RNA-seq derived identification of coronatine-regulated genes putatively involved in terpenoid biosynthetic pathway in the rubber tree *Hevea brasiliensis*

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**Abstract.** Rubber tree (*Hevea brasiliensis*) is a woody plant of the Spurge family (*Euphorbiaceae*) and the most economically important member of the genus *Hevea*. Rubber trees are often damaged and exposed to the attack of pathogens through wounds during and after tapping periods. Jasmonic acid (JA) and methyl jasmonate (MeJA), collectively termed jasmonates (JAs), are signaling molecules that are produced by plants, especially when subjected to environmental stresses. JA/MeJA treatment triggers the biosynthesis of many specialized metabolites including terpenoids through transcriptional reprogramming. To gain molecular insights into terpenoid metabolism and mechanism underlying rubber tree’s responses to stress signals such as wounding and pathogenicity, we performed an RNA-seq analysis of *H. brasiliensis* treated with either water or coronatine (COR) - a structural and functional mimic of JAs. We reconstructed the transcriptomes from the published RNA-sequencing data under the guidance of the newly released high-quality chromosome-level genome assembly of *H. brasiliensis* cultivar GT1 and screened for genes that are differently expressed between the two treatments. The putative functions and pathways of the differently expressed genes (DEGs) were obtained by using BLASTX search against NCBI non-redundant database, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) mapping. As a result, six out of the 446 identified DEGs were mapped to terpenoid metabolism pathway, five of which were up-regulated by coronatine treatment. This result provides a clue to gain insight into the genetic basis underlying terpenoid-related yield and resistance traits in the rubber tree.

1. Introduction
The rubber tree *Hevea brasiliensis* is a popular plant in Asia that has highly economic values. Its latex is used for rubber production. Rubber trees also provide timber for the construction industry, and its logging residues are useful for power generation [1,2]. Many physiological or biochemical studies on increasing the yield of latex from the rubber tree have been performed, through supplying soil nutrients, managing tapping cuts, or improving plant abiotic stress tolerance [3–5]. Recently, the development of next generation sequencing (NGS) technology has paved the way for major research
on molecular basis underlying desirable characteristics of the rubber tree, such as high latex yield or resistance to environmental stresses [6–9]. In addition to genome assemblies of *H. brasiliensis* constructed via the whole-genome shotgun sequencing approach [10–14], various RNA-seq studies have been conducted in this species to profile transcriptomes and explore genes, the expression of which is changed under specific conditions such as low temperature, drought, tapping cut dryness or leaf fungus diseases [8,15–20].

The rubber tree is rich in isoprenoids that serve many functions including as primary metabolites involved in plant growth and development, or as specialized metabolites that optimize the interaction between plants and their environment. Particularly, natural rubber, also produced from the isoprenoid pathway, makes up about one-third of the volume of latex derived from the inner bark of the rubber tree.

Plant isoprenoids are synthesized from C5-units isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) that originate from two distinct routes: the cytoplasmic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway [21,22]. It has been reported that the *H. brasiliensis* genome contains a greater number of genes involved in the MVA and MEP pathways than *A. thaliana* [11]. This significant expansion of isoprenoid biosynthesis-related genes in the *H. brasiliensis* genome correlates with the capacity to produce high levels of natural rubber in the latex and the resilience of the rubber tree, which often carries wounds and is exposed to disease-causing insects and pathogens through openings or wounds.

Defense responses, including rubber biosynthesis, have been shown to be affected by wounding and jasmonates (JA) [23–25]. Given that coronatine functionally mimics the active form of jasmonate [26,27], the discovery of coronatine-regulated genes involved in isoprenoid biosynthesis serves as a valuable starting point for studying the role of these genes in respect of the latex yield and the defense responses of rubber trees. Here we reconstructed the transcriptomes from the published RNA-sequencing data of *H. brasiliensis* treated with either coronatine (COR) or water as control [28] under the guidance of the newly released high-quality chromosome-level genome assembly of *H. brasiliensis* cultivar GT1 [14] and screened for genes that were differently expressed between the two treatments. Next, analysis of Gene Ontology (GO) [29,30] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [31,32] was performed to explore the functions and pathways of DEGs from which to identify a list of DEGs involved in terpenoid metabolism of *H. brasiliensis*.

2. Methods and Materials

2.1. Genome and RNA-sequencing data

The raw RNA sequencing data of two inner bark samples of the *H. brasiliensis* clone CATAS7-33-97 treated with coronatine (COR) and water (Control) deposited in the NCBI Sequence Read Archive (SRA) under accession number SRP073752 was used for analysis in this study [28]. The reference genome and its annotation were retrieved from the genomic assembly of the *H. brasiliensis* cultivar GT1 (Assembly accession GCA_010458925.1).

