, organic acids, nucleic acids and proteins/enzymes [3]. Tupý reported a positive correlation between sucrose content and latex yield [9, 10, 11]. A sucrose transporter, HbSUT3, is responsible for sucrose loading into laticifers, and its expression has been found to be induced by the treatments of ethylene and tapping, both bolstering the latex yield [12]. A kind of alkaline/neutral invertase cleaves sucrose in the latex into glucose and fructose that are then exploited in subsequent latex production [11]. Liu et al. identified the responsible invertase gene, *HbNIN2*, and found its expression and enzymatic activity in the latex are positively correlated with latex yield [13]. NR biosynthesis occurs on rubber particles, a kind of organelle where the proteins of rubber elongation factor (REF)/small rubber particle protein (SRPP) are important components of the rubber biosynthesis machinery [14–17]. Priya et al. observed a positive correlation between REF mRNA abundance and the yielding levels of *Hevea* clones [18]. Amalou et al. revealed that a marked increase in transtonoplast ΔpH within *Hevea* laticifers, which consists of one of the major mechanisms of ethylene stimulation on latex yield [19]. Therefore, latex regeneration, with the biosynthesis of rubber hydrocarbon as a major activity, involves a complex regulatory network of gene expression, multi-enzyme reaction and physio-biochemical processes. Virgin *Hevea* trees produce very little latex at the first tapping, and the latex yield increases significantly with subsequent tappings with regular intervals of 2 to 3 days, and reaches a relatively stable level after 7–10 tappings [12, 20]. Adiwilaga revealed that in the latex the accumulation of farnesyl diphosphate synthase transcripts is induced by tapping [21]. Tang *et al.* concluded that in virgin *Hevea* trees the metabolic activity of laticifers is mediocre, and gets activated with the tapping treatment and maintains a high level of dynamic equilibrium for latex regeneration in regularly tapped trees [12]. Therefore, virgin *Hevea* trees could be an ideal material to study the mechanisms of latex regeneration and to identify the genes involved.

Genome-wide transcript profiling techniques play an important role in large-scale screening and characterization of the genes involved in various biological processes. Since the advent of the cDNA-AFLP transcript-profiling technique in 1996 [22], owing to its advantages of good repeatability, high sensitivity and high throughput, this technique has been successfully applied to the field of plant biology, such as abiotic stress response [23], plant-microorganism interaction [24], hormone signal [25] and development [26].

In this study, the profiles of latex transcriptome in virgin *Hevea* trees were systematically compared for the first five tappings using a silver staining cDNA-AFLP protocol we previously established for *Hevea* latex transcriptome [27]. A total of 505 non-redundant TDFs were identified as differentially expressed (DE) with the five tappings. These DE-TDFs were further verified for their expression patterns by semi-

quantitative RT-PCR (sqRT-PCR) and quantitative RT-PCR (qRT-PCR). The results highlight the importance of sucrose transport and sugar metabolism as well as rubber biosynthesis in tapping-activated latex regeneration in virgin *Hevea* trees.

Results

Screening of DE-TDFs

To extensively identify the differentially expressed TDFs (DE-TDFs) responding to the tapping treatment, all the 128 selective primer combinations of *Apo*Ⅰ/*Mse*Ⅰenzyme restriction system were screened in the latex for the first five tappings in virgin *Hevea* trees (Table 1). On average, about 70 TDFs greater than 100 bp were discernable on the silver-stained polyacrylamide gels for each pair of primer combination (Fig. 1). Therefore, nearly 9, 000 TDFs were expected to be profiled for each latex RNA sample when all the 128 *Apo*Ⅰ/*Mse*Ⅰselective primer pairs are examined. In total, 651 DE-TDFs were identified and successfully cloned and sequenced. Taking the gene expression level at the first tapping as a reference, the DE-TDFs identified were classified into three types according to their patterns of expression along with the five successive tappings: up-regulation, down-regulation and bell-type regulation (Fig. 1). The up-regulation type includes three subtypes: (i) increase successively; (ii) increase first and then stabilize; (iii) increase first and then decrease, but still higher than the first tapping. The down-regulation type also includes three subtypes: (i) decrease successively; (ii) decrease first and then stabilizes; (iii) decrease first and then increase, but still lower than the first tapping. The bell-type regulation includes two subtypes: (i) increase first, reaching a high threshold, and then decrease to a level lower than the first tapping (upward-bell); (ii) decrease first, reaching a low threshold, and then increase to a level higher than the first tapping (downward-bell).

