Transcriptome analysis of Pará rubber tree (H. brasiliensis) seedlings under ethylene stimulation

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Abstract

Background: Natural rubber (cis-1,4-polyisoprene, NR) is an indispensable industrial raw material obtained from the Pará rubber tree (H. brasiliensis). Natural rubber cannot be replaced by synthetic rubber compounds because of the superior resilience, elasticity, abrasion resistance, efficient heat dispersion, and impact resistance of NR. In NR production, latex is harvested by periodical tapping of the trunk bark. Ethylene enhances and prolongs latex flow and latex regeneration. Ethephon, which is an ethylene-releasing compound, applied to the trunk before tapping usually results in a 1.5- to 2-fold increase in latex yield. However, intense mechanical damage to bark tissues by excessive tapping and/or over-stimulation with ethephon induces severe oxidative stress in laticifer cells, which often causes tapping panel dryness (TPD) syndrome. To enhance NR production without causing TPD, an improved understanding of the molecular mechanism of the ethylene response in the Pará rubber tree is required. Therefore, we investigated gene expression in response to ethephon treatment using Pará rubber tree seedlings as a model system.

Results: After ethephon treatment, 3270 genes showed significant differences in expression compared with the mock treatment. Genes associated with carotenoids, flavonoids, and abscisic acid biosynthesis were significantly upregulated by ethephon treatment, which might contribute to an increase in latex flow. Genes associated with secondary cell wall formation were downregulated, which might be because of the reduced sugar supply. Given that sucrose is an important molecule for NR production, a trade-off may arise between NR production and cell wall formation for plant growth and for wound healing at the tapping panel.

Conclusions: Dynamic changes in gene expression occur specifically in response to ethephon treatment. Certain genes identified may potentially contribute to latex production or TPD suppression. These data provide valuable information to understand the mechanism of ethylene stimulation, and will contribute to improved management practices and/or molecular breeding to attain higher yields of latex from Pará rubber trees.

Keywords: Ethylene, Microarray, Natural rubber, Pará rubber tree, H. brasiliensis

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Background

Natural rubber (cis-1,4-polyisoprene; NR) is a vitally important industrial raw material because it cannot be replaced by synthetic rubbers on account of the superior resilience, elasticity, abrasion resistance, efficient heat dispersion, and impact resistance of NR [1]. NR accounted for 47.2% of the world rubber production in 2019 (more than 13.6 million tonnes, from Statista website [2]). Virtually all commercial NR is derived from the Para rubber tree (H. brasiliensis), which is cultivated in tropical and subtropical regions worldwide but predominantly in Southeast Asia. Increased production of NR has been prompted by the increasing global demand for rubber. Given that it is difficult to expand the cultivation area because of competition with production of other important crops and conservation of natural rainforests, further improvement in NR yield per area is desired.

Latex, a rubber-containing cytoplasmic component, is produced in laticifers, which are highly differentiated cells that synthesize and store latex in the inner bark of Para rubber trees. In NR production, latex is harvested by periodical tapping of the trunk bark. Wounding of the bark caused by tapping induces endogenous ethylene production. Ethylene is a gaseous plant hormone involved in the regulation of diverse biochemical, physiological, and developmental processes in plants. The role of ethylene in defense responses to wounding, herbivory, and pathogen infection has been widely studied in plants [3, 4]. Ethephon, an ethylene releaser, effectively increases the latex yield in Para rubber trees and ethylene stimulation is commonly practiced in rubber tree plantations worldwide [5]. Ethephon application to the bark enhances and prolongs latex flow and latex regeneration, usually resulting in a 1.5- to 2-fold increase in latex yield [5]. However, intense mechanical damage to bark tissues by excessive tapping and/or over-stimulation with ethephon induces severe oxidative stress in laticifer cells, which often causes the tapping panel dryness (TPD) syndrome in Para rubber tree. Under TPD, latex flow ceases and in situ coagulation of rubber particles or deep degeneration of tissues are observed [6–9]. The TPD syndrome is characterized by two types of physiological symptoms: a temporary halt in latex flow and histological deformation of the bark. The halt in latex flow is induced by reactive oxygen species (ROS) in laticifer cells and is mitigated during a resting period [10]. Deformation of the bark severely impairs latex flow [11]. Ethylene stimulation is certainly effective to enhance latex production, whereas TPD is undesirable because it leads to severe reduction in latex yield. Susceptibility to TPD is variable and clone dependent; clones that show low latex production may be effectively stimulated by ethylene application and are TPD tolerant, whereas clones that show high latex metabolism are more susceptible to TPD [11]. Therefore, a means of tapping and ethylene stimulation for maximal induction of latex yield without causing TPD is desired. To this end, further studies are required to improve knowledge of the molecular mechanism of the ethylene response in Para rubber tree.

Several types of approaches, including transcriptome analyses, have been conducted previously to explore the molecular mechanisms involved in responses to ethylene of the Para rubber tree [11–18]. Transcriptome analysis is a powerful approach to understand gene regulatory mechanisms. However, previous studies have provided only limited information on ethylene-specific events in Para rubber trees because ethephon was applied to tapping panels of mature trees in a plantation before or after tapping and, therefore, the effects of wounding were not excluded [15, 19]. Thus, an experimental system suitable for analysis of the ethylene-specific response must be established. In the present study, we used young seedlings of Para rubber tree for ethephon and mock treatments to obtain a time-dependent gene expression profile as a model system. A large number of seedlings of Para rubber tree can be readily cultivated in a greenhouse and an experimental garden, and can be uniformly treated, in contrast to mature trees in plantations. Dynamic and specific changes in gene expression profiles were revealed in response to ethephon treatment. The study provides valuable information on the biochemical and metabolic responses to ethylene in Para rubber trees, in addition to the establishment of a model experimental system useful for studying latex biology and rubber biosynthesis.