2.2. RNA-seq data cleaning and transcriptome assembly

The same procedure from pre-processing to the transcriptome assembly was applied for each RNA library. The raw data gathered from RNA-seq reads of inner bark samples were filtered to remove low quality reads by Trimmomatic v0.38 [33] according to these conditions: (1) reads with the adaptor sequences, (2) reads with 3' and 5' low-quality sequences (Phred quality score ≤ 3), (3) the percentage of low-quality bases (Phred quality score ≤ 20) was >50% in a read, (4) reads shorter than 50% of the read length. The quality of reads before and after being trimmed was checked by FASTQC v0.72. The trimmed reads were then sent to HISAT2 v2.1.0 in order to map RNA reads against the reference genome of rubber tree *H. brasiliensis* clone GT1, identify genomic positions of reads and generate spliced alignments [34]. The alignment file of each RNA-seq sample then was sent to StringTie v1.3.6 [35] for transcriptome construction. StringTie merge [35] were imported in this pipeline to combine all transcripts across the two transcriptomes representing the two RNA-seq samples and the reference
annotation of genome of *H. brasiliensis* clone *GT 1* into a transcriptome database. *GFFcompare* v0.9.8 [36] was used to remove the redundant transcripts in this newly created database and make the unique set of transcripts that can be used for the annotation in the following steps (Figure 1). All of these tools were implemented in the accessible and reproducible Galaxy platform [37].

![Figure 1. Pipeline of genome-guided assembly and analysis of *H. brasiliensis* RNA-seq](image)

- **a)** Genome guided-transcriptome assembly
- **b)** Identification of DEGs
- **c)** Functional annotation and pathway enrichment of DEGs

2.3. **Identification of differentially expressed genes (DEGs) in response to coronatine (COR)**

*FeatureCounts* v1.6.3 [37,38] was used to assign the aligned reads from *HISAT2* output file of each sample to the transcriptome database and identify the number of mapped reads for each gene. The minimum quality of reads to be counted was set at 12, while other parameters were set at default. The raw counts of mapped reads were normalized with library size (total number of reads) by *DESeq2* v2.11.40.2 using the default mode [37,39]. The normalized read counts for each gene were proportional to the expression of the gene and thus differently expressed genes (DEGs) were identified by comparing the normalized read counts for a given gene between COR-treated samples and control samples. The criteria for genes to be considered as DEGs were $|\log_{2} \text{FC}| \geq 1$ and p-value $\leq 0.05$. These DEGs were then sent to downstream analysis to retrieve their functional annotation.

2.4. **Functional annotation and screening for DEGs involved in terpenoid biosynthesis pathway**
The coding sequences (CDS) of DEGs were extracted from the *H. brasiliensis* genome database using BEDTools v2.29.0 [37,40]. The CDSs of DEGs were searched against Non-Redundant (NR) protein database from NCBI (16-05-2020) using BLASTX program with an E-value cut-off set to 1e-5, limitation in Eudicots and the top 10 hits was obtained. BLASTX results of NR were sent to Blast2GO v5.2 [41], combined with annotations from InterProScan [42-44] and Gene Ontology (GO) [29,30] to identify functional annotations. GO mapping and KEGG pathway mapping [31,32] in Blast2GO were used to obtain pathway annotation for each DEG. All parameters in Blast2GO were set at default.

2.5. Amino acid sequence alignment and phylogenetic analysis

Phylogenetic trees were constructed using the neighbor-joining algorithm and visualized using Molecular Evolution Genetics Analysis (MEGA X) [45]. The selected terpene synthases used in the phylogenetic analysis were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/).

3. Results

3.1. Genome-guided transcriptome assembly

After removing low quality reads and reads having adaptor sequences by Trimmomatic, each library contains more than 70 million clean reads, ranging from 45 to 90 bp (Table 1). Clean reads of each library were mapped to the reference genome of rubber trees *H. brasiliensis* cultivar GT1 by HISAT2 [34]. The overall alignment rates for the two RNA-seq libraries, Control and COR, were 93.98% and 94.06%, respectively. The mapped reads were passed to StringTie for transcript assembly [35], and as a result, two transcriptomes of the Control and COR treatment were generated, with 57,798 and 58,786 transcripts, respectively. Using StringTie merge to combine redundant transcript structures across the two transcriptomes and the genomic reference, we constructed a transcriptome database with a total of 89,815 transcripts. The transcripts of the newly created transcriptome were annotated using GFFcompare, with 45,669 identified as novel transcripts. The transcripts’ length ranged from 102 to 15,787 bp (Figure 2), with an average length of 1,168 bp and N50 length of 1,554 bp (Table 2).