DE-TDFs annotation and redundancy removal

The DE-TDFs were made clean by removing the sequences of vector, primer and adaptor at both ends and then annotated by Blastx online searching (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI non-redundant protein database (nr), with a threshold of E value $<10^{-4}$ and score >50. According to the blasting against the *Hevea* latex transcriptome database [17], the DE-TDFs belonging to the same transcript and sharing similar expression pattern in the cDNA-AFLP analysis were clustered together, and only the longest TDF was retained. As a result, a total of 505 non-redundant DE-TDFs were obtained, including 217 (43.0%) up-regulated (Table S1), 180 (35.6%) down-regulated (Table S2) and 108 (21.4%) bell-regulated (Table S3).

Functional classification of non-redundant DE-TDFs

According to the results of Blastx searching, the 505 non-redundant DE-TDFs were divided into four categories: (i) Proteins with clear functional annotation; (ii) Unclassified proteins, the functional

annotation of the proteins being multiple; (iii) Predicted protein, showing high homology with a predicted protein in the database; (iv) No hit, no homologous sequence in the database. Most (366, 72.5%) of these DE-TDFs had homology to genes with known functions, whereas 30 (5.9%) and 59 (11.7%), respectively, belonged to unclassified proteins and predicted proteins and the remaining 50 (9.9%) were with no hit (Table 2).

With reference to the functional categories of plant genes defined by Bevan et al. [28], the 366 DE-TDFs with known functions were classified into 11 functional categories, of which a new category, rubber biosynthesis, had been singled out from “secondary metabolism” (Fig. 2 and Table 2). Of these categories, five, *i.e.* cell growth and division, protein degradation and storage, cellular structure, secondary metabolism, and rubber biosynthesis had a higher portion of up-regulated DE-TDFs than that of the down- and bell-regulated DE-TDFs together (Table 2). Strikingly, nine of the ten DE-TDFs implicated in rubber biosynthesis were up-regulated, and the remaining one is upward bell-regulated.

Validation of expression pattern by sqRT-PCR

To determine the reliability of the cDNA-AFLP results, 80 DE-TDFs, covering >10% of the DE-TDFs we identified and sequenced, were randomly selected from each functional category and subjected to sqRT-PCR analysis using specific primers for TDFs with *18S rRNA* as the reference gene (Table 3). About 84% (67 TDFs) showed the expression pattern consistent with their cDNA-AFLP gel profiles, indicating the high reliability of the cDNA-AFLP screening.

qRT-PCR analysis of latex regeneration-related DE-TDFs

According to the metabolism pathways reported to be involved in latex regeneration, 29 latex-regeneration related DE-TDFs were further investigated by qRT-PCR analysis for their expression patterns across the five successive tappings (Table S4). About 90% of the qRT-PCR results were consistent with their original cDNA-AFLP expression profiles (Fig. 3; Table 4). The genes of these DE-TDFs are putatively involved in the pathways of primary metabolism, rubber biosynthesis and regulation, transporters and intracellular transport. Of the ten rubber biosynthesis pathway DE-TDFs, nine revealed qRT-PCR patterns similar to their cDNA-AFLP results (Table 4 and Fig. 3).

Discussion

Functional categories with reference to tapping-stimulated latex regeneration

Tapping can stimulate the regeneration of latex, especially in virgin *Hevea* trees [12, 20]. A number of early studies have shown that the first few tappings greatly stimulate the metabolism of laticifers, accompanied by the enhanced expression of several specific genes involved in latex regeneration [18, 21,

29-30]. The latex flows out of laticifers after tapping, and in order to compensate for the loss of cytoplasm (latex) and maintain the balance of intracellular metabolism, the laticifers require large amounts of RNA and proteins to be synthesized before the next tapping. Of the 366 DE-TDFs identified with known function (Table 2), 26.2% were classified into the functional category of transcription and protein synthesis (Fig. 2), representing the largest category, 42.7% of which were up-regulated by the tapping treatment. These results indicated that tapping significantly affects the ways of laticifers to synthesize RNA and proteins, thus laying a foundation for their subsequent physiological response to the tapping treatment [3]. Laticifers are believed to be a defense system for *Hevea* trees to cope with biological and abiotic stresses, and the latex exuded after bark wounding has been found to play roles in resisting pathogen infection, insect feeding and abiotic stress [31]. The tapping itself is a kind of abiotic stress upon *Hevea* trees. Therefore, it is reasonable that “stress and defense” also accounted for a large portion of the functional DE-TDFs identified responsive to tapping (Table 2; Fig. 2), ranking the second place in functional categories. The harvesting stress response has been suggested to be one of the key factors affecting latex regeneration and rubber productivity in *Hevea* trees [32].