Materials and methods

Plant materials

Para rubber tree (H. brasiliensis clone PB260) seeds were collected in the field of PT. Bridgestone Sumatra Rubber Estate, Serbalawan, North Sumatra, Indonesia. These seeds were germinated and the seedlings cultivated in the experimental greenhouse of the Agency for the Assessment and Application of Technology (BPPT), Serpong, Tangerang Selatan, Indonesia. Approximately 6-week-old seedlings, which were about 50 cm in height, were used for experiments. The stem region 2–5 cm below the shoot apex was swabbed with either 2.5% Ethrel® (containing 24% ethephon; Bayer CropScience, Inc.) or distilled water (mock control) using small brushes at around 09:00 in the greenhouse. At three time points (6, 24, and 48 h) after treatment, the treated stem region (3 cm long) was collected and placed in small plastic bags, immediately frozen in liquid nitrogen, and stored at –80 °C until used for RNA extraction.
RNA extraction, reverse transcription (RT), and quantitative RT-PCR

For RNA extraction, the stem segment was ground with a mortar and pestle in liquid nitrogen. The ground sample was homogenized in cetyltrimethylammonium bromide (CTAB) reagent containing 2% (w/v) CTAB, 2.5% (w/v) PVP-40, 100 mM Tris-HCl (pH 7.5), 25 mM EDTA (pH 8.0), 2 M NaCl, and 2% 2-mercaptoethanol, then treated with chloroform:isoamyl alcohol (24:1, v/v). The aqueous phase was incubated with 3 M LiCl at 4 °C overnight, then RNA was precipitated and the solution was centrifuged at 13000 rpm for 20 min at 4 °C. The pellet was purified with the Plant RNeasy Mini Kit (QIAGEN) following the manufacturer’s instructions. To degrade genomic DNA, the RNA was treated with recombinant DNase I (TAKARA Bio). The quality of the RNA was analyzed with an Agilent 2100 Bioanalyzer using the RNA 6000 Nano Kit (Agilent). One microgram of RNA and 2.5 μM oligo(dT) primer were used for reverse transcription with the PrimeScript® RT Reagent Kit (TAKARA Bio). The cDNA was appropriately diluted and used for quantitative RT-PCR analysis with the Power SYBR® Green PCR Master Mix and Applied Biosystems 7300 Real Time PCR System (Thermo Fisher Scientific). Primer sets used for each gene are listed in Table S1.

Microarray and data analysis

Two hundred nanograms of RNA was used for microarray analysis using the custom microarray for H. brasiliensis clone PB260 (8 × 60 K) in accordance with the manufacturer’s instructions (Agilent). Three biological replicates of each sample were analyzed using the one-color method. The total signal value was divided by the median value of all probes for global normalization of the microarray data. The statistical significance of the difference between the ethephon and mock treatments was examined using Welch’s t-test. The differentially expressed genes (DEGs) were selected as the genes induced more than 10-fold in the ethephon-treated sample with \( P < 0.01 \) at either time point. The corresponding \( Q \) value [20] for each time point was 0.0279, 0.0155, and 0.0506; therefore, we consider the false discovery rate to have been adequately controlled. The collected 3270 genes were classified into 6 groups by \( K \)-means clustering of Cluster3 software with default setting [21]. For the enrichment analysis, the collected 3270 genes were mapped to non-redundant 1775 Arabidopsis loci by BLASTX search against Arabidopsis TAIR10 coding sequence dataset with the cut-off E-value = 0.00001 (https://www.arabidopsis.org/) because gene annotation information in rubber tree is not sufficient. Then, enrichment of particular Arabidopsis gene ontology term was assessed one by one with a self-made program installed within an in-house database system, which uses the binomial test function of R software (https://www.r-project.org/). The \( P \) value less than 0.05 was considered as statistically significant. All custom microarray data including platform data has been deposited in Gene Expression Omnibus of NCBI under the accession number GSE174832.

Results and discussion

Sample preparation

Gene expression in response to ethylene or ethephon, an ethylene-releasing compound, has been analyzed previously in latex or bark of mature Pará rubber trees subject to tapping [11, 13, 14]. However, ethylene- or ethephon-specific gene expression in virginal mature trees has not been reported. By contrast, Duan et al. (2010) treated 3-month-old young trees with ethylene gas without wounding to analyze the expression of a set of 25 selected genes [12]. In the present study, we used approximately 6-week-old seedlings to examine changes in gene expression profiles in response to ethylene treatment by means of microarray analysis. Rubber tree stems were swabbed with ethephon or water (mock control). At 6 and 24 h after treatment, no visible change was observed between the treatments, whereas some leaves had become yellow and abscised at 48 h after ethephon treatment. This response was interpreted as ethephon-induced senescence and is a typical ethylene response in plants [22]. At 6, 24, and 48 h after treatment, the treated stem segments were harvested for microarray analysis. The amount of latex exudate from the ethephon-treated stems was much higher than that of the mock control (data not shown). Taken together, these results suggested that the experimental system successfully mimicked, at least in part, the physiological and biochemical responses to ethylene stimulation.

Differentially expressed gene set

In this study, we used a custom microarray that contained 61,657 probes corresponding to each transcript, of which 41,656 showed sequence homology to Arabidopsis genes. We compared the expression level between the ethephon and mock treatments at each sampling time point to assess changes in the gene expression profile in response to ethephon treatment. As a result, 3270 genes showed a significant difference in expression between the ethephon and mock treatments at any time point; \( p < 0.01 \). To characterize the DEGs, we categorized the genes into six groups (groups G0 to G5) using \( K \)-means clustering and performed enrichment analysis using gene ontology terms (Fig. 1, Table S2). Approximately one-third of the DEGs were upregulated after ethephon treatment and were categorized as groups G0 or G1. Genes in G0 showed
continuous upregulation from 6 or 24 h to 48 h after ethephon treatment, whereas genes in G1 showed up-regulation from 6 h but expression subsequently declined almost to the control level at 48 h. Genes for protein kinases, stress responses, and transcription factors were enriched in these groups. Genes that showed continuous downregulation until the end of the experimental period were categorized as G2 or G3. These groups contained many genes associated with chloroplasts and involved in cell growth. Genes that showed striking downregulation at 48 h after treatment were categorized as G4. Genes involved in secondary cell wall biosynthesis or cuticle development were enriched in this group. The smallest group was G5. Genes in this group showed downregulation immediately after ethephon treatment, and thereafter expression declined almost to the control level at 24 or 48 h after treatment. Genes involved in amino acid transport and metabolic processes were enriched in G5. We examined the expression of the DEGs more extensively in the following analyses.

Rubber biosynthesis genes
Natural rubber is predominantly composed of cis-1,4-polyisoprene, which is a polymer of an isoprenyl unit derived from isopentenyl diphosphate (IPP). IPP is synthesized by the cytosolic mevalonate (MVA) pathway and the plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway in plants [23]. The former pathway is considered to mainly supply IPP for rubber biosynthesis in Pará rubber tree [18, 24, 25]. Ethephon treatment is reported not to stimulate the MVA pathway and therefore the contribution of this pathway to the increase in yield in response to ethephon treatment would be minimal [5, 26]. As expected, no gene encoding an enzyme involved in the MVA pathway was included among the DEGs. Certain genes, including 3-hydroxy-3-methylglutaryl coenzyme A synthase reductase, were slightly decreased by ethephon treatment, which is consistent with a previous report [16]. In the MEP pathway, however, genes encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMEK) were significantly
upregulated by ethephon treatment (Fig. 2). The MEP pathway is considered to contribute to the supply of IPP for carotenoids biosynthesis [24, 27] and DXS plays an important role in this process [28]. Upregulation of DXS and CMEK was observed in rubber trees suffering from TPD, under which ROS are accumulated [17]. Carotenoids are believed to act as antioxidants and have been proposed to be sensors or signals of oxidative stress induced by ROS [27]. The upregulation of genes in the MEP pathway in response to ethephon treatment may be for production of carotenoids to scavenge ROS. In addition, plants produce other antioxidants and control the ROS-scavenging system to maintain redox homeostasis [29]. The present microarray data revealed the downregulation of superoxide dismutase (SOD), and the phi and theta families of glutathione S-transferase (GST) genes (Table 1). To overcome oxidative stress, GSTs are important and should be abundant. Thus, genes that encode members of the plant-specific tau family of GSTs were highly induced in response to ethephon treatment (Table 1). The tau family of GSTs is considered to be involved in abiotic stress responses and overexpression of these genes confers salt and osmotic tolerance [30, 31]. These results suggest that specific pathways for oxidative stress tolerance were induced in response to ethephon treatment.