![Table 1. Output statistics from sequencing reads of RNA-seq samples](image)

**Table 1.** Output statistics from sequencing reads of RNA-seq samples

| Library | Total raw reads | Length of raw reads | Total clean reads | Length of clean reads | GC percentage | Q30 (%) |
|---------|----------------|---------------------|-------------------|-----------------------|---------------|---------|
| Control | 80.8 x 10^6    | 90                  | 75 x 10^6         | 45 – 90               | 44.0%         | 99.50%  |
| COR     | 81.4 x 10^6    | 90                  | 76.8 x 10^6       | 45 – 90               | 43.5%         | 99.59%  |

*Percentage of reads in the library with a Phred score > 30*

![Figure 2. Plot of length distribution and number of transcripts in transcriptome database](image)

3.2. Identification and annotation of the DEGs
The normalized read counts of each transcript from control and coronatine-treated samples were compared to identify genes that show differences in expression level between samples. Under the screening criteria of $|\log_{2}\text{FC}| \geq 1$ and $p$-value $\leq 0.05$, 446 transcripts were identified as differentially expressed gene (DEG), including 177 up-regulated genes and 269 down-regulated genes after coronatine treatment.

For functional annotations, the coding sequences of 446 DEGs were searched against the NR protein databases by using BLASTX (E-value cut-off $1\text{e}^{-5}$) and 407 DEGs (91.26%) were annotated by significantly similar sequences. BLASTX results of NR were sent to Blast2GO for GO mapping and annotation. The annotation was then combined with GO terms retrieved from InterProScan, resulting in 320 DEGs with at least one GO term. Based on GO term analysis, there were 252 DEGs (56.50%) involved in molecular function (MF), 238 DEGs (53.36%) in biological process (BP), and 212 DEGs (47.53%) in cellular component (CC) categories. For the biological processes, the organic substance metabolic process, the cellular metabolic process and the primary metabolic process were highly represented (150, 145 and 140, respectively). In the cellular component category, many DEGs were identified to encode proteins in membrane (114). In the molecular function category, a great number of DEGs were involved in binding activities (heterocyclic compound binding: 96, organic cyclic compound binding: 96, ion binding 82, and protein binding: 49) (Figure 3).

| Table 2. Statistics of transcriptome database |
|---------------------------------------------|
| Number of genes                              | 59,588     |
| Number of transcripts                        | 89,815     |
| Total length of sequences (bp)               | 104,697,625|
| Average transcript length (bp)               | 1,168      |
| N50 length (bp)                              | 1,554      |
| Maximum transcript length (bp)               | 15,787     |
| Minimum transcript length (bp)               | 102        |

![GO Enrichment of DEGs](image)
Figure 3. The GO enrichment of the annotated *H. brasiliensis* DEGs. The functionally annotated DEGs were assigned to three main GO categories: biological process (BP), molecular function (MF) and cellular component (CC).

Table 3. DEGs of terpenoid biosynthesis pathway in *H. brasiliensis* in response to coronatine treatment

| ID      | Predicted function                                      | Log2FC | p-value | GO term                                           | KEGG Pathway                       |
|---------|----------------------------------------------------------|--------|---------|--------------------------------------------------|------------------------------------|
| CDS_063 | α-copaene synthase-like                                  | 1.992  | 0.0056  | GO:0000287; GO:0005506; GO:0016114; GO:0016705; GO:0047461; GO:0055114; GO:1901928 | Sesquiterpenoid and triterpenoid biosynthesis |
| CDS_198 | probable terpene synthase 9                             | 1.578  | 0.0232  | GO:0000287; GO:0019288; GO:0046872; GO:0050992; GO:0051745; GO:0055114 | ---NA---                           |
| CDS_199 | 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, chloroplastic | -1.225 | 0.0465  | GO:0000287; GO:0019288; GO:0046872; GO:0050992; GO:0051745; GO:0055114 | Terpenoid backbone biosynthesis    |
| CDS_234 | probable 1-deoxy-D-xylulose-5-phosphate synthase 2, chloroplastic | 1.911  | 0.0022  | GO:0006694; GO:0008661; GO:0016114 | Terpenoid backbone biosynthesis    |
| CDS_340 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1         | 1.276  | 0.0237  | GO:0004420; GO:0005515; GO:0005778; GO:0005783; GO:0005789; GO:0008299; GO:0015936; GO:0016021; GO:0016126; GO:0042282; GO:0050662; GO:0055114 | Terpenoid backbone biosynthesis    |
| CDS_379 | gibberellin 2-β-dioxygenase 1-like                       | 1.171  | 0.0165  | GO:0009416; GO:0045487; GO:0046872; GO:0052634; GO:0055114 | Diterpenoid biosynthesis           |