The category of transporters and intracellular was the third largest among the 11 functional categories, accounting for 12.3% of the total functional DE-TDFs (Fig. 2). This corresponds well to the sink effect caused by the large loss of latex after tapping. The process of regenerating the expelled latex involves the synthesis, transport, loading and subcellular localization of a large number of organelles, proteins, nucleic acids, sugars, etc., all of which require the active involvement of transporters and intracellular transport-related proteins [3, 33]. DE-TDFs involved in signal transduction were also highly represented, accounting for a proportion of 11.5% for the total DE-TDFs (Fig. 2). A variety of signaling pathways within *Hevea* laticifers, including ethylene, jasmonic acid and wound signaling, have been reported to be extensively participate in latex regeneration and regulation [4, 17, 29, 32, 34-35]. The proportions for the two categories, protein degradation and storage and primary metabolism were also high, covering, respectively, 9.3% and 8.2% of the total functional DE-TDFs. Their high representation suggested that with the progress of tapping, in order to meet the balance of supply and demand of all substances in latex regeneration, protein turnover rate becomes faster and primary metabolism gets active. In a word, these results indicated that the latex regeneration regulated by tapping involves a complex multi-gene regulatory network, as well as a physiological and biochemical response process.

Sugar metabolism and rubber biosynthesis in tapping-stimulated latex production

In regularly tapped *Hevea* trees, the main metabolic activity of the laticifers is latex regeneration, which centers on the biosynthesis of rubber hydrocarbon that accounts for about 90% of the dry weight of fresh latex [3]. Sucrose has been identified as the precursor material for rubber biosynthesis in laticifers, providing the carbon skeleton and energy required for latex regeneration [11, 36]. In *Hevea* trees normally tapped at intervals of 2-4 days, each tree produces dozens to hundreds of milliliters of fresh latex, and the removed latex could be effectively recovered before the next tapping to ensure the sustained productivity

of the tree [3, 12]. Therefore, the laticifers are an active carbon sink, and the effective supply of sucrose is a key factor to determine the yield of latex [10, 37]. In this study, the genes of a sucrose transporter and a sugar transporter were among the DE-TDFs identified, both of which were significantly up-regulated with the increase of tappings (Table S1). Interestingly, the former is just the sucrose transporter HbSUT3 we previously identified to be critical in sucrose uptake into laticifers and rubber production in exploited *Hevea* trees [12]. The up-regulation of these two transporters indicated an active involvement of sucrose and sugar transport in tapping-stimulated latex regeneration. Sucrose catabolism and the following pathways of glycolysis, tricarboxylic acid cycle and pentose phosphate provide essential components, *i.e.* the carbon skeleton (acetyl CoA), the reducing power (NADPH) and the energy (ATP) for the final rubber biosynthesis pathway [3, 11]. Therefore, sugar metabolism becomes one of the core metabolic pathways contributing to latex regeneration and rubber biosynthesis in *Hevea* [3, 11, 38, 40-41]. This study identified multiple DE-TDFs involved in sucrose cleavage and the three above mentioned sugar metabolism pathways (Table 4; Tables S1 to 3), including those encoding neutral/alkaline invertase, fructokinase, phosphofructokinase, glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase, pyruvate dehydrogenase, and glucose-6-phosphate dehydrogenase, etc. Most of them were up-regulated in the latex for the first few tappings (Table S1; Fig. 3). It is worth noting that the up-regulated DE-TDF (M16-A7-1) as identified by both cDNA-AFLP (Table S1) and qRT-PCR (Fig. 3) turned out to be *HbNIN2*, the neutral/alkaline invertase that is responsible for sucrose catabolism in *Hevea* laticifers [13].