![Fig. 2](image_url)

**Fig. 2** Differentially expressed genes (DEGs) involved in the MEP pathway and ABA biosynthesis. Fold change in gene expression for DEGs involved in the MEP pathway and ABA biosynthesis is displayed as color bars (from left to right; 6, 24, and 48 h after ethephon treatment). Graphs show actual gene expression for these genes, which was confirmed by quantitative RT-PCR analysis. Gray and black bars represent the expression level in mock and ethephon samples, respectively.

| Table 1 DEGs of SOD and GST family |
|------------------------------------|
| **Locus** | **Short description** | **Et/Mock (Ln)** |
| **SOD; superoxide dismutase from SALAD** |
| 2 | bx020280 | AT1G08830 CSD1 | −3.44 | −1.75 | −3.72 |
| 3 | bx066811 | AT1G08830 CSD1 | −1.08 | −3.23 | −3.10 |
| **Glutathione S-transferase family from TAIR** |
| **Phi family** |
| 3 | bx063742 | AT3G03190 ATGSTF6, ATGSTF11 | −1.09 | −1.67 | −2.51 |
| **Tau family** |
| 0 | bx026931 | AT2G29420 ATGSTU7 | 3.66 | 5.29 | 2.77 |
| 1 | bx031189 | AT2G29420 ATGSTU7 | 5.78 | 6.35 | 3.88 |
| 1 | bx037937 | AT2G29420 ATGSTU7 | 1.87 | 3.27 | 0.26 |
| 0 | bx038747 | AT2G29420 ATGSTU7 | 4.03 | 5.20 | 4.91 |
| 4 | bx045475 | AT2G29420 ATGSTU7 | −0.88 | −0.95 | −2.38 |
| 1 | bx038733 | AT3G09270 ATGSTU8 | 3.12 | 3.70 | 0.98 |
| 1 | bx001704 | AT1G78380 ATGSTU19 | 6.10 | 5.91 | 4.14 |
| 2 | bx019040 | AT1G17180 ATGSTU25 | −2.66 | −2.13 | −2.79 |
| **Theta family** |
| 5 | bx012945 | AT5G41210 ATGSTT1 | −3.78 | −2.51 | −0.97 |

*Clustering group
Polymerization of IPP derived from MVA into cis-1,4-polyisopren e is catalyzed by cis-prenyltransferases (CPTs). It has been demonstrated that CPTs are localized on the surface of rubber particles [32]. In addition, two important protein groups for rubber biosynthesis are localized on the surface of rubber particles: small rubber particle proteins (SRPPs) and rubber elongation factors (REFs) [33]. Two rubber tree genomes have been sequenced to date (Reyan7–33-97 in Tang et al. 2016; RRIM 600 in Lau et al. 2016) [18, 25], and all members of each protein group have been revealed [18, 25]. We first confirmed that the probe sets corresponding to these genes were included in the present microarray because we designed the probe sets based on the comprehensive cDNA sequencing data derived from rubber tree clone PB260, which differs from the clones used for genome-sequencing projects. In our probe set, probes corresponding to CPT1, CPT2, CPT9, CPT11, REF2, REF4, REF6, and SRPP10 were not included because the expression levels of these genes were relatively low [25]. We observed that only one gene for SRPP4 [25] was induced by ethephon treatment, whereas no other genes that encode CPT, SRPP, or REF proteins were detected among the DEGs (Table S3). The basal expression level of SRPP4 was low and laticifer-specific genes (REF1, REF3, REF7, and SRPP1), for which transcripts were abundant in latex and accounted for 96.8% of the expression of REF/SRPP genes in latex [25], were not induced by ethephon treatment. Taken together, the results suggested that ethephon treatment might have little direct effect on rubber biosynthesis, which is consistent with previous reports [5, 19, 25].

Ethylene biosynthesis and signaling

In plants, ethylene induces expression of genes involved in ethylene biosynthesis. For instance, expression of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase gene is reported to be regulated by a positive feedback mechanism [34]. The present microarray data revealed the upregulation of genes for S-adenosyl-methionine synthetase and ACC synthase after ethephon treatment, and that expression of these genes returned to the control level at 48 h after treatment. The present data supported the claim for positive feedback regulation in ethylene biosynthesis after ethephon treatment (Table 2).

ABA biosynthesis

The present results indicated that the MEP pathway is activated and supplied IPP for carotenoids biosynthesis in response to ethephon treatment, and that these carotenoids may contribute to protection from ROS. Abscisic acid (ABA) is derived from carotenoids [27]. We observed that ethephon treatment induced the expression of genes involved in ABA biosynthesis and repressed expression of genes that participate in ABA catabolism (Fig. 2), which suggests de novo ABA biosynthesis and accumulation. Previous studies have demonstrated the induction of ABA biosynthesis by ethylene [35, 36]. Thus, carotenoids production induced by ethylene treatment would supply not only antioxidants but also precursors of ABA. It is well known that ABA induces leaf abscission and ethylene accelerates this process in the presence of ABA [37, 38]. We observed that some leaves abscised at 48 h after ethephon treatment (data not shown), which might be initiated by the induced ABA.

Transporters

Abscisic acid is synthesized in response to drought stress and can induce stomatal closure to prevent water loss by transpiration. Previous experiments have shown that ABA signaling induces certain aquaporins, which results in increased hydraulic conductivity and water potential [39–42]. A remarkable response to ethephon treatment is the prolonged flow of latex. The prolonged latex flow may be because water influx into laticifer cells mediated by aquaporins is increased [14, 43–45]. In the present study, expression of genes that encode plasma membrane intrinsic protein (PIP) 1;2 and 1;5 is induced, whereas the expression of PIP2;7 and tonoplast intrinsic protein (TIP) 1;1 is decreased by ethephon treatment (Table 3). PIPs import water into laticifer cells, thus the turgor pressure is increased in laticifer cells [44], whereas TIPs maintain the stability of lutoid membranes and/or cell osmotic balance [46]. The present findings suggested that water status may be dramatically changed in the cells in response to ethephon treatment.