3.3. Identification of the DEGs involved in the terpenoid biosynthesis pathway

Based on GO terms (GO:0010333 - terpene synthase activity, GO:0016114 - terpenoid biosynthetic process, GO:0019288 - isopentenyl diphosphate biosynthetic process, GO:0008299 - isoprenoid biosynthetic process) [29,30], we were able to identify five DEGs involved in terpenoid biosynthesis pathway. Those DEGs were annotated as α-copaene synthase-like (CDS_063), probable terpene
synthase 9 (CDS_198), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (CDS_199), probable 1-deoxy-D-xylulose-5-phosphate synthase 2 (CDS_234) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (CDS_340), which we designated as HbTPS063, HbTPS198, HbHDR, HbDXS and HbHMGR, respectively. Based on KEGG pathway annotation by Blast2GO, one more DEG (CDS_379) was predicted to be gibberellin 2-β-dioxygenase 1-like participating in diterpenoid metabolism and was renamed as HbGD. The log2 fold change (log2FC) and functional annotations of these DEGs were shown in the Table 3. Of the six DEGs, only one gene encoding for 4-hydroxy-3-methylbut-2-enyl diphosphate reductase- like (CDS_199) was found to be down-regulated, the other five genes showed up-regulation after coronatine treatment. Noticeably, genes putatively encoding HbDXS (CDS_234) and HbTPS063 (CDS_063) were induced about 4-fold (log2 FC ~ 2) following application of coronatine.

3.4. HbTPS063 is a putative sesquiterpene synthase

Based on the sequence comparison with previously characterized homologs from other species, HbTPS063, HbDXS, HbHMGR and HbGD appear to contain complete open reading frames, whereas HbTPS198 and HbHDR are incomplete. The amino acid sequence deduced from the complete ORF of HbTPS063 consists of 598 amino acids with a calculated molecular mass of 68.82 kD and contains an evolutionarily conserved aspartate-rich motif, DDXXD. A phylogenetic tree based on TPS sequences from many different angiosperm plant species indicates that the HbTPS063 protein belongs to the TPS-a clade, which consists of sesquiterpene synthases (Figure 4).

![Figure 4](image-url)

**Figure 4.** Maximum-likelihood phylogenetic tree of HbTPS063 and functionally characterized TPSs representing different TPS clades from angiosperm plants, including carrot *Daucus carota* L. (Dc), bay laurel *Laurus nobilis* (Ln), Taiwan cherry *Prunus campanulate* (Pc), and the cultivated tomato *Solanum lycopersicum* (Sl). HbTPS063 is marked with an asterisk. Bootstrap values were obtained from 1000 replicates. Accession numbers are as follow: DcTPS01, XP_017214942.1; DcTPS23, XP_017217934.1; DcTPS25-X1, XP_017242829.1; DcTPS25-X2, XP_017242830.1; DcTPS26, XP_017245927.1; DcTPS28, XP_017248749.1; LnTPS1, AKQ19357.1; LnTPS2, AKQ19358.1;
LnTPS3, AKQ19359.1, PcTPS1, AIC76493.2; PcTPS2, AIC76494.2; PcTPS4, AIC76496.1; PcTPS9, AIC76501.1; SITPS3, NP_001295307.1; SITPS12, NP_001234766.1; SITPS20, NP_001310383.1; SITPS37, XP_010327235.1; SITPS40, NP_001234008.2.

4. Discussion
Under the guidance of the newly released high-quality chromosome-level genome assembly of H. brasiliensis cultivar GT1 [14], we reconstructed the transcriptomes from the published RNA-sequencing data [28] and identified 446 genes that are differently expressed between the control and coronatine-treated samples. Six out of those DEGs, including CDS_063, CDS_198, CDS_199, CDS_234, CDS_340, and CDS_379, were involved in the terpenoid biosynthesis pathway according to their functional annotations, and the full-length protein-coding sequences were obtained for four of them (CDS_063, CDS_234, CDS_340, and CDS_379).