There are 20 gene families directly involved in the rubber hydrocarbon biosynthesis and termed as rubber biosynthesis genes [17, 31]. The DE-TDFs identified in this study involved six of these families, including cis-prenyltransferase, hydroxymethylglutaryl coenzyme A synthase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, farnesyl diphosphate synthase, rubber elongation factor and small rubber particle protein (Table 4). Among the nine DE-TDFs, eight were demonstrated by qRT-PCR to be up-regulated with the tappings (Fig. 3). In addition, a DE-TDF (M8-A5-6) annotated as inorganic pyrophosphatase was also bolstered by the tapping treatment (Fig. 3). A vacuolar type of inorganic pyrophosphatase has been found to locate on rubber particles and essential for the incorporation of IPP monomers into elongating rubber molecules [40].

Strength and weakness of the cDNA -AFLP technique

The cDNA-AFLP technique has been widely applied in various eukaryotes including the *Hevea* tree for transcript profiling due to its advantages of stringency, reproducibility, cost-effectiveness, genome-wide coverage and the ability to distinguish among highly homologous genes [24, 39, 41-43]. In this study, as determined by sqRT-PCR and qRT-PCR, about 84% and 90%, respectively, of the selected DE-TDFs were verified for their cDNA-AFLP profiles (Tables 3 & 4; Fig. 3), reflecting a high reliability of this technique in screening tapping-responsive DE-TDFs in *Hevea* latex. According to a previous in-silico estimation [27], about 84% of the genes expressed in *Hevea* latex could be visualized using the silver-staining cDNA-AFLP technique with the restriction enzyme pair of *Apo* I and *Mse* I exploited here. The sucrose transporter HbSUT3 [12] and the neutral/alkaline invertase HbNIN2 [13] that have been reported to be up-regulated in

the latex of virgin *Hevea* trees by the tapping treatment were among the DE-TDFs identified in this study (Table 4; Fig. 3; Table S1), demonstrating a high transcript coverage of this technique. However, compared with the currently popularly used next generation RNA-sequencing technique that relies on expensive DNA sequencers and specialized bioinformatics [44], the cDNA-AFLP is labor-intensive. Nevertheless, the cDNA-AFLP technique still have its niche among the various transcript profiling techniques, and can be readily established in a mediocrely equipped and stringently funded lab to fulfill its customized transcript profiling task.

Conclusions

A genome-wide cDNA-AFLP transcript profiling identified a total of 505 tapping-responsive DE-TDFs in the rubber-producing laticifers of virgin *Hevea* trees. According to the 366 DE-TDFs with definite functional annotation, the tapping treatment brought about extensive physiological and molecular changes in laticifers. The integration of these changes upgraded the mediocre level of laticifer metabolism in virgin trees to a high dynamic equilibrium of latex regeneration in regularly tapped trees. Further combined studies of transcriptomics, proteomics and metabolomics will benefit a deeper insight into the exact relationships (synergy or antagonism) among the vast number of biological pathways implicated in tapping stimulated latex production.

Materials And Methods

Plant materials

Hevea trees of Reyan7-33-97 clone, planted for eight years in the experimental field of Chinese Academy of Tropical Agricultural Sciences (Danzhou, Hainan), were first brought to tapping with a system of half spiral, every 3 days, no ethylene stimulation (S/2, d/3).

Extraction of latex total RNA

Five rubber trees attaining the tapping standard (trunk girth >= 50 cm at 1 m above the ground) were selected for latex collection. Twenty seconds after tapping, about five mL of latex was allowed to flow into a centrifuge tube containing 5ml 2×RNA extraction buffer (0.3 M LiCl, 10 mM EDTA, 10% SDS, 100 mM Tris-HCl, pH8.0). The collected latex was placed in ice box and brought to laboratory for RNA extraction as described in Tang et al. [45]. Electrophoresis on a 1.5% formaldehyde denaturing agarose gel was used to detect the integrity of RNA samples.

Semi-quantitative reverse transcription PCR (sqRT-PCR)

The first strand of cDNA was synthesized by reverse transcriptase kit (RevertAid^{TM} First Srtand cDNA Synthesis Kit, Thermo), and then diluted ten times as the template for sqRT-PCR with *18S rRNA* used as

the reference. The amount of cDNA samples used in sqRT-PCR was adjusted to be the same for the five tappings based on the level of *18S rRNA* expression.

cDNA-AFLP analysis

The manipulations were conducted according to the procedure which we previously established for transcript profiling in *Hevea* latex [27]. A total of 50 μg latex total RNA taken from each of the five tapping samples was subjected to cDNA-AFLP analysis. The synthesized double-stranded cDNA was cut by the restriction enzymes of *Apo* I and *Mse* I, and all the 128 possible selective primer combinations with 8 *Apo* I primers and 16 *Mse* I primers (Table 1) were applied in screening DE-TDFs affected by the tapping treatment in mature virgin *Hevea* trees.