Sucrose is an important molecule for rubber production because it is the unique precursor of IPP and is expected to be imported into laticifers cells from surrounding cells. Previous studies indicate that expression of sugar transporters is induced by ethylene, which contributes to high latex production [47–49]. Six genes encoding sucrose transporters (SUTs) have been cloned from Pará rubber tree [47–49]. We observed that the probe sets shared high nucleotide sequence identities (~99%) with these genes. Although previous studies have reported upregulation of certain SUT genes by ethylene [47–49], the present microarray analysis detected down-regulation of two such genes (Table 3). Moreover, several genes encoding glucose and/or sucrose efflux transporter family proteins (SWEET) were downregulated (Table 3). In addition to these transporters, genes encoding a sugar transport protein (STP) and one polyol transporter (PLT) were upregulated by ethephon treatment. The STPs are monosaccharide/H+ symporters and uptake hexoses from the apoplastic space into cells...
through the plasma membrane [50]. Sucrose is the predominant molecule utilized for carbon partitioning between sources and sinks in higher plants, and is transported directly into the sink cells. Sucrose is otherwise cleaved into glucose and fructose by cell wall-type invertases (INVs), and these monosaccharides are transported into the cell by the STPs [51, 52]. The present microarray data revealed the upregulation of a gene that encodes a cell wall-type INV (Table 3), which suggests possible co-upregulation of STPs and INVs for monosaccharide uptake. Substantially higher and continuous induction of a gene encoding a PLT, which is a polyol/H+ symporter localized to the plasma membrane or tonoplast, was observed in response to ethephon treatment (Table 3). Two HbPLT genes (HbPLT1 and HbPLT2) have been identified and their expression pattern in response to ethephon has been demonstrated [53], but the present microarray data detected no significant changes in the expression of these genes. These data suggested the possibility of a tissue- and/or age-dependent mechanism for expression of PLT genes in response to ethylene in Pará rubber tree.

The ABC transporters are among the largest protein families in plants and 130 genes have been predicted in the Arabidopsis genome [54]. Given that ABC transporters translocate diverse types of substances, including inorganic compounds, phytohormones, primary products, and lipids, plant ABC transporters are considered to play an important role in plant growth and development, detoxification, response to abiotic stress, and pathogen resistance [54, 55]. Expression of many members of the ABC transporter family is induced in a rubber tree suffering TPD [17]. We detected 46 genes for ABC transporters in the Pará rubber tree transcriptome,

Table 2 DEGs of ERF family and ethylene biosynthesis

| G0 | Gene ID | Arabidopsis homologue | Short description | Et/Mock (Ln) |
|----|---------|-----------------------|-------------------|-------------|
|    |         | Locus | Short description | 6 h | 24 h | 48 h |
| ERF family |         | ERF group | ERF No |         |         |         |
| 2 | bx047672 | AT2G44940 | llld | ERF34 | −3.57 | −3.48 | −1.37 |
| 2 | bx024041 | AT5G11590 | lle | ERF41 | −2.40 | −2.48 | −2.27 |
| 3 | bx038544 | AT5G25190 | Va | ERF3 | −2.21 | −1.39 | −2.65 |
| 3 | bx046468 | AT5G25190 | Va | ERF3 | −1.20 | −1.38 | −2.49 |
| 0 | bx023675 | AT4G11140 | VI | ERF63 | 2.35 | 2.94 | 2.71 |
| 0 | bx017330 | AT3G16770 | VII | ERF72 | 1.83 | 2.48 | 2.47 |
| 0 | bx022445 | AT3G16770 | VII | ERF72 | 2.81 | 3.05 | 2.14 |
| 0 | bx020251 | AT3G15210 | Vilia | ERF78 | 2.18 | 2.32 | 1.96 |
| 0 | bx039197 | AT4G7500 | IXa | ERF100 | 5.74 | 5.22 | 4.50 |
| 0 | bx039254 | AT4G7500 | IXa | ERF100 | 2.03 | 2.69 | 1.64 |
| 0 | bx046661 | AT4G7500 | IXa | ERF100 | 3.80 | 6.04 | 4.57 |
| 1 | bx046659 | AT5G51190 | IXb | ERF105 | 2.69 | 2.70 | 1.40 |
| 1 | bx071140 | AT3G23230 | IXc | ERF98 | 5.86 | 5.63 | 4.11 |
| 0 | bx038100 | AT3G23240 | IXc | ERF92 | 5.92 | 7.62 | 7.16 |
| 0 | bx040001 | AT3G23240 | IXc | ERF92 | 3.81 | 7.49 | 8.04 |
| 0 | bx058590 | AT3G23240 | IXc | ERF92 | 4.22 | 3.35 | 4.40 |
| 1 | bx027059 | AT3G23240 | IXc | ERF92 | 5.24 | 4.95 | 3.18 |
| 0 | bx011862 | AT5G43410 | IXc | ERF96 | 4.48 | 5.01 | 4.10 |
| 1 | bx033374 | AT5G50080 | Xa | ERF110 | 4.36 | 3.87 | 2.47 |
| 0 | bx037951 | AT5G61890 | Xa | ERF114 | 1.90 | 3.62 | 3.89 |
| 3 | bx059815 | AT4G37750 | – | – | −2.35 | −2.46 | −3.53 |
| 3 | bx007515 | AT5G10510 | – | – | −2.37 | −2.32 | −3.60 |

Ethylene biosynthesis gene

| G0 | Gene ID | Arabidopsis homologue | Short description | Et/Mock (Ln) |
|----|---------|-----------------------|-------------------|-------------|
|    |         | Locus | Short description | 6 h | 24 h | 48 h |
| 1 | bx000299 | AT4G01850 | SAMS2 | 2.69 | 2.50 | 0.14 |
| 1 | bx063180 | AT3G61510 | ACS1 | 3.19 | 2.62 | 0.09 |
| 1 | bx040584 | AT4G11280 | ACS6 | 3.27 | 3.66 | −0.41 |