Figure 5. The mevalonate (MVA) and methyerythriol phosphate (MEP) pathways in terpenoid biosynthesis. HMG, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl diphosphate; GA-3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate synthase; ME-cPP, 2-C-methyl-d-erythritol-2,4-cyclopyrophosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate

CDS_340, one of the transcripts that were up-regulated in the bark of H. brasiliensis following the coronatine treatment, putatively encodes 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (HMGR), a rate-limiting enzyme of the mevalonate (MVA) pathway. Given that coronatine functionally mimics the active form of jasmonate [26,46], this is consistent with previous findings where validation of RNA-seq results from the bark of rubber seedlings by reverse transcription quantitative PCR (RT-qPCR) shows that HMGR transcript was significantly higher in the JA-treated sample than in the control [46]. The classical MVA pathway, which is mainly localized in the cytosol, gives rise to IPP and via enzymatic isomerization to DMAPP, both of which act as substrates (at a
ratio of 2:1) for cytosolic farnesyl dipiphosphate (FPP) synthase (FPPS) to form FPP (Figure 5). FPP in the cytosol then serves as a precursor for sesquiterpene biosynthesis. Interestingly, our RNA-seq analysis also identified at least one coratine-induced terpene synthase (TPS) gene (CDS_063 or HbTPS063), and this gene is likely to encode a sesquiterpene synthase. In addition to this, in silico analysis of a co-functional network database for H. brasiliensis at the Rubber Database RIKEN using HbTPS063 as a guide gene identified a co-expressed gene that is predicted to encode a bHLH transcription factor, a key regulator of the JA-mediated wound signalling pathway in the rubber tree H. brasiliensis [47,48].

The expression of the MEP pathway genes such as HbDXS (1-deoxy-D-xylulose-5-phosphate synthase) and HbHDR (4-hydroxy-3-methylbut-2-enyl diphosphate reductase) is also changed upon coronatine treatment. This suggests that coratine is likely an important regulator of not only the MVA pathway but also of the MEP pathway in the rubber tree H. brasiliensis. Consistent with the study by Liu et al. (2016), we showed that the expression of HbDXS increased after coronatine treatment. More interestingly, this expression pattern of HbDXS was similar to that of HbTPS198, which encodes a putative terpene synthase within the TPS-b clade. Although additional TPS gene transcript analysis and enzymatic characterization are needed to confirm the function of HbTPS063 and HbTPS198 in terpenoid-mediated defense response of the rubber tree, there is a strong possibility that initiation of JA signalling during wounding, pathogen infection or insect attack could elevate the emissions of the volatile terpenes formed by these TPSs in H. brasiliensis. This study thus opens up an opportunity to predict and study the function of genes that define terpenoid-related yield and resistance traits in the rubber tree, as a basis for developing effective methods for selecting rubber clones that have desirable characteristics.

5. Conclusion

Based on RNA sequencing analysis using control and coronatine-treated H. brasiliensis, we identified six DEGs involved in terpenoid biosynthesis pathway. Four full-length protein-coding sequences (CDS_063, CDS_234, CDS_340, and CDS_379) were obtained, one of which (CDS_063) encodes a putative terpene synthase. It is predicted by in silico analysis that the protein encoded by CDS_063 belongs to the TPS-a clade, which consists of sesquiterpene syntheses. This study provides a clue to the role of those six putative terpenoid biosynthetic genes, the expression of which is regulated by coratine treatment. It would be of interest to further characterize the function of these genes to confirm their functionality in the terpenoid metabolism and defense responses of the rubber tree.

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References

[1] Barlow C 1983 The natural rubber industry Outlook Agric. 12 2–11
[2] Ratnasingam J, Ramasamy G, Tau Wai L, Senin A L and Muttilah N 2015 The Prospects of Rubberwood Biomass Energy Production in Malaysia BioResources 10 2526–48
[3] Lestari R, Rio M, Martin F, Leclercq J, Woraاثhasin N, Roques S, Dessailly F, Clément-Vidal A, Sanier C, Fabre D, Melliti S, Suharsono S and Montoro P 2018 Overexpression of Hevea brasiliensis ethylene response factor HbERF-Ixc5 enhances growth and tolerance to abiotic stress and affects laticifer differentiation Plant Biotechnol. J. 16 322–36
[4] Chantuma P, Lacote R, Leconte A and Gohet É 2011 An innovative tapping system, the double cut alternative, to improve the yield of Hevea brasiliensis in Thai rubber plantations F. Crop. Res. 121 416–22
[5] Vrignon-Brenas S, Gay F, Ricard S, Snoeck D, Perron T, Mareschal L, Laclau J-P, Gohet É and Malagoli P 2019 Nutrient management of immature rubber plantations. A review Agron. Sustain. Dev. 39 11
[6] Li D, Wang X, Deng Z, Liu H, Yang H and He G 2016 Transcriptome analyses reveal molecular mechanism underlying tapping panel dryness of rubber tree (Hevea brasiliensis) Sci. Rep. 6 23540