DE-TDFs extraction and amplification

The DE-TDFs was scraped from the polyacrylamide gel with a surgical blade, put into a sterile PCR tube containing 30 μl 0.1× TE buffer (10 mM Tris-HCl, 1mM EDTA, pH8.0), heated at 95℃ for 15min, kept overnight at 4℃, and centrifuged at 10, 000 g for 5 min to collect the dissolved DNA solution for PCR amplification. A total of 25 μl PCR reaction mixture includes: 2μl DNA solution, 2.5 μl 10× PCR buffer (plus Mg^{2+}), 1.2 μl 2.5 mM dNTPs Mix, 0.5 μl 50 ng/μl *Mse*□selective primer, 0.5μl 50 ng/μl *Apo*□selective primer, 0.2 μl 5U/μl *Taq* DNA Polymerase (Takara) and 18.1 μl ddH_2O. The PCR amplification procedure is the same as the pre-amplification in cDNA-AFLP analysis [27]. PCR products were fractionated by 1.2% agarose gel electrophoresis, and the target band was sliced and purified using AxyPrep™ DNA Gel Extraction Kit (AxyGen).

DE-TDFs cloning and sequencing

The purified DE-TDFs were ligated with the T-vectors using the pMD18-T Vector Kit (Takara) in accordance with the manufacture's manual. The ligation mixture was used to transform *E. coli* JM109 competent cells and the transformants were sent to BGI Genomics Co., Ltd for sequencing.

Bioinformatics analysis

For DE-TDFs analysis, sequences of vectors and adaptors were first trimmed off by using the VecScreen program on the NCBI website (https://www.ncbi.nlm.nih.gov/tools/VecScreen). Then, the clean DE-TDF sequences were subjected for homology analysis to publicly available GenBank non-redundant sequences databases (http://www.ncbi.nlm.nih.gov) using the BLASTX program. Also, the Gene Ontology (http://amigo1.geneontology.org/cgi-bin/amigo/go.cgi) database was used to investigate the molecular function of each DE-TDF in the cell, which was used as the basis for functional classification.

Quantitative RT-PCR (qRT-PCR)

The expression pattern of candidate genes was detected by qRT-PCR. The first strand of cDNA was diluted 20 times as the template for qRT-PCR with *HbYLS8* as the reference gene as recommended in our previous study [46]. The PCR reaction mixture includes: 2μl template, 0.3 μl each for 10 μM forward and reverse primers, 10 μl 2×SYBR® Premix Ex *Taq*™ (Takara) and 7.4 μl ddH_2O. Roche's LightCycler 2.0 system was used for qRT-PCR analysis with the program as follows: 95℃ 30 sec; 94℃ 5 sec, 60℃ 20 sec, 72℃ 20 sec, 45cycles. Three technical replicates were analyzed for each of the three biological samples. All the cycle threshold (Ct) values from one gene were determined at the same threshold fluorescence value of 0.2 using the $2^{-\Delta\Delta Ct}$ method. The primers of target and reference genes were listed in Table S4. Statistical analysis was performed using Student's *t*-test.

Abbreviations

NR: Natural rubber; **AFLP:** Amplified fragment length polymorphism; **TDF:** Transcript derived fragment; **DE-TDF:** Differentially expressed transcript derived fragment; **EDTA:** Ethylene diamine tetraacetic acid; **dNTP:** Deoxy-ribonucleoside triphosphate; **DNA:** Deoxyribonucleic acid; **ATP:** Adenosine triphosphate; **PCR:** Polymerase chain reaction; **sqRT-PCR:** Semi-quantitative reverse transcription PCR; **qRT-PCR:** Quantitative reverse transcription PCR; **SUT:** Sucrose transporter; **NIN:** Alkaline/neutral invertase; **REF:** Rubber elongation factor; **SRPP:** Small rubber particle protein; **NADPH:** Reduced form of nicotinamide-adenine dinucleotide phosphate; **CoA:** Coenzyme A.