*Clustering group

*ERF group and No. from Nakano et al. 2006
### Table 3 DEGs of transporters

| Gene ID   | Arabidopsis homologue | Locus | Short description | Et/Mock (Ln) |
|-----------|------------------------|-------|-------------------|-------------|
|           |                        |       |                   | 6H  | 24H  | 48H  |
| **Aquaporin** |                       |       |                   |     |      |      |
| 0         | bx000682               | AT2G45960 | PIP1;2           | 0.69 | 2.50 | 2.64 |
| 0         | bx000130               | AT2G45960 | PIP1;2           | 0.76 | 3.43 | 1.91 |
| 0         | bx013984               | AT2G45960 | PIP1;2           | 0.60 | 3.33 | 1.80 |
| 0         | bx081354               | AT4G23400 | PIP1;5           | 0.98 | 2.42 | 2.86 |
| 5         | bx005180               | AT4G35100 | PIP2;7           | −2.65| −2.61| −0.06|
| 5         | bx071027               | AT4G35100 | PIP2;7           | −2.64| −2.58| −0.04|
| 5         | bx013710               | At2g36830 | TIP1;1           | −3.17| −1.19| −1.00|
| 5         | bx000052               | At2g36830 | TIP1;1           | −3.63| −1.28| −1.25|
| 5         | bx079866               | At2g36830 | TIP1;1           | −3.33| −1.30| −1.11|
| **Monosaccharide transporter** | |       |                   |     |      |      |
| **Monosaccharide (hexoses / pentoses)-H+ symporter family (plasma membrane)** | |       |                   |     |      |      |
| 4         | bx001787               | AT1G11260 | AtSTP1         | −0.25| −0.39| −2.77|
| 1         | bx025866               | AT5G61520 | AtSTP3         | 1.85 | 2.64 | 1.17 |
| 1         | bx012963               | AT5G61520 | AtSTP3         | 2.52 | 1.96 | 0.72 |
| 0         | bx006914               | AT5G26250 | AtSTP8         | 1.85 | 0.26 | 2.76 |
| 1         | bx027564               | AT5G26340 | AtSTP13        | 5.35 | 5.31 | 2.58 |
| 2         | bx055460               | AT1G77210 | AtSTP14        | −3.04| −3.23| −2.81|
| **H + -Symporter family for polyols and monosaccharides (plasma membrane)** | |       |                   |     |      |      |
| 0         | bx046600               | AT2G18480 | AtPLT3         | 6.25 | 6.26 | 6.34 |
| **Putative monosaccharide transporter family (ERD-group = induced by early dehydration)** | |       |                   |     |      |      |
| 0         | bx021636               | AT2G48020 | Major facilitator superfamily protein | 2.01 | 2.93 | 2.45 |
| 0         | bx037209               | AT2G48020 | Major facilitator superfamily protein | 2.01 | 2.98 | 2.49 |
| 2         | bx019596               | AT3G05160 | Major facilitator superfamily protein | −1.84| −2.66| −1.41|
| **Sucrose-proton symporter, SUS, SWEET family** | |       |                   |     |      |      |
| 4         | bx012374               | AT1G22710 | SUT1           | −0.83| −1.54| −2.59|
| 4         | bx013239               | AT1G22710 | SUT1           | −0.83| −1.30| −2.41|
| 5         | bx049225               | AT5G07970 | SWEET10        | −3.89| −1.96| 0.16 |
| 5         | bx019194               | AT5G07970 | SWEET10        | −4.13| −1.95| 0.18 |
| 2         | bx038944               | AT5G13170 | SWEET15        | −2.05| −2.70| −0.85|
| 2         | bx055911               | AT4G15920 | SWEET17        | −2.49| −1.72| −1.35|
| **ABC transporter** | |       |                   |     |      |      |
| 4         | bx013345               | AT2G36910 | AtABC1         | −0.93| −1.00| −2.56|
| 3         | bx063540               | AT4G18050 | AtABC9         | −0.91| −1.89| −2.62|
| 5         | bx008871               | AT3G28345 | AtABC15        | −2.65| −0.18| −1.20|
| 5         | bx047965               | AT3G28345 | AtABC15        | −2.62| −0.08| −1.16|
| 0         | bx059322               | AT3G13080 | AtABC3         | 3.02 | 2.49 | 2.46 |
| 0         | bx053101               | AT2G47800 | AtABC4         | 2.06 | 2.47 | 1.64 |
| 1         | bx013696               | AT2G47800 | AtABC4         | 2.71 | 2.83 | 1.75 |
| 1         | bx050933               | AT3G21250 | AtABC8         | 2.74 | 2.29 | 2.02 |
| 0         | bx002770               | AT3G21250 | AtABC8         | 3.43 | 3.44 | 2.73 |
| 0         | bx029139               | AT3G21250 | AtABC8         | 3.39 | 3.38 | 2.67 |
| 0         | bx014388               | AT3G21250 | AtABC8         | 3.21 | 2.77 | 2.44 |
of which four are known to be upregulated by ethephon treatment [56]. Although no significant changes in expression of these genes was observed, the present results revealed that expression of other ABC transporters was highly responsive to ethephon treatment of seedlings (Table 3). Several genes encoding ABCB proteins putatively localized in the plasma membrane were downregulated, whereas genes encoding ABCC proteins putatively localized in the vacuolar membrane were upregulated (Table 3). Transporters belonging to the ABCC group transport chlorophyll catabolites into the tonoplast [57, 58]. The present microarray data revealed strong upregulation of a gene encoding chlorophyllase, which catalyzes the first step of chlorophyll degradation, and downregulation (or no induction) of genes involved in photosystem and chlorophyll biosynthesis (Fig. 3, Table 4). Quantitative RT-PCR analysis showed that transcripts corresponding to those of chlorophyllase in all mock samples were below the limit of detection. These data indicated that chlorophyll degradation and suppression of photosynthesis occurred in response to ethephon treatment (Fig. 3). Increased quantities of these chlorophyll catabolites would be transported into the tonoplast by ABCC proteins. The co-regulation of genes encoding ABCC proteins and chlorophyllase may contribute to efficient degradation of chlorophylls during ethylene-induced senescence.

Several genes encoding ABCG proteins were upregulated or downregulated (Table 3). Arabidopsis ABCG40 (At1g15520), which is involved in ABA uptake [59], is induced by ethylene [60]. Certain rubber genes corresponding to ABCG40 genes were upregulated by ethephon treatment; this response may be correlated with the upregulation of genes involved in ABA biosynthesis and accumulation (Fig. 2).

### Flavonoid biosynthesis

In the flavonoid biosynthesis pathway, genes for flavonol synthase and dihydroflavonol 4-reductase were downregulated (Fig. 4). To confirm the microarray data, expression of these genes was examined by quantitative RT-PCR analysis. Expression of flavonol synthase in the mock samples increased with time after the mock treatment, whereas expression was maintained at a low level and showed little increase at 48 h after ethephon treatment. These results suggested that ethephon application may increase dihydroquercetin (DHQ) production. DHQ is a strong antioxidant and increases the stability of the tonoplast membrane, on account of its antioxidant properties, and decreases membrane permeability by suppressing ion channels [61]. Therefore, DHQ may play a role in stabilization of lutoid membranes, which are particularly sensitive to osmotic stress. Water influx into the laticifer cells caused by tapping leads to rupture of lutoids and the release of their contents; subsequently,
coagulation of rubber particles and damaged lutoids occurs, which would cause the arrest of latex flow for wound healing and recovery in Pará rubber trees. Thus, lutoid stability influences latex flow [62]. However, inhibition of membrane oxidation by DHQ leads to activation of H^+-ATPase [61]. In the present microarray analysis, upregulation of several genes encoding H^+-ATPases was observed (Table 5). Given that tonoplast H^+-ATPase is activated by ethephon treatment [63], the current results are reasonable. H^+-ATPase in the lutoid membrane plays an important role by pumping protons from the cytosol into the lutoid during active