[7] Liu J-P, Zhuang Y-F, Guo X-L and Li Y-J 2016 Molecular mechanism of ethylene stimulation of latex yield in rubber tree (Hevea brasiliensis) revealed by de novo sequencing and transcriptome analysis BMC Genomics 17 257

[8] Campos Mantello C, Boatwright L, da Silva C C, Scaloppi E J, de Souza Goncalves P, Barbazuk W B and Pereira de Souza A 2019 Deep expression analysis reveals distinct cold-response strategies in rubber tree (Hevea brasiliensis) BMC Genomics 20 455

[9] Roy C B, Liu H, Rajamani A and Saha T 2019 Transcriptome profiling reveals genetic basis of disease resistance against Corynespora cassiicola in rubber tree (Hevea brasiliensis) Curr. Plant Biol. 17 2–16

[10] Rahman A Y A, Usharraj A O, Misra B B, Thottathil G P, Jayasekaran K, Feng Y, Hou S, Ong S Y, Ng F L, Lee L S, Tan H S, Sakaff M K L M, Teh B S, Khoo B, Badai S S, Aziz N A, Yuryev A, Knudsen B, Dionne-Laporte A, Mchunu N P, Yu Q, Langston B J, Freitas T A K, Young A G, Chen R, Wang L, Najimudin N, Saito J A and Alam M 2013 Draft genome sequence of the rubber tree Hevea brasiliensis BMC Genomics 14 75

[11] Lau N-S, Makita Y, Kawashima M, Taylor T D, Kondo S, Othman A S, Shu-Chien A C and Matsui M 2016 The rubber tree genome shows expansion of gene family associated with rubber biosynthesis Sci. Rep. 6 28594

[12] Tang C, Yang M, Fang Y, Luo Y, Gao S, Xiao X, An Z, Zhou B, Zhang B, Tan X, Yeang H-Y, Qin Y, Yang J, Lin Q, Mei H, Montoro P, Long X, Qi J, Hua Y, He Z, Sun M, Li W, Zeng X, Cheng H, Liu Y, Yang J, Tian W, Zhuang N, Zeng R, Li D, He P, Li Z, Zou Z, Li S, Li C, Wang J, Wei D, Lai C-Q, Luo W, Yu J, Hu S and Huang H 2016 The rubber tree genome reveals new insights into rubber production and species adaptation Nat. Plants 2 16073

[13] Pootakham W, Sonthirod C, Naktae C, Rung-Areee B, Yoocha T, Sangsrakru D, Theerawattanasuk K, Rattanawong R, Lekawipat N and Tangphatsornruang S 2017 De novo hybrid assembly of the rubber tree genome reveals evidence of paleotetraploidy in Hevea species Sci. Rep. 7 41457

[14] Liu J, Shi C, Shi C-C, Li W, Zhang Q-J, Zhang Y, Li K, Hu H-F, Shi C, Zhu S-T, Xiao Z-Y, Nan H, Yue Y, Zhu X-G, Wu Y, Hong X-N, Fan G-Y, Tong Y, Zhang D, Mao C-L, Liu Y-L, Hao S-J, Liu W-Q, Lv M-Q, Zhang H-B, Liu Y, Hu-tang G-R, Wang J-P, Wang J-H, Sun Y-H, Ni S-B, Chen W-B, Zhang X-C, Jiao Y-N, Eichler E E, Li G-H, Liu X and Gao L-Z 2020 The Chromosome-Based Rubber Tree Genome Provides New Insights into Spurge Genome Evolution and Rubber Biosynthesis Mol. Plant 13 336–50

[15] Cheng H, Chen X, Fang J, An Z, Hu Y and Huang H 2018 Comparative transcriptome analysis reveals an early gene expression profile that contributes to cold resistance in Hevea brasiliensis (the Para rubber tree) ed C-J Tsai Tree Physiol. 38 1409–23

[16] Gong X-X, Yan B-Y, Hu J, Yang C-P, Li Y-J, Liu J-P and Liao W-B 2018 Transcriptome profiling of rubber tree (Hevea brasiliensis) discovers candidate regulators of the cold stress response Genes Genomics 40 1181–97