Declarations

Ethics approval, guidelines and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets measured and analyzed during the current study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

C.T. designed the research; Y.F., X.X., J.Y. and J.Q. performed the research; Y.F., X.X., C.T., Y.Z., S.Z. and J.T. analyzed the data; Y.F. and C.T. wrote the manuscript. The authors have read and approved the manuscript.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

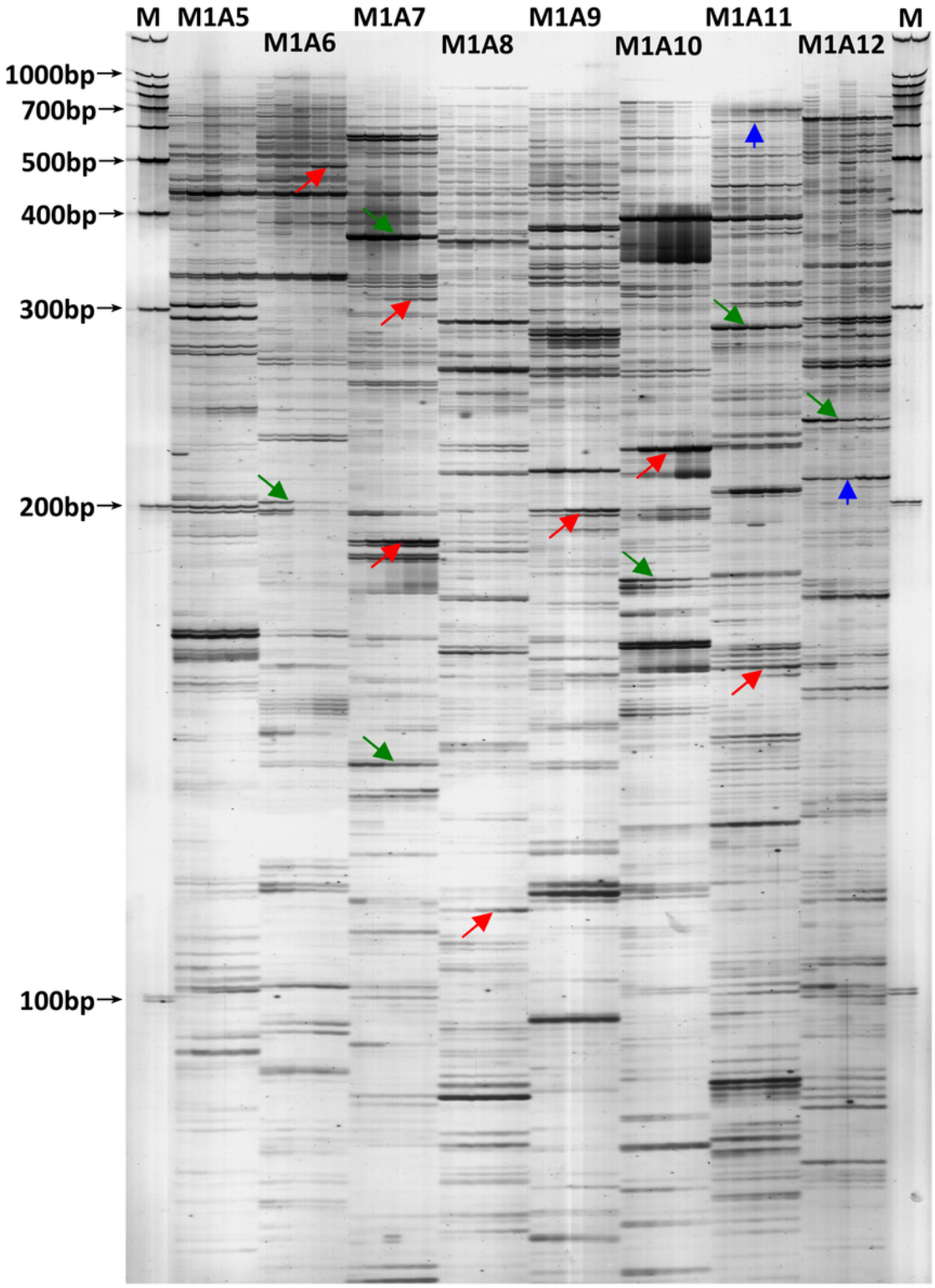

Figure 1

A typical cDNA-AFLP silver-stained polyacrylamide gel revealing latex transcript profiles in the latex of virgin Hevea trees for the first five tappings. M: 100bp DNA ladder molecular weight standard; M1A5 to M1A12: selective primer combinations. Under each primer pair, the five lanes (from left to right) represent the latex transcript profiles for the first five tappings. The arrows marked in red indicate the DE-TDFs up-regulated by tapping, the green arrows indicate down-regulated, and the blue arrows indicate bell-shaped.