Table 4 DEGs of photosystem and chlorophyll biosynthesis

| G^a | Probe ID | Arabidopsis homologue^b | Locus | Short description | Et/Mock (Ln) |
|-----|----------|-------------------------|-------|------------------|-------------|
| 5   | bx045370 | AT5G66190 | ferredoxin-NADP(+) oxidoreductase I | bx049388 | −3.65 | −2.52 | −1.41 |
| 2   | bx052092 | AT4G03280 | photosynthetic electron transfer C | bx041863 | −3.06 | −2.18 | −1.51 |
| 2   | bx006007 | AT4G12800 | photosystem I subunit I | bx050932 | −2.88 | −2.06 | −1.94 |
| 2   | bx024863 | AT1G08380 | photosystem I subunit O | bx051011 | −2.67 | −2.67 | −1.90 |
| 2   | bx079126 | AT1G08380 | photosystem I subunit O | bx051011 | −1.68 | −2.47 | −1.53 |
| 5   | bx022553 | AT2G30570 | photosystem II reaction center W | bx027740 | −3.24 | −2.66 | −1.15 |
| 2   | bx020447 | AT3G50820 | photosystem II subunit O-2 | bx039366 | −2.31 | −2.19 | −1.38 |
| 3   | bx039366 | AT3G30790 | photosystem II subunit P-2 | bx049802 | −2.33 | −3.74 | −3.22 |
| 2   | bx049802 | AT3G01440 | photosystem II oxygen-evolving enhancer protein 3 | bx039366 | −4.14 | −3.51 | −1.81 |
| 3   | bx039366 | AT5G54190 | protochlorophyllide oxidoreductase A | bx049802 | −1.94 | −2.34 | −3.21 |
| 1   | bx041863 | AT1G19670 | Chlorophyllase 1 | bx020447 | 7.33 | 5.62 | 2.24 |

*aClustering group
*bGenes from KEGG pathway
carbohydrate metabolism to supply carbon sources for latex biosynthesis by maintaining a cell pH suitable for pH-dependent enzymes, such as INV [63, 64]. High-yielding rubber clones show high activity of H⁺-ATPase in lutoids [64]. These responses would be natural mechanisms for defense and recovery from wounding caused by tapping. Taken together, ethephon treatment-induced enhancement of DHQ biosynthesis might be involved in enhanced latex yield via activation of the substrate supply for rubber biosynthesis and the extension of latex exudation by stabilizing lutoid membranes and consequent restraint of coagulation. Based on this hypothesis, excess tapping and/or ethylene stimulation may cause catastrophic changes resulting in TPD.

Transcription factors
During the ethylene response, dynamic changes in the expression of a diverse array of genes occur in plants [65]. Transcription factors control gene transcription in response to various environmental cues and developmental factors. In the present microarray analysis, 2641 probes detected transcription factor genes, of which 236 were among the DEGs (Table S4). Certain transcription factor families, including the ERF, NAC, and WRKY families, are involved in stress responses and ethephon treatment affected expression of these genes.

The ERF family, which is a major constituent of the AP2/ERF superfamily, is a large and plant-specific transcription factor family and members are classified into 10 groups [66]. The microarray data revealed that genes belonging to group IX were highly upregulated by ethephon treatment (Table 2). In addition, genes classified in groups VI, VII, VIII, and X were upregulated, whereas genes of groups III and V were downregulated.

The WRKY transcription factor family is a plant-specific transcription factor family that participates in abiotic/biotic stresses responses, and diverse developmental and physiological processes [67, 68]. Two WRKY transcription factors (WRKY18 and 40) are induced by ABA, and physically and functionally interact under biotic and abiotic stresses in Arabidopsis [69, 70]. In the current study, expression of genes corresponding

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**Table 5** DEGs of ATPase family

| G* | Gene ID | Arabidopsis homologue | Short description | Et/Mock (Ln) | 6 h | 24 h | 48 h |
|----|---------|-----------------------|------------------|-------------|-----|-----|-----|
| 1  | bx014049| AT2G24520 | H⁺-ATPase 5 | 3.32 | 2.37 | 2.10 |
| 1  | bx041129| AT2G22950 | Autoregulated Ca²⁺-ATPase 7 | 3.60 | 2.89 | 0.35 |
| 0  | bx047876| AT5G57110 | Autoinhibited Ca²⁺-ATPase 8 | 1.49 | 1.13 | 2.97 |
| 1  | bx038015| AT3G63380 | ATPase E1-E2 type family protein | 4.82 | 4.40 | 2.58 |
| 0  | bx021597| AT3G01390 | Vacuolar membrane ATPase 10 | 1.71 | 2.43 | 1.70 |

*aClustering group
WRKY18 and 40 was induced by ethephon treatment (Table 6). This finding is consistent with the gene expression changes suggesting that ABA content might be increased in Pará rubber trees in response to ethephon treatment (Fig. 2). Previous studies of Arabidopsis have shown that several WRKY transcription factors are involved in the promotion (WRKY6, 53, and 75) or delay (WRKY54 and 70) of leaf senescence.

WRKY75 expression is induced in senescent leaves and knockout of WRKY75 results in a delayed-senescence phenotype in Arabidopsis, which indicates that WRKY75 is a positive regulator of leaf senescence [71]. The present microarray analysis detected upregulation of genes corresponding to WRKY53, 70, and 75. In particular, expression of the gene corresponding to WRKY75 was highly increased by ethephon treatment and the upregulation was sustained for 48 h (Table 6).