[17] Deng X, Wang J, Li Y, Wu S, Yang S, Chao J, Chen Y, Zhang S, Shi M and Tian W 2018 Comparative transcriptome analysis reveals phytohormone signalings, heat shock module and ROS scavenger mediate the cold-tolerance of rubber tree Sci. Rep. 8 4931

[18] Mohamed Sathik M B, Luke L P, Rajamani A, Kuruvilla L, Sumesh K V and Thomas M 2018 De novo transcriptome analysis of abiotic stress-responsive transcripts of Hevea brasiliensis Mol. Breed. 38 32

[19] Liu J-P, Xia Z-Q, Tian X-Y and Li Y-J 2015 Transcriptome sequencing and analysis of rubber tree (Hevea brasiliensis Muell.) to discover putative genes associated with tapping panel dryness (TPD) BMC Genomics 16 398

[20] Fang Y, Mei H, Zhou B, Xiao X, Yang M, Huang Y, Long X, Hu S and Tang C 2016 De novo
Transcriptome Analysis Reveals Distinct Defense Mechanisms by Young and Mature Leaves of Hevea brasiliensis (Para Rubber Tree) Sci. Rep. 6 33151

[21] McGarvey D J and Croteau R 1995 Terpenoid metabolism. Plant Cell 7 1015–26

[22] Rodriguez-Concepcion M 2002 Elucidation of the Methylenedioxytritol Phosphate Pathway for Isoprenoid Biosynthesis in Bacteria and Plastids. A Metabolic Milestone Achieved through Genomics Plant Physiol. 130 1079–89

[23] Deng X, Guo D, Yang S, Shi M, Chao J, Li H, Peng S and Tian W 2018 Jasmonate signalling in the regulation of rubber biosynthesis in laticifer cells of rubber tree, Hevea brasiliensis J. Exp. Bot. 69 3559–71

[24] Duan C, Rio M, Leclercq J, Bonnot F, Oliver G and Montoro P 2010 Gene expression pattern in response to wounding, methyl jasmonate and ethylene in the bark of Hevea brasiliensis Tree Physiol. 30 1349–59

[25] Pirrello J, Leclercq J, Dessailly F, Rio M, Piyatrakul P, Kuswanhabi K, Tang C and Montoro P 2014 Transcriptional and post-transcriptional regulation of the jasmonate signalling pathway in response to abiotic and harvesting stress in Hevea brasiliensis BMC Plant Biol. 14 341

[26] Fonseca S, Chini A, Hamberg M, Adie B, Porzel A, Kramell R, Miersch O, Wasternack C and Solano R 2009 (+)-7-isoojasmonoyl-L-isoleucine is the endogenous bioactive jasmonate Nat. Chem. Biol. 5 344–50

[27] Feys B, Benedetti C E, Penfold C N and Turner J G 1994 Arabidopsis Mutants Selected for Resistance to the Phytotoxin Coronatine Are Male Sterile, Insensitive to Methyl Jasmonate, and Resistant to a Bacterial Pathogen. Plant Cell 751–9

[28] Wu S, Zhang S, Chao J, Deng X, Chen Y, Shi M and Tian W-M 2016 Transcriptome Analysis of the Signalling Networks in Coronatine-Induced Secondary Laticifer Differentiation from Vascular Cambia in Rubber Trees Sci. Rep. 6 36384

[29] Ashburner M, Ball C A, Blake J A, Botstein D, Butler H, Cherry J M, Davis A P, Dolinski K, Dwight S S, Eppig J T, Harris M A, Hill D P, Issel-Tarver L, Kasarskis A, Lewis S, Matese J C, Richard J E, Ringwald M, Rubin G M and Sherlock G 2000 Gene Ontology: tool for the unification of biology Nat. Genet. 25 25–9