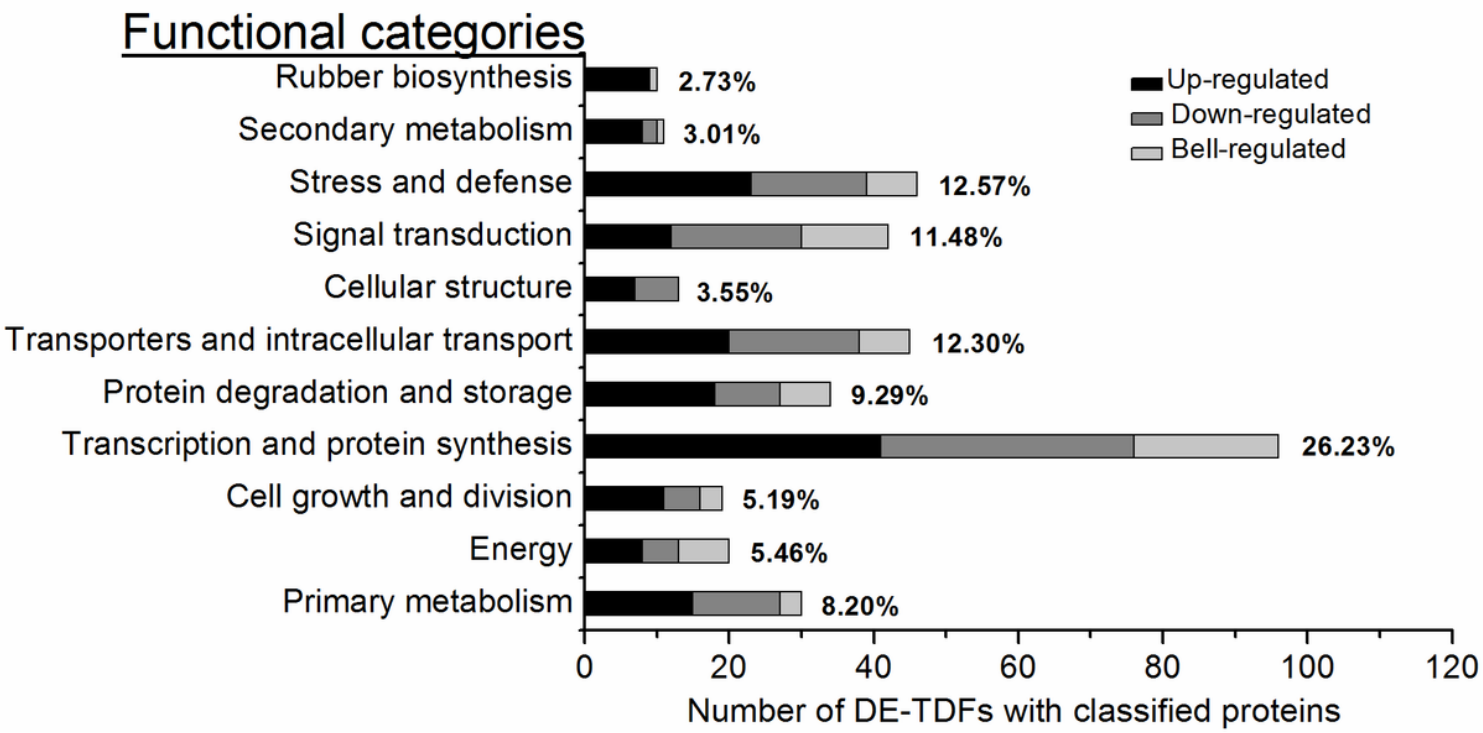

Figure 2

Functional category and percentage of the DE-TDFs with known function. The 366 non-redundant DE-TDFs of annotation proteins with known function were identified in the latex for the first five tappings in virgin trees. These DE-TDFs were classified into 11 functional categories. In each category, the percentage covering the total know functional DE-TDFs was placed at its right whereas the three types of expression were shown in differing gray bars.