The NAC transcription factor family is a plant-specific transcription factor family that comprises many members. The NAC transcription factors are involved in many developmental processes, including stress response and secondary cell wall formation [72]. Expression of ANAC029 is induced by leaf senescence and the gene directly regulates AAO3 expression in Arabidopsis [73, 74]. Enhanced expression of AAO3 increases ABA content, which may induce chlorophyll degradation during leaf senescence [74]. We observed a similar expression profile in response to ethephon treatment (Figs. 2 and 3, Table 7). Genes encoding NST1 and SND2, which regulate secondary cell wall formation [75], were

### Table 6 DEGs of WRKY family

| G* | Gene ID | Arabidopsis homologue | Locus | WRKY group | WRKY No | Et/Mock (Ln) |
|----|---------|-----------------------|-------|------------|---------|-------------|
|    | bx007583 | AT1G13960 | I     | WRKY4      | 2.95    | 3.80        | 2.79        |
| 0  | bx005246 | AT1G29960 | I     | WRKY71     | 1.40    | 1.98        | 2.70        |
| 0  | bx049005 | AT1G29860 | I     | WRKY71     | 0.80    | 2.42        | 1.26        |
| 0  | bx051016 | AT1G29860 | I     | WRKY71     | 1.08    | 1.72        | 2.45        |
| 1  | bx029622 | AT2G30250 | I     | WRKY25     | 2.85    | 2.31        | 1.53        |
| 1  | bx026110 | AT2G38470 | I     | WRKY33     | 3.80    | 3.35        | 0.50        |
| 1  | bx026599 | AT2G38470 | I     | WRKY33     | 2.81    | 2.34        | 1.11        |
| 1  | bx050263 | AT2G38470 | I     | WRKY33     | 2.76    | 2.81        | 1.96        |
| 1  | bx066153 | AT2G38470 | I     | WRKY33     | 3.58    | 2.95        | 0.15        |
| 1  | bx081907 | AT4G31800 | II-a  | WRKY18     | 3.81    | 3.79        | 1.60        |
| 1  | bx023458 | AT1G80840 | II-a  | WRKY40     | 2.95    | 3.67        | −0.47       |
| 1  | bx038227 | AT1G80840 | II-a  | WRKY40     | 5.96    | 4.82        | 3.37        |
| 1  | bx047918 | AT1G80840 | II-a  | WRKY40     | 3.78    | 2.80        | 0.65        |
| 1  | bx059684 | AT1G80840 | II-a  | WRKY40     | 3.83    | 3.77        | 1.45        |
| 0  | bx077810 | AT4G22070 | II-b  | WRKY31     | 1.79    | 3.12        | 3.73        |
| 0  | bx08303 | AT1G62300 | II-b  | WRKY6      | 2.62    | 2.78        | 2.68        |
| 1  | bx042142 | AT1G62300 | II-b  | WRKY6      | 2.41    | 1.88        | 1.58        |
| 0  | bx06204 | AT5G13080 | II-c  | WRKY75     | 5.38    | 6.97        | 6.83        |
| 0  | bx046970 | AT5G13080 | II-c  | WRKY75     | 4.74    | 4.91        | 4.52        |
| 0  | bx054083 | AT5G13080 | II-c  | WRKY75     | 6.34    | 7.10        | 6.22        |
| 0  | bx061693 | AT5G13080 | II-c  | WRKY75     | 5.31    | 5.97        | 5.82        |
| 1  | bx032062 | AT5G64810 | II-c  | WRKY51     | 4.48    | 4.55        | 1.34        |
| 2  | bx065502 | AT5G43290 | II-c  | WRKY49     | −4.24   | −2.96       | −4.20       |
| 2  | bx009275 | AT5G28650 | II-d  | WRKY74     | −2.78   | −1.44       | −2.05       |
| 1  | bx026667 | AT4G11070 | III   | WRKY41     | 2.80    | 3.67        | 0.27        |
| 1  | bx062508 | AT4G23810 | III   | WRKY53     | 2.84    | 2.87        | 0.01        |
| 1  | bx002887 | AT3G56400 | III   | WRKY70     | 2.15    | 2.95        | −0.09       |
| 1  | bx023296 | AT3G56400 | III   | WRKY70     | 2.02    | 2.74        | −0.36       |

*Clustering group

bWRKY group and number from TAIR
Table 7 DEGs of NAC family

| G^ | Gene ID | Arabidopsis homologue^b | Locus No | Other name | Et/Mock (Ln) |
|----|---------|-------------------------|----------|------------|--------------|
|    |         |                         |          |            | 6 h  | 24 h  | 48 h  |
| 0  | bx038862| AT1G69490 ANAC029       | 0        | NAP        | 1.24 | 2.30  | 3.09  |
| 0  | bx074452| AT1G69490 ANAC029       | 0        | NAP        | 1.12 | 2.19  | 2.97  |
| 1  | bx024871| AT2G17040 ANAC036       | 1        | NAP        | 2.97 | 3.35  | 1.88  |
| 1  | bx071860| AT5G22380 ANAC090       | 1        | NAP        | 3.52 | 1.96  | 0.51  |
| 3  | bx064839| AT5G22380 ANAC090       | 3        |            | −0.29| −1.86 | −2.55 |
| 4  | bx009169| AT2G6770 ANAC043 NST1   | 4        |            | 1.12 | −2.46 | −2.68 |
| 4  | bx029117| AT2G6770 ANAC043 NST1   | 4        |            | −1.35| −2.06 | −4.05 |
| 4  | bx03373 | AT4G28500 ANAC073 SND2  | 4        |            | −1.57| −0.69 | −3.11 |
| 4  | bx041479| AT4G28500 ANAC073 SND2  | 4        |            | −1.53| −0.91 | −4.57 |
| 4  | bx065762| AT4G28500 ANAC073 SND2  | 4        |            | −1.45| −1.10 | −4.37 |
| 4  | bx065953| AT4G28500 ANAC073 SND2  | 4        |            | −1.26| −0.46 | −3.48 |
| 5  | bx026186| AT3G15510 ANAC056       | 5        |            | −2.75| −1.62 | −1.52 |
| 5  | bx036393| AT3G15510 ANAC056       | 5        |            | −2.41| −1.41 | −1.39 |
| 5  | bx040482| AT3G15510 ANAC056       | 5        |            | −3.06| −0.89 | −2.12 |
| 5  | bx052893| AT3G15510 ANAC056       | 5        |            | −2.54| −1.45 | −1.36 |
| 5  | bx070347| AT3G15510 ANAC056       | 5        |            | −2.45| −1.38 | −1.32 |