[30] Carbon S, Douglass E, Dunn N, Good B, Harris N L, Lewis S E, Mungall C J, Basu S, Chisholm R L, Dodson R J, Hartline E, Fey P, Thomas P D, Albou L P, Ebert D, Kesling M J, Mi H, Muruganujan A, Huang X, Poudel S, Mushayahama T, Hu J C, LaBonte S A, Siegele D A, Antonazzo G, Attrill H, Brown N H, Fexova S, Garapati P, Jones T E M, Marygold S J, Millburn G H, Rey A J, Trouvise V, Dos Santos G, Emmert D B, Falls K, Zhou P, Goodman J L, Strelets V B, Thurmond J, Courtot M, Osumi D S, Parkinson H, Ronaglia P, Accenio M L, Kuiper M, Reid A, Logie C, Lovering R C, Huntley R P, Denny P, Campbell N H, Kramarz B, Acquah V, Ahmad S H, Chen H, Rawson J H, Chibucos M C, Giglio M, Nadendla S, Tauber R, Duesbury M J, Del N T, Meldal B H M, Perfetto L, Porras P, Orchard S, Shrivastava A, Xie Z, Chang H Y, Finn R D, Mitchell A L, Rawlings N D, Richardson L, Sangrador-Vegas A, Blake J A, Christie K R, Dolan M E, Drabkin H J, Hill D P, Ni L, Sitnikov D, Harris M A, Oliver S G, Rutherford K, Wood V, Hayles J, Bahler J, Lock A, Bolton E R, De Pons J, Dwinell M, Hayman G T, Laulelerkind S J F, Shimoyama M, Tutaj M, Wang S J, et al 2019 The Gene Ontology Resource: 20 years and still GOing strong Nucleic Acids Res. 47 D330–8

[31] Kancheha M and Sato Y 2020 KEGG Mapper for inferring cellular functions from protein sequences Protein Sci. 29 28–35

[32] Kancheha M 2000 KEGG: Kyoto Encyclopedia of Genes and Genomes Nucleic Acids Res. 28 27–30

[33] Bolger A M, Lahse M and Usadel B 2014 Trimmomatic: a flexible trimer for Illumina sequence data Bioinformatics 30 2114–20

[34] Kim D, Langmead B and Salzberg S L 2015 HISAT: a fast spliced aligner with low memory requirements Nat. Methods 12 357–60

[35] Pertea M, Pertea G M, Antonescu C M, Chang T-C, Mendell J T and Salzberg S L 2015
StringTie enables improved reconstruction of a transcriptome from RNA-seq reads *Nat. Biotechnol.* **33** 290–5

[36] Pertea M, Kim D, Pertea G M, Leek J T and Salzberg S L 2016 Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown *Nat. Protoc.* **11** 1650–67

[37] Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, Taylor J and Nekrutenko A 2020 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update *Nucleic Acids Res.* **48** 8205–7

[38] Liao Y, Smyth G K and Shi W 2014 featureCounts: an efficient general purpose program for assigning sequence reads to genomic features *Bioinformatics* **30** 923–30

[39] Love M I, Huber W and Anders S 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2 *Genome Biol.* **15** 550

[40] Quinlan A R and Hall I M 2010 BEDTools: a flexible suite of utilities for comparing genomic features *Bioinformatics* **26** 841–2

[41] Conesa A, Gotz S, Garcia-Gomez J M, Terol J, Talon M and Robles M 2005 Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research *Bioinformatics* **21** 3674–6

[42] Zdobnov E M and Apweiler R 2001 InterProScan - an integration platform for the signature-recognition methods in InterPro *Bioinformatics* **17** 847–8

[43] Quevillon E, Silvestro V, Pillai S, Harte N, Mulder N, Apweiler R and Lopez R 2005 InterProScan: protein domains identifier *Nucleic Acids Res.* **33** W116–20

[44] Hunter S, Apweiler R, Attwood T K, Bairoch A, Bateman A, Binns D, Bork P, Das U, Daugherty L, Duquenne L, Finn R D, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Lauagraud A, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orengo C, Quinn A F, Selengut J D, Sigrist C J A, Thimma M, Thomas P D, Valentin F, Wilson D, Wu C H and Yeats C 2009 InterPro: the integrative protein signature database *Nucleic Acids Res.* **37** D211–5

[45] Kumar S, Stecher G, Li M, Knyaz C and Tamura K 2018 MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms ed F U Battistuzzi *Mol. Biol. Evol.* **35** 1547–9

[46] Loh S C, Thottathil G P and Othman A S 2016 Identification of differentially expressed genes and signalling pathways in bark of Hevea brasiliensis seedlings associated with secondary laticifer differentiation using gene expression microarray *Plant Physiol. Biochem.* **107** 45–55

[47] Zhai J, Hao H, Xiao H, Cao Y, Lin X and Huang X 2018 Identification of JAZ-interacting MYC transcription factors involved in latex drainage in Hevea brasiliensis *Sci. Rep.* **8** 909

[48] Loh S C, Othman A S and Veera Singham G 2019 Identification and characterization of jasmonic acid- and linolenic acid-mediated transcriptional regulation of secondary laticifer differentiation in Hevea brasiliensis *Sci. Rep.* **9** 14296