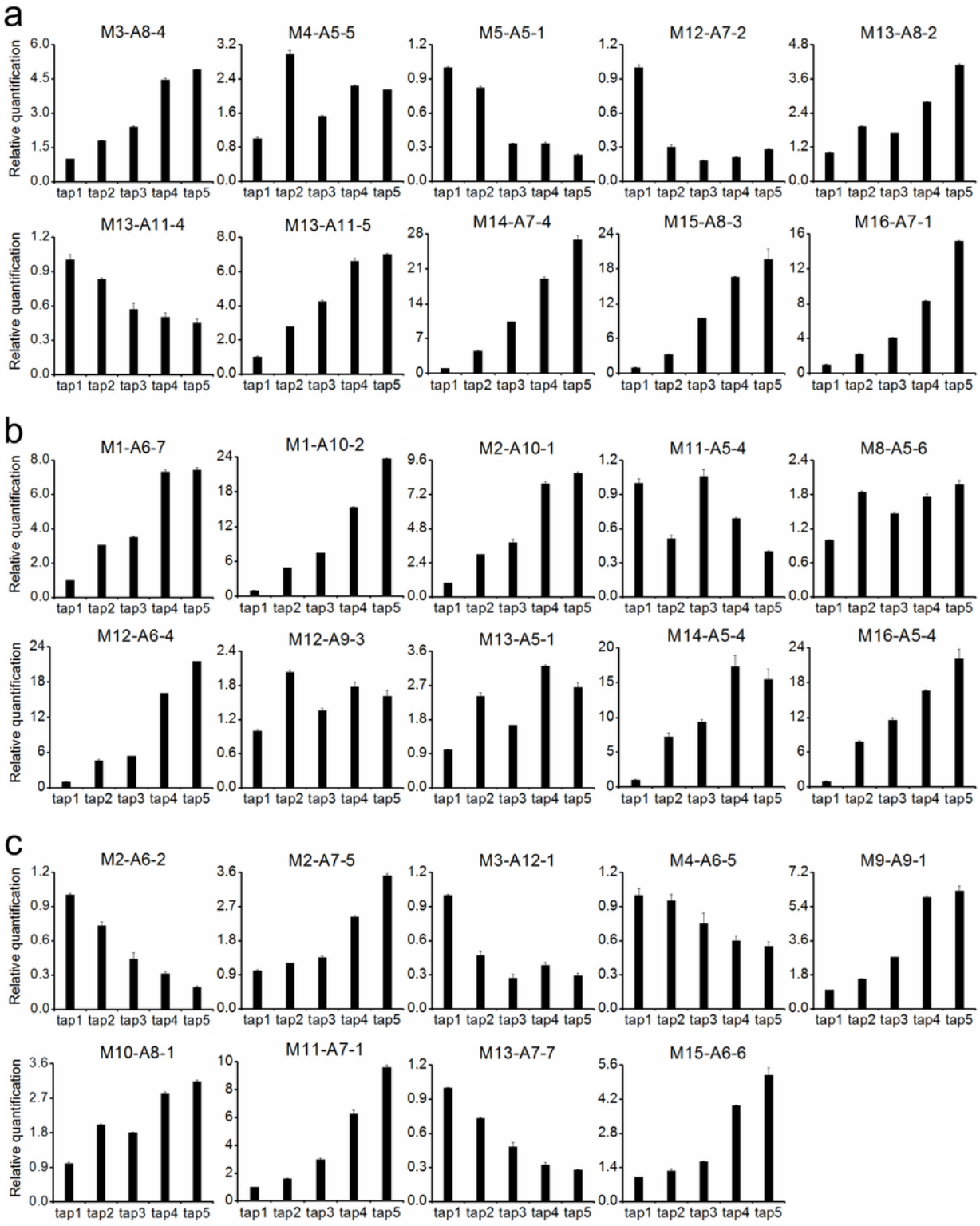

Figure 3

qRT-PCR analysis for expression of latex regeneration genes in the latex of the first five tappings. a: primary metabolism pathway (ten DE-TDFs); b: rubber biosynthesis and regulation pathway (ten DE-TDFs); c: transporters and intracellular transport pathway (nine DE-TDFs). Except for M3-A8-4, M11-A5-4and M16-A7-1, 26 of the 29 DE-TDFs shows the results consistent with their original cDNA-AFLP

expression patterns and were presented here. All datas were normalized to the expression level of HbYLS8 gene. Error bars indicate s.d. of three technical repeats.

Supplementary Files

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