^Clustering group
^NAC group and number from TAIR

Table 8 DEGs of IRX genes

| G^ | Gene ID | Arabidopsis homologue | Locus No | Short description | Et/Mock (Ln) |
|----|---------|-----------------------|----------|------------------|--------------|
|    |         |                       |          |                  | 6 h  | 24 h  | 48 h  |
| 4  | bx048993| AT4G18780 IRX1 CesA8  | 4        |                  | −1.26| −1.49 | −5.17 |
| 4  | bx059326| AT4G18780 IRX1 CesA8  | 4        |                  | −1.16| −1.22 | −4.90 |
| 4  | bx02167 | AT5G17420 IRX3 CesA7  | 4        |                  | −1.11| −1.62 | −4.41 |
| 4  | bx025747| AT5G44030 IRX5 CesA4  | 4        |                  | −1.56| −2.00 | −4.44 |
| 4  | bx073220| AT5G44030 IRX5 CesA4  | 4        |                  | −1.69| −1.72 | −5.59 |
| 3  | bx06479 | AT5G15630 IRX6 COBRA-LIKE4 | 3   |                  | −2.02| −1.82 | −2.69 |
| 4  | bx07065 | AT5G15630 IRX6 COBRA-LIKE4 | 4   |                  | −1.35| −1.66 | −5.85 |
| 4  | bx06479 | AT5G15630 IRX6 COBRA-LIKE4 | 4   |                  | −1.11| −1.61 | −5.64 |
| 3  | bx007251| AT2G37090 IRX9 GT43   | 3        |                  | −0.77| −2.40 | −3.57 |
| 2  | bx048506| AT1G27600 IRX9-L GT43 | 2        |                  | −3.20| −2.60 | −2.58 |
| 4  | bx004786| AT1G27440 IRX10 GT47  | 4        |                  | −1.37| −1.40 | −5.06 |
| 4  | bx041075| AT1G27440 IRX10 GT47  | 4        |                  | −1.69| −1.84 | −4.79 |
| 4  | bx080788| AT1G27440 IRX10 GT47  | 4        |                  | −1.06| −1.18 | −4.54 |
| 4  | bx007461| AT2G38080 IRX12 LACCASE 4 | 4   |                  | −1.83| −1.80 | −5.35 |
| 4  | bx023195| AT2G38080 IRX12 LACCASE 4 | 4   |                  | −1.13| −1.47 | −5.28 |
| 4  | bx072483| AT2G38080 IRX12 LACCASE 4 | 4   |                  | −1.05| −2.22 | −5.01 |
| 4  | bx03921 | AT5G03170 IRX13 FLA11 | 4        |                  | −1.53| −1.39 | −5.14 |
| 3  | bx022936| AT5G67210 IRX15-L DUF579 | 3   |                  | −2.50| −2.37 | −2.91 |
| 4  | bx025175| AT5G67210 IRX15-L DUF579 | 4   |                  | −1.38| −1.79 | −6.14 |

^Clustering group
downregulated following ethephon treatment (Table 7). Their downstream genes, including *Irregular Xylem* (*IRX*), were also downregulated (Table 8). Thus, ethephon treatment may prevent stem growth, including development of xylem and/or fiber cells.

**Conclusion**

The aim of this study was to assess the molecular mechanism of the response to ethylene stimulation in Pará rubber tree with the goal of improving latex production by inhibition of TPD. We established an experimental system using young seedlings and obtained comprehensive transcriptome data during the treatment response. Based on the present results, we propose a schematic model of the events in response to ethephon treatment (Fig. 5). The ethephon treatment induces changes in gene expression associated with carotenoids production. Carotenoids and a downstream product, ABA, may induce expression of PIP genes [27, 38–40], which serve to import water into laticifer cells, resulting in enhanced latex flow. In addition, changes in expression of genes that participate in flavonoids biosynthesis, especially genes associated with DHQ production, were observed. Given that DHQ is believed to contribute to stabilization of lutoid membranes [61], latex exudation would be prolonged, which may suppress coagulation of rubber particles. This series of molecular events may be involved in the increase in latex yield caused by ethylene stimulation. Given that carotenoids and DHQ are known ROS scavengers, ethephon-induced upregulation of genes involved in their biosynthesis may enhance defense responses and contribute to prevention of TPD. It should be noted that ethylene is produced in Pará rubber tree following wounding; therefore, these events may naturally occur during tapping as defense responses or for wound healing.

Ethephon treatment induced chlorophyll degradation, which is the most typical event of leaf senescence [76]. In the present study, some leaves became yellow and abscised at 48 h after ethylene stimulation; thus, ethephon treatment might induce leaf senescence. The induction of many senescence-associated genes is observed in TPD-affected trees subject to excessive tapping or over-stimulation with ethylene [9, 77]. Therefore, upregulation of genes involved in chlorophyll degradation might be an indicator of the TPD syndrome derived from over-stimulation with ethylene. To prevent chlorophyll degradation and maintain photosynthesis is important for sucrose production, which influences NR biosynthesis.

The present results indicate that ethephon treatment may affect the growth and/or differentiation of cambium and/or laticifer cells. In this study, we observed that genes associated with secondary cell wall formation were highly downregulated at 48 h after ethephon treatment. In woody plants, secondary cell walls are an important carbon sink [78]. Thus, these results suggest that there is a trade-off in carbon supply between rubber biosynthesis and secondary cell wall synthesis after tapping and ethylene stimulation. Otherwise, secondary cell wall formation or biosynthesis might be aberrantly regulated in the secondary phloem because phloem necrosis and abnormal cell layers in the secondary phloem and parenchyma tissues are also observed as symptoms of irreversible TPD [79, 80].
In this study, we have demonstrated that ethephon treatment caused dramatic positive and negative changes in expression of a diverse array of genes involved in metabolism, defense responses, growth, and development. It is suggested that ethephon treatment induced the changes negatively associated with rubber production and regulation of the trade-off between rubber production and other events, in addition to the changes positively associated with rubber production. These changes may cause induction of TPD, which can be triggered by excess tapping and/or ethylene stimulation. The results provide valuable information to understand the mechanism of ethylene stimulation, and will contribute to improved management practices and/or molecular breeding to attain higher yields of latex from Pará rubber trees.

Abbreviations
ABA: Abscisic acid; ACC: 1-aminoacyclopropene-1-carboxylic acid; CMEK: 4-cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; CPT: cis-prenyltransferase; DEG: Dihydroquercetin; DSX: 1-deoxy-D-xylolose 5-phosphate synthase; GGPPS: Geranylgeranyl diphosphate synthase; GST: Glutathione S-transferase; INV: Invertase; IPP: Isopentenyl diphosphate; MPT: 2-C-methyl-D-erythritol 4-phosphate; MVA: Mevalonate; NR: Natural rubber; PIP: Plasma membrane intrinsic protein; PLT: Polyol transporter; REF: Rubber elongation factor; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SUT: Sucrose transporter; TIP: Tonoplast intrinsic protein; TPD: Tapping panel dryness

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-021-03196-y.

Additional file 1: Table S1. Primer sets used for quantitative RT-PCR analysis.
Additional file 2: Table S2. Probe list and fold change of 3270 genes that showed a significant difference in expression between the ethephon and mock treatments.
Additional file 3: Table S3. Corresponding probe sets for CPT, REF, and SODP genes.
Additional file 4: Table S4. Probe lists for 234 differentially expressed genes encoding transcription factors.

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Authors’ contributions
YN, KL, FRM, SR, NW, and KS designed the study. YN, KL, and FRM performed ethylene treatment and collecting samples. KL, TM, and NW designed the microarray. YN prepared materials and YN and NM performed microarray analysis and data analysis. YN, NM, and KS wrote the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials
All data analyzed in this study is available upon request. All custom microarray data including platform data has been deposited in Gene Expression Omnibus of NCBI under the accession number GSE174832.

Declarations
Ethics approval and consent to participate
All methods were in compliance with